STUDIES OF METAL IONS INTERACTION WITH PURINE - PYRIMIDINE BASE PAIR

A
REPORT ON
Laboratory Project submitted for the partial
fulfilment of the degree
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in
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By

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled "**Studies Of Metal Ions Interaction With Purine-Pyrimidine Base Pair**" in partial fulfilment of the requirement for the award of the Degree of **Master of Philosophy** submitted in the **Department of Chemistry** of the University is an authentic record of my own work carried out during a period from January, **98** to **July 98** under the supervision of **Dr. Udai P. Singh**, Asstt. Professor, Department of Chemistry, University of Roorkee, Roorkee.

The matter embodied in this thesis has not been submitted by me for the award of any other degree.

Lokesh Kumar

Date : 30 · 07 · 98 **(LOKESH KUMAR)**

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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Abstract

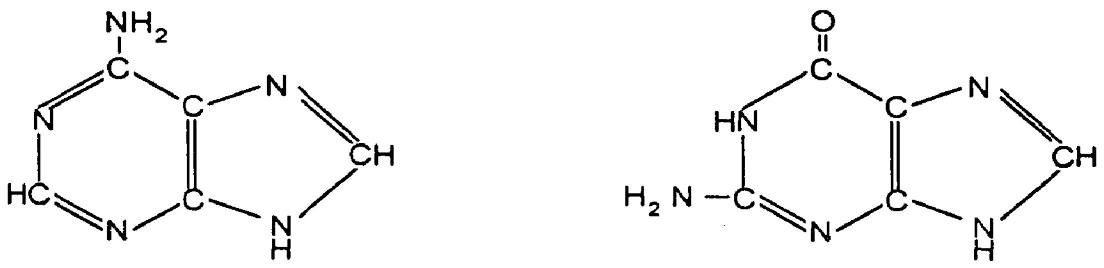
ABSTRACT

The interaction of some divalent transition metal ions, viz. Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) with adenine (A), uracil (U) and 5 bromouracil (5 BrU) separately in binary (1:1) and in presence of each other in ternary (1:1:1) metal-ligand systems has been studied pH-metrically at 25 ± 0.1°C temperature and a constant ionic strength of 0.1 M $NaNO_3$ in aqueous medium. The stability constant of binary (MA / ML) and ternary (MAL) complexes formed in solution were determined by using Bjerrum-Calvin's method as adopted by Irving and Rossotti for binary, and Chidambaram and Bhattacharya for ternary systems respectively. For the studies of ternary complexes, adenine (A) has been taken as primary ligand and uracil (U) as well as 5 bromouracil (5 BrU) as secondary ligand. The $\Delta \log K$ values of the ternary systems are positive in comparison to binary metal-A systems and those with respect to binary metal-U or 5 BrU systems are mostly negative. This suggests that the ternary complexes of U or 5 BrU are more stable than the metal-A binary systems but are less stable than corresponding metal-U or 5 BrU binary complexes.

INTRODUCTION

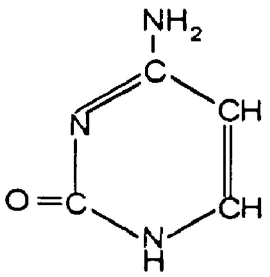
INTRODUCTION

The nucleic acids are the naturally occurring polymers like proteins and other biomolecules. On complete hydrolysis, the nucleic acids afford a mixture containing phosphoric acid, a sugar (ribose in the case of RNA or deoxyribose in the case of DNA), two purines and three pyrimidines in general. The purines are adenine and guanine and the pyrimidines are uracil, cytosine and thymine, abbreviated as A, G, U, C and T respectively. The structures of these nucleobases are shown in Fig. 1.



Adenine **Guanine**

Uracil **Thymine**



Cytosine

Fig. 1

The nucleic acids are conveniently classified into two types, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), depending upon the presence of base as well as presence or absence of 2′-hydroxyl group in sugar moiety. The RNA consists of four most common bases – adenine, guanine, cytosine and uracil in combination with ribose sugar and phosphate moiety, while DNA lacks 2′-hydroxyl group in sugar molecules (2′-deoxyribose) and uracil is substituted by thymine. Adenine always pairs with thymine in DNA and with uracil in RNA by two hydrogen bonds whereas guanine forms three hydrogen bonds with cytosine in both RNA and DNA (1). The purine and pyrimidine bases of DNA carry genetic information, whereas the sugar and phosphate groups perform a structural role. The sequence of nucleotides (or heterocyclic bases) along a polynucleotide chain determines the biological properties of the polymer. The double-helix structure with the complementary base pairs is the basis for the genetic information transfer action of the nucleic acids. Each strand of the double helix contains a form of genetic code. The replication process is known to be assisted by the presence of metal-ions (2), and can also be thwarted by metal ions (3), thus illustrating the significance of metal complexation in the biochemistry of the nucleic acids.

Not only the replication of DNA, but virtually every step in the utilization of the genetic code for the eventual production of the protein specified by the code is governed in some way by the presence of metal ions (4 - 7). Transcription of DNA into mRNA, which in turn carries the genetic information from the nucleus of ribosomes in the cytoplasm for the protein synthesis is influenced by some of the metal ions (8, 9). The interpretation of such DNA code to synthesize the protein is known as translation phenomenon, have also been found to be very much influenced by the presence of metal ions. Though, the presence of metal ions is of great significance in the normal course of protein systhesis, but the metal ions in wrong amounts or of the wrong kind can cause this processes to show anamalous behaviour (10, 11).

Base pairs as well as mispair formation and their possible incorporation in the DNA helix has also been observed to be assisted by the presence of metal ions (2, 5, 9, 12). Metal ions in general stimulate the initiation of the reaction, possibly by affecting the organisation of the subunits of which the enzyme is composed as it is not required to achieve binding of enzyme to the DNA (13). Sorokin et al. (14) study the UV and CD spectra of polyadenine in the presence of Mn (II) at different ionic strength of sodium salt in solution and reported that at low concentration, Mn (II) binds to phosphate groups of the polymer, increasing the degree of its helicity while at higher concentrations Mn (II) start to bind with base moiety of the polynucleotide leading to a helix to random coil transition. Anwander et al. (15) studied the effect of Zn (II) on DNA and RNA by means of quantum chemical calculations and reported that hydrogen bonding ability between the molecules in base pairs (i.e. A-T and G-C) as well as in mispairs is largely influenced by the presence of Zn (II). For example, when G-C pairs bound to Zn (II) via N_3 and N_7 of G, the hydrogen bonds are stabilized and also when Zn (II) is coordinated to $C_2=O$ of T(U), the hydrogen bonds in A-T(U) are stabilized. However, for N_3 coordination in A, the A-T base pairs are destabilized, while for N_7 coordination in A, it was assumed that the coordination does not significantly change the interaction of A and T (U).

In brief, the biological functioning of nucleic acids involve the participation of metal ions either directly or indirectly. Hence it is desirable to study the biochemical aspects concerned with metal-nucleic acids interactions. In recent years considerable interest has been evinced in the field of bioinorganic chemistry. Though the structures of nucleic acids and the primary roles of nucleic acids and their derivatives as hereditary determinants in biological reproduction and growth has been well established. But in order to propose the structure and reactions of nucleic acid and their constituents, it is necessary to have the knowledge of sites and thermodynamic quantities associated with interaction of protons and metal ions with these substances. In an attempt to obtain an insight concerning the metal ion interactions with the purine-pyrimidine base pair, a potentiometric study has been performed in the present work to investigate the stability of the complexes which will help towards understanding the driving forces that lead to the formation of such complexes in biological systems. In the present project, the interaction of U as well as 5 BrU and adenine with various metal ions, viz., Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) have been studied potentiometrically at an ionic strength of 0.1M $NaNO_3$ and at 25 ± 0.1°C .

Further the study of ternary complexes involving purine and pyrimidine (5-substituted) base pairs and mispairs may provide models for the more complicated processes involving nucleic acid, viz., replication, transcription, translation and mutations (5), since these heterocyclic ring systems, e.g., purines and pyrimidines play a significant role in many biological systems. Among the various substituted purines the most useful compound is 6-aminopurine (A) which has been extensively investigated as potential anticancer drug in man as well as in animals (16, 17). Besides their anticancer property, A serves manifold role in biological systems as it is a major component of nucleic acids, a large number of antibiotics and various coenzyme systems (16). It is also found in all the cells as mono-, di- and tri-phosphates of adenine nucleosides, viz., AMP (adenosine monophosphate), ADP (adenosine diphosphate) and ATP (adenosine triphosphate) which play vital roles in many metabolic processes (17). Due to the presence of suitable donor groups in the molecule, A has the capacity to form metal chelates. A has been reported to possess chelating property and also lowest pK value as compared with other nucleobases chosen for the study, therefore, it is used as a primary ligand.

LITERATURE REVIEW

LITERATURE REVIEW

Formation of ternary complexes depend on the electronic structure and ionic radius of the central atom. In addition to these most important requirements, the mutual polarizability of the central atom and the ligands, π-acceptor ability of the ligand and the symmetry relation of the electronic orbitals of central atom and ligands affect the formation of ternary complexes considerably. Ternary complexes play important role in biological processes as examplified by many instances in which enzyme, nucleic acids and other biocomponents are known to be activated by metal ions (18, 19). The ternary complexes of nucleosides and nucleotides have attracted the attention of many scientists since they provide the model for various metal activated reaction in biological system (20 - 22) and the study emerges as the current interest of investigations throughout the world.

One of the important aspects concern with the ternary complexes is the influence of secondary ligands on the binary (1:1) metal ligand complexes. This influence becomes much more important when the secondary ligands are capable of participating in electrostatic, hydrophobic and stacking interaction.

Number of workers have studied the mixed - ligand complexes of various metal ions with nitrogen and /or oxygen donors as ligand both in solid state as well as in solution (23 - 29). Kitagawa et al. (30) reported the effect of the pH on the ^{13}C NMR spectra of mixed - ligand complexes of Mn (II), Co (II), Ni (II) and Cu (II). Another group of workers (31-34) have studied the ternary complexes of the type [M-A-(ATP)] [where A is some biologically important ligand molecule behaving as secondary ligand in solutions] by potentiometric, calorimetric as well as ion-exchange methods. Complexes involving the interaction of divalent metal ions with purine, pyrimidine bases and their derivatives have been studied by other workers (35, 36) in solid state. They reported that purine and pyrimidine bases sometimes behave as monodentate ligand coordinating to metal through N_7 or N_9 of purine (37) and N_3 or N_1 of pyrimidine moiety (38). But in some other cases (39) it has been reported that purine and pyrimidine bases act as bidentate ligand. Purine binds with metal ions by C_6-NH_2 / C_6-O-N_7 or by N_3-N_9 sites while pyrimidine utilize one of the carbonyl oxygen (C=O) and one amino nitrogen (N-H) or both carbonyl oxygen atom while coordinating with metal ions.

Studies on ternary complexes of purine and pyrimidine bases with Mn (II), Co (II), Ni (II) and Zn (II) metal ions have been carried out by various workers (40, 41). Dey et al. (42) reported pH-metric studies on mixed-ligand complexes of Co (II), Ni (II) and Zn (II) with various purine derivatives viz., adenine, hypoxanthine, xanthine, guanosine 6-mercaptopurine, thioguanine and xanthosine using nitriloacetic acid, 1,10-phenanthroline and 2,2′-bipyridine as primary ligands. Mital and coworkers (43) determined the formation constants for the various metal complexation equilibria involved in M-A-L type of ternary systems [L = 8-azaguanine and A = 2,2′-bipyridine, 1,10-phenanthroline and nitriloacetic acid]. Mishra et al. (44) studied the ternary complexes of UO_2 (II), Ni (II), Cu (II), Zn (II), Hg (II) and Be (II) with nitriloacetic acid as primary ligand and various purine and pyrimidine bases as secondary ligands in aqueous solution by ionophoretic method and evaluated the stability constants. Bernard et al. (45) calculated the protonation constant of U, C and various amino acids at 25°C and ionic strength of 0.1 M $NaNO_3$. The stability constant of their Cu (II), Hg (II) and Pt (II) simple complexes (binary) were determined and then the mixed complexation of several U homologos (T, thymidine, orotic acid and C etc.) was also investigated under the same experimental conditions by potentiometric method.

Rao and Srinivas (46) determined the stability constant of ternary complexes of Co (II), Ni (II), Zn (II), Cd (II), Mg (II) and Ca (II) with adenosine-5′-triphosphate as the primary ligand and a series of amino acids along with U and

T as the secondary ligand using potentiometric method and reported the formation constant at 30°C and $\mu = 0.2M\ KNO_3$. A number of workers have also studied the interaction of divalent metal cations, viz. Mg (II), Ca (II), Ba (II) and Cd (II) with DNA by Laser-Raman spectroscopy. Raman difference spectra revealed that transition metal cations induce the greatest structural changes in B-DNA. They reported that the Raman (vibrational) band differences are extensive and indicate the partial disordering of B-form of DNA back bone, reduction in base stacking, reduction in base pairing and specific metal interaction with acceptor sites on the purine (N_7) and pyrimidine (N_3) rings.

Khan and Satyanarayan (47) determined the stability constants of Mg (II), Co (II), Ni (II), Cu (II) and Zn (II) mixed-ligand complexes with A and C as a primary ligand and L-tryptophan and L-histidine as secondary ligand potentiometrically at 25°C and 0.1 M KNO_3. Reddy and Reddy (48) used potentiometric titrations at 35°C to study the formation of ternary complexes of xanthosine and Mn (II), Co (II), Ni (II), Cu (II), Zn (II), Mg (II) and Ca (II) with glycine, histidine, catechol, oxalic acid and 2, 2' - bipyridine in a 1:1:1 mixture. They reported that the ternary complexes of these systems are more stable than the corresponding binary complexes and also the ternary metal xanthosine systems involving aromatic secondary ligands are more stable than the corresponding complexes with aliphatic ligands. Reddy and Rao (49) studied and reported the role of histidine, histamine and other amino acids as ligands in the structure and stability of metal-cytidine complexes in solution. Potentiometric equilibrium measurements have been made by Reddy and Coworkers at 35°C for the interaction of 5'-cytidine monophosphate and Mn (II), Co (II), Ni (II), Cu (II), Zn (II), Mg (II) and Ca (II) with biologically important secondary ligands, especially amino acids and C in a 1:1:1 ratio (50). The stability constants of Co (II), Ni (II), Cu (II) and Zn (II) binary complexes (1:1) with 5'-guanosine monophosphate (5'-GMP) as the primary ligand and the corresponding ternary complexes (1:1:1) with various amino acids as the secondary ligands have been determined potentiometrically at 35°C and an ionic strength of 0.1 M KNO_3 (51).

Though a huge amount of works have been reported on metal purine or pyrimidine interaction in binary as well as ternary complexing system with a number of biologically important secondary ligands but a very few work on metal base pair interaction is available in the literature (18-22). Henceforth in the present work the interaction of U as well as 5 BrU and A with divalent transition metal ions Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) have been studied potentiometrically at an ionic strength of 0.1 M $NaNO_3$ and at 25 ± 0.1°C.

EXPERIMENTAL DETAILS

EXPERIMENTAL DETAILS

General Procedure and Techniques

Metal ligand equlibria and stability constants of various species for the metal ions, viz. Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) with adenine-uracil and adenine-5 bromouracil base pairs have been investigated pH-metrically in aqueous media, at constant ionic strength (0.1 M) maintained by sodium nitrate and at 25 ± 0.1°C temperature. The Calvin-Bjerrum's pH-metric technique (52, 53) as modified by Irving and Rossotti (54) has been used for the present investigations.

Materials

Water :- Distilled water was redistilled over alkaline permagnate and the resulting distillate was boiled to expel carbon dioxide and was cooled in stoppered pyrex flasks. The pH of this water was ~ 6.8.

Sodium Hydroxide :- 250 ml carbon dioxide free sodium hydroxide (E. Merck) solution of ~ 5 N concentration was prepared in distilled water (55). The solution was standardized against a standard oxalic acid solution and subsequently diluted to prepare the stock solution of 0.2 N. The concentration of alkali was rechecked by standard oxalic acid solution before starting the experiment and also during the experimental work.

Sodium Nitrate :- Aqueous stock solution of sodium nitrate (Sarabhai M. Chemicals) of 1.0 M concentration was prepared by direct weighing and used for maintaining the ionic strength of the titrating mixtures.

Nitric Acid :- The stock and standard solution of 0.02 N nitric acid (E. Merck) was prepared by diluting a suitable volume of concentrated (~16 N) nitric acid. The solution was standardized against a standard sodium hydroxide solution.

Metal Nitrate Solutions :- The metal nitrates (all E. Merck except Zn from Loba Chemie) were used for preparing the solutions (0.01 M) of metal ions viz. Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) and were standardized by EDTA titration method as discussed elsewhere in literature (56).

Ligand Solutions :- 100 ml fresh solution (0.01M) of adenine (SRL) was prepared by dissolving accurately weighed amount in known volume of alkali (0.2 N). The final concentration of alkali being 0.02 N.

100 ml fresh solutions (0.01 M) of uracil (SRL) and 5-bromouracil (Aldrich) were prepared by dissolving accurately weighed amounts in minimum volumes of freshly distilled ethyl alcohol by heating it about 40°C with vigorous shaking, the volumes were made by adding CO_2 free double distilled water.

Instruments

pH-meter and Accessories :- ANALAB digital pH-meter with combined pH electrode was employed throughout the present work for pH measurements. The pH-meter was standardized against buffers of potassium hydrogen phthalate of pH 4.0 and of borax of pH 9.2.

Experimental Design

The following mixtures were prepared and the total volume of each mixture was kept constant (i.e. 50 ml) by adding distilled water and the ionic strength of each mixture was also kept constant i.e. $\mu = 0.1M\ NaNO_3$ by adding appropriate volume of 1.0 M $NaNO_3$ solution.

(I) Nitric acid (0.02 M, 5.0 ml) + Sodium nitrate solution (1.0 M, 5.0 ml)

(II) Mixture I + Primary ligand solution (0.01 M, 5.0 ml) + Nitric acid (0.02 M, 5.0 ml).

(III) Mixture II + Metal nitrate solution (0.01 M, 5.0 ml)

(IV) Mixture I + Secondary ligand solution (0.01 M, 5.0 ml)

(V) Mixture III + Secondary ligand solution (0.01 M, 5.0 ml)

Each set was titrated pH-metrically against a standard alkali (0.2 N, NaOH) solution. In almost all the cases precipitation appeared at higher pH-range.

Calculation of the Parameter $\bar{n}_A$, $\bar{n}$ and pL

Bjerrum emphasized the importance of all the potentiometric methods which make it possible to determine either the concentration of the free metal ion or that of the free ligand in the solution. The method used in the present project is the Bjerrum-Calvin pH titration technique (53) as adopted by Irving and Rossotti (54). This method is also suitable for the determination of the average number of protons attached to the ligand ($\bar{n}_A$) and degree of formation ($\bar{n}$) of a complex. The following mixtures (i) Nitric acid + Sodium nitrate solution, (ii) Mixture I + Primary ligand solution, and (iii) Mixture II + Metal ion solution were prepared and titrated separately against a standard sodium hydroxide solution by pH-metric method using a digital pH-meter. Thus, three curves, (I = acid titration curve, II = ligand titration curve and III = chelate titration curve) obtained by plotting the pH-meter reading against the volume of alkali added, were used for further calculations.

(I) Acid Titration Curve

The stoichiometric hydrogen ion concentration C_H^i at any point of acid titration curve (I) can be given by

$$C_H^i = E^i + C_{OH}^i + C_{Na}^i \qquad (1)$$

where E^i is the concentration of acid, C_{Na}^i is the concentration of Na^+ ions from sodium hydroxide added. The term C_{OH}^i results from hydrolysis which nullifies the effect of addition of alkali and hence is positive.

(II) Ligand Titration Curve

For the ligand titration curve (II), the corresponding equation becomes

$$C_H^{ii} = E^{ii} + yTC_L^{ii} + C_{OH}^{ii} - C_{Na}^{ii} - \bar{n}_A^{ii} \,.\, TC_L^{ii} \qquad (2)$$

Here, the terms yTC_L^{ii} represents the number of titrable hydrogen ions in gram-ions per litre arising from the ligand, where it is an acid HyL (y is zero for a base). The last term $\bar{n}_A^{ii} . TC_L^{ii}$ gives the number of hydrogen ions removed as a result of formation of proton-ligand complexes and obtained from the definition of $\bar{n}_A$ given earlier.

For two solutions having the same ionic strength at identical pH values (B), the corresponding titration curve have

$$C_H^{i} = C_H^{ii} \qquad (3)$$

$$C_{OH}^{i} = C_{OH}^{ii} \qquad (4)$$

Since, C_H and C_{OH} are related with ionic product of water which is constant at constant ionic strength and temeperature. Therefore from equation 1 and 2, the following relation is obtained.

$$\bar{n}_A = \frac{(E^{ii} - E^{i}) - (C_{Na}^{ii} - C_{Na}^{i}) + y.TC_L^{ii}}{TC_L^{ii}} \qquad (5)$$

Under the condition in which the initial volume of the solution (V^o), the initial concentration of the acid (E^o) are same in two titrations, the initial ligand concentration TC_{L^o} and the alkali used is of the same concentration N^o, the equation given below would be obtained, when the volume V^i and V^{ii} of the alkali are required to be added for reaching the same pH (B) values :

$$E^{ii} = E^o V^o / (V^o + V^{ii}) \qquad (6)$$

$$C_{Na}^{ii} = N^o V^{ii} / (V^o + V^{ii}) \qquad (7)$$

$$TC_L^{ii} = V^o . TC_{L^o} / (V^o + V^{ii}) \qquad (8)$$

Similar equation would represent E^i, C_{Na}^i and TC_L^i.

Substitution of these values in equation (5) would lead to the following equation :

$$\bar{n}_A = y - \frac{(V^{ii} - V^{i})}{(V^o - V^{i})} \times \frac{(N^o + E^o)}{TC_{L^o}} \qquad (9)$$

(III) Chelate Titration Curve

The chelate titration curve (III) gives relation similar to equation 3 and 4.

$$C_H^{iii} = E^{iii} + y.\,TC_L^{iii} + C_{OH}^{iii} - C_{Na}^{iii} - \bar{n}_A^{iii}\,(TC_L^{iii} - \bar{n}^{iii}.\,TC_M^{iii}) \quad (10)$$

in which the last term has same significance as the last term in equation 2, with the difference that the former takes into account of the decrease in concentration of the unchelated ligand after the formation of the necessary amount of the metal-ligand chelate.

Since, the ionic strength in a set of experiments is constant, at a particular pH value corresponding to the curve II and III:

$$C_H^{ii} = C_H^{iii},\ C_{OH}^{ii} = C_{OH}^{iii} \text{ and } \bar{n}_A^{ii} = \bar{n}_A^{iii} \quad (11)$$

Hence,

$$\bar{n}^{iii} = \frac{(E^{ii} - E^{iii}) + (TC_L^{ii} + TC_L^{iii})\,(y - \bar{n}_A^{ii}) - (C_{Na}^{ii} - C_{Na}^{iii})}{\bar{n}_A^{iii}.\,TC_M^{iii}} \quad (12)$$

Considering equations similar to 6, 7 and 8 the following expression can be obtained :

$$\bar{n}^{iii} = \frac{(V^{iii} - V^{ii})[(N^o + E^o) + TC_{L^o}(y - \bar{n}_A^{ii})]}{(V^o + V^{ii})\bar{n}_A^{iii}.\,TC_{M^o}} \quad (13)$$

Under the conditions given below;

(i) Values of V^o, E^o, $TC_L{}^o$ and N^o are the same for the reagent,

(ii) V^{iii} is the volume of alkali required to reach the same pH value in the metal-chelate titration, and

(iii) $TC_M{}^o$ is the initial concentration of the metal ion,

for practical purposes equation 13 can be modified as

$$\bar{n} = \frac{(V^{iii} - V^{ii})}{(V^o + V^i)} \times \frac{(N^o + E^o)}{(\bar{n}_A.\,TC_{M^o})} \quad (14)$$

V^{iii} is the volume of alkali used with acid + ligand + metal.

Further the free ligand exponent (pL) may be calculated by using the equation

$$pL = \log\left[\frac{1+\text{antilog}(\log K_1 - B) + \text{antilog}(\log K_1 + \log K_2 - 2B)}{TC_{L^o} - \bar{n}.TC_{M^o}} \times \frac{V^o + V^{iii}}{V^o}\right] \quad (15)$$

where B = pH value.

The curve plotted between the calculated values of pL at every $\bar{n}$ against $\bar{n}$ is known as the formation curve, the formation constants are calculated by graphical method.

The formation constants for binary system are also calculated by average value method using following equation

$$\log K_1 = pL + \log\left(\frac{\bar{n}}{1-\bar{n}}\right) \quad (16)$$

For different values of $\bar{n}$ and pL, $\log K_1$ can be calculated and its mean value can be taken as reliable value.

The formation of a mixed ligand complex can be described by the equilibrium :

$$M + iA + nL \rightleftharpoons MAi\,Ln \quad (17)$$

where A and L are two different ligands.

Martell proposed various equilibria in which either (i) both the ligands combine with metal ion simultaneously, or (ii) the two ligands may combine at different pH (57). He studied the mixed ligand complexes and developed a method for the evaluation of solution stability constant in mixed ligand systems where two ligands combined at different pH-ranges (58). Chidambaram and Bhattacharya (21) extended the Irving and Rossotti method (54) to obtain the formation constants in mixed ligand complexes of the type studied by Martell. The necessary condition in such studies is that 1:1 metal-primary ligand complex (MA) formed at low pH, should not undergo dissociation or disproportionation at higher pH-range and the secondary ligand should commence combining with MA species at higher pH only after the complete formation of MA. Here it is assumed that the secondary ligand combines with MA species similarly as it does with $M(H_2O)_n$ in simple mononuclear binary systems. The formation of ternary complexes can be described by the following equilibria :

$$M + A \rightleftharpoons MA \quad (18)$$

$$MA + L \rightleftharpoons MAL \tag{19}$$

whereby the formation constant of the mixed ligand (ternary) complex K^{M}_{MAL} would be :

$$K^{M}_{MAL} = [MAL] / [MA][L] \tag{20}$$

The procedure adopted by Chidambaram et al. (21) has been fully employed for the evaluation of formation constants of ternary complexes in solution.

Calculation of the Parameter $\bar{n}_{mix}$ and pL_{mix}

The average number of secondary ligand L attach per metal–primary ligand complex (MA), $\bar{n}_{mix}$ and the free secondary ligand exponent, pL_{mix} can be evaluated by carrying out the following set of pH titrations against standard alkali:

(I) Acid + Sodium nitrate solution
(II) Mixture (I) + Primary ligand
(III) Mixture (II) + Metal ion solution
(IV) Mixture (I) + Secondary ligand solution
(V) Mixture (III) + Secondary ligand solution

Five titration curves are thus obtained by plotting pH vs. volume of alkali added. From the curves I, II and III, the range of pH in which the metal – primary ligand complex (MA) is stable could be ascertained by using Irving and Rossotti's calculation (54). The additional hydrogen of L with MA species are determined from the horizontal distance between curve IV and V. This distance $(V^{V} - V^{IV})$ was further employed for the calculation of $\bar{n}_{mix}$ using the following form of Irving-Rossotti's equation

$$\bar{n}_{mix} = \frac{(V^{V} - V^{IV})}{(V^{o} + V^{I})} \times \frac{(N^{o} + E^{o})}{\bar{n}_{L}.TC_{MA^{o}}} \tag{21}$$

where V^{V} and V^{IV} are the volume of alkali consumed to reach the same pH value in the curve V and IV respectively. $TC_{MA^{o}}$ is the total concentration of MA species which in turn is equivalent to the total initial metal ion concentration taken in mixture V, $\bar{n}_{L}$ is the average number of protons attached with the secondary ligand L. The other symbols have their usual meanings.

Free ligand exponent (pL_{mix}) can also be deduced using the following equation :

$$pL_{mix} = \log\left[\frac{1 + \text{anti}\log(\log K_1 - B) + \text{anti}\log(\log K_1 + \log K_2 - 2B)}{TC_{L^o} - \bar{n}_{mix}.TC_{MA^o}} \times \frac{V^o + V^V}{V^o}\right] \quad (22)$$

The values of $\bar{n}_{mix}$ and pL_{mix} calculated at different pH values for each systems can then be further treated in a similar manner as described for the simple mononuclear binary complex systems to determine the mixed ligand formation constants. The formation constants for mixed ligand complexes are also calculated by average value method using following equation :

$$\log K_{mix} = pL_{mix} + \log\left(\frac{\bar{n}_{mix}}{1 - \bar{n}_{mix}}\right) \quad (23)$$

Thus, for different values of $\bar{n}_{mix}$ and pL_{mix}, log K_{mix} can be calculated and its mean value can be taken as a reliable value.

RESULTS & DISCUSSION

RESULTS AND DISCUSSION

The interactions of purine and pyrimidine bases with metal ions, separately as well as in the presence of each other (purine-pyrimidine base pairs) are well evident from the titration curves (Figs. 2-6 and Figs. 8-12) obtained by plotting the pH against the volume of alkali added, for various systems under investigation. The literature value of the proton-ligand stability constants for A, U and 5 BrU were utilized for the evaluation of stability constants of ternary (1:1:1) metal-ligand complexes using the modified approach of Irving and Rossotti (22) method modified by Chidambaram and Bhattacharya (21) described in literature. Formation curves for various systems under study (Fig. 7 and 13) were also plotted between $\bar{n}_{mix}$ and pL_{mix} and used further for the evaluation of graphical values of log K.

The stability constants of the ternary complexes of A with U and 5 BrU and metal ions under investigation are summarized in Table 1 and 2 along with its graphical values and the overall stability constants of corresponding binary (1:1) metal ligand systems. The comparison of the stability constants of binary and their corresponding ternary metal ligand complexes, with respect to both primary as well as secondary ligands have also been evaluated and given in the Table 1 and 2 in terms of $\Delta \log K$ (where $\Delta \log K$ is the difference between the stabilities of the binary and ternary metal ligand complexes), which provides an insight into the various factors responsible for the formation and stabilization of ternary complexes in solution. When a solution contains two different ligands and a metal ion, there may exist the equilibria in which either

(i) both the ligands may combine with the metal ions simultaneously or

(ii) the two ligands may combine at different pH.

The formation of the ternary complex takes place according to the following equilibria (charges are omitted for clarity) :

$$M + HA \rightleftharpoons MA + H$$

$$MA + HL \rightleftharpoons MAL + H$$

and the equilibria used for calculation is

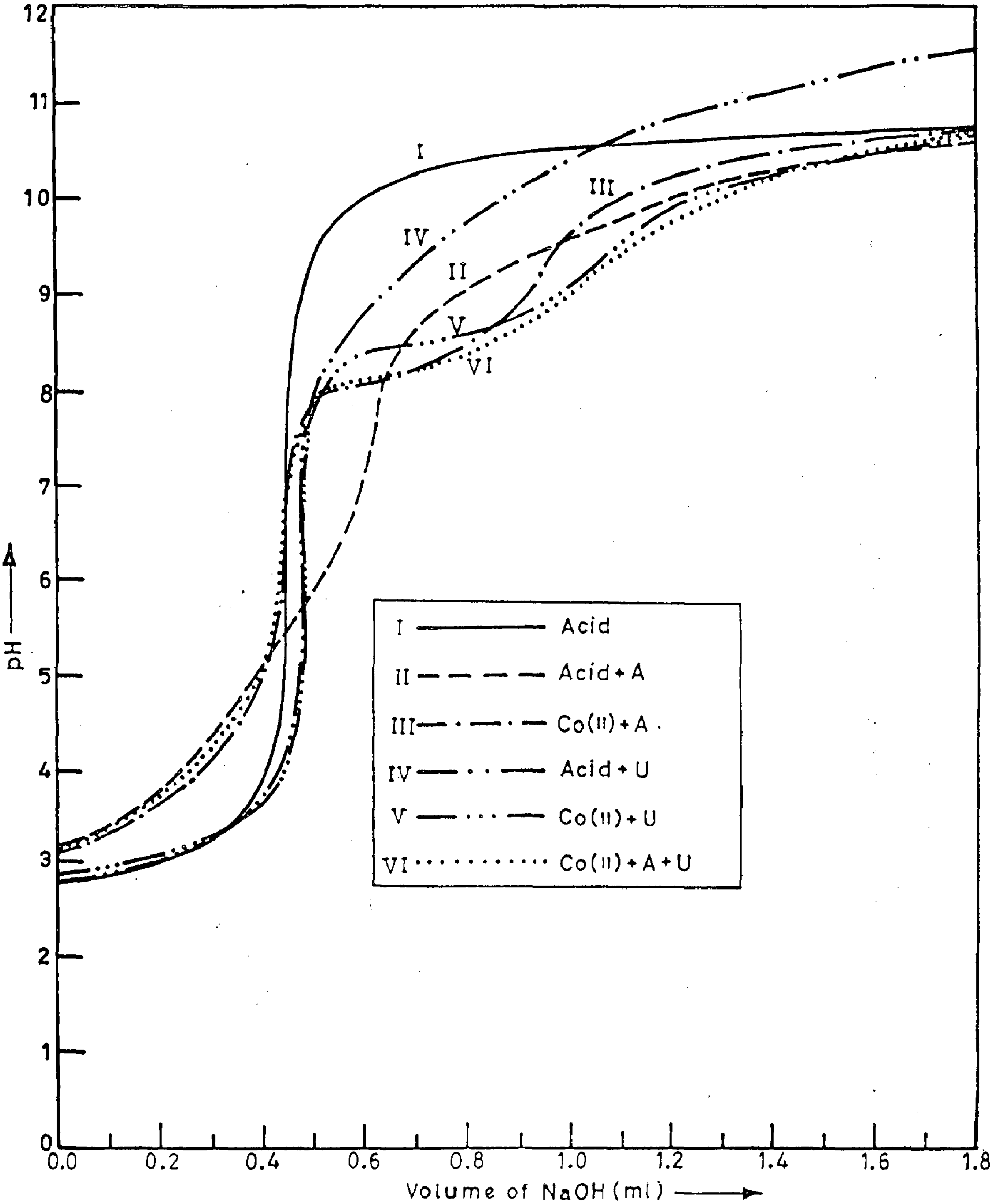


Fig. 2 : Potentiometric titration curves of Ternary (1:1:1) Co(II)-A-U system in aqueous medium at T = 25 ± 0.1°C and μ = 0.1 M $NaNO_3$.

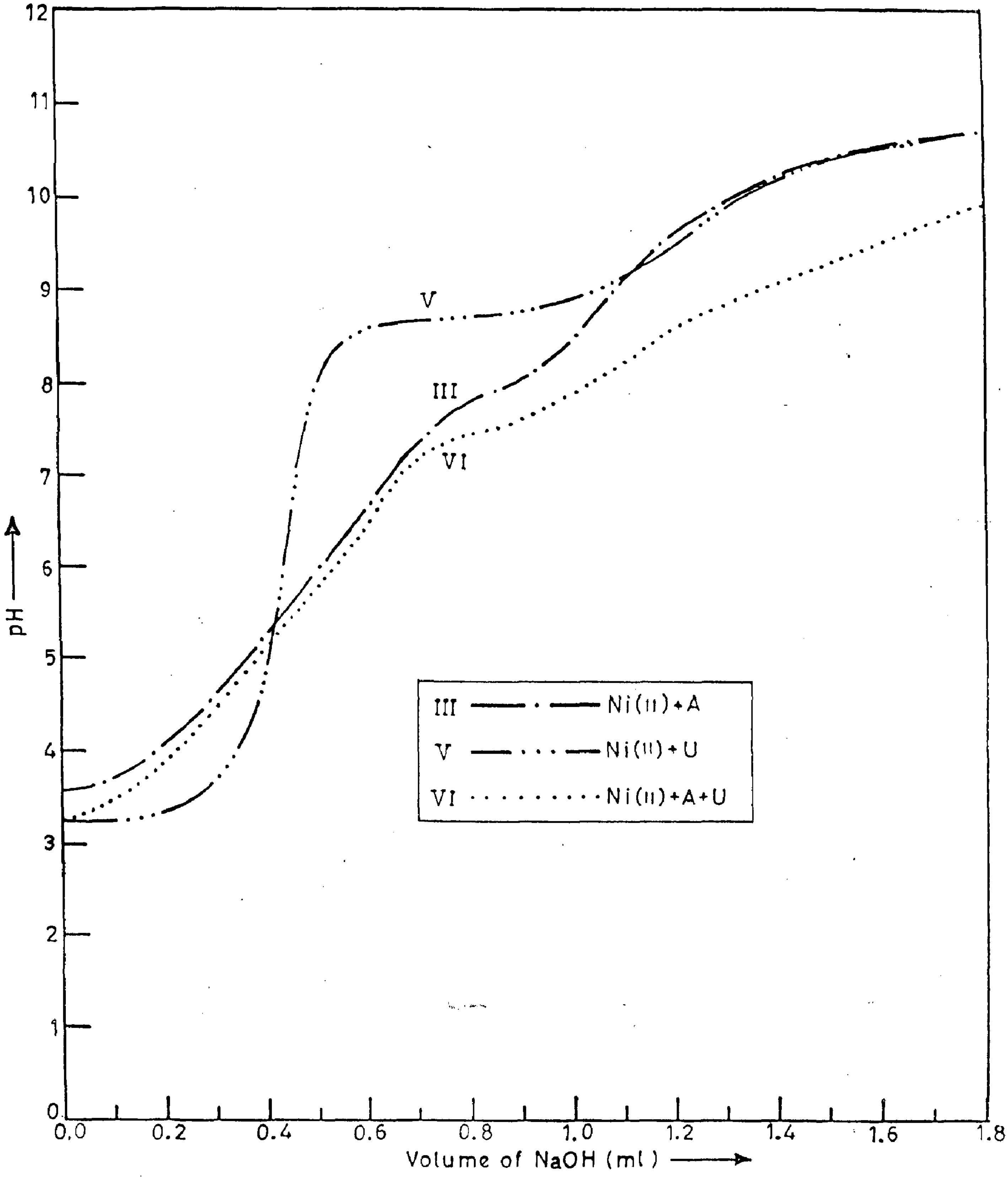


Fig. 3 : Potentiometric titration curves of Ternary (1:1:1) Ni(II)-A-U system (I, II and IV are omitted for clarity) in aqueous medium at T = 25 ± 0.1°C and μ = 0.1 M $NaNO_3$. The reading for curves I, II and IV are taken from Fig. 2 for calculation.

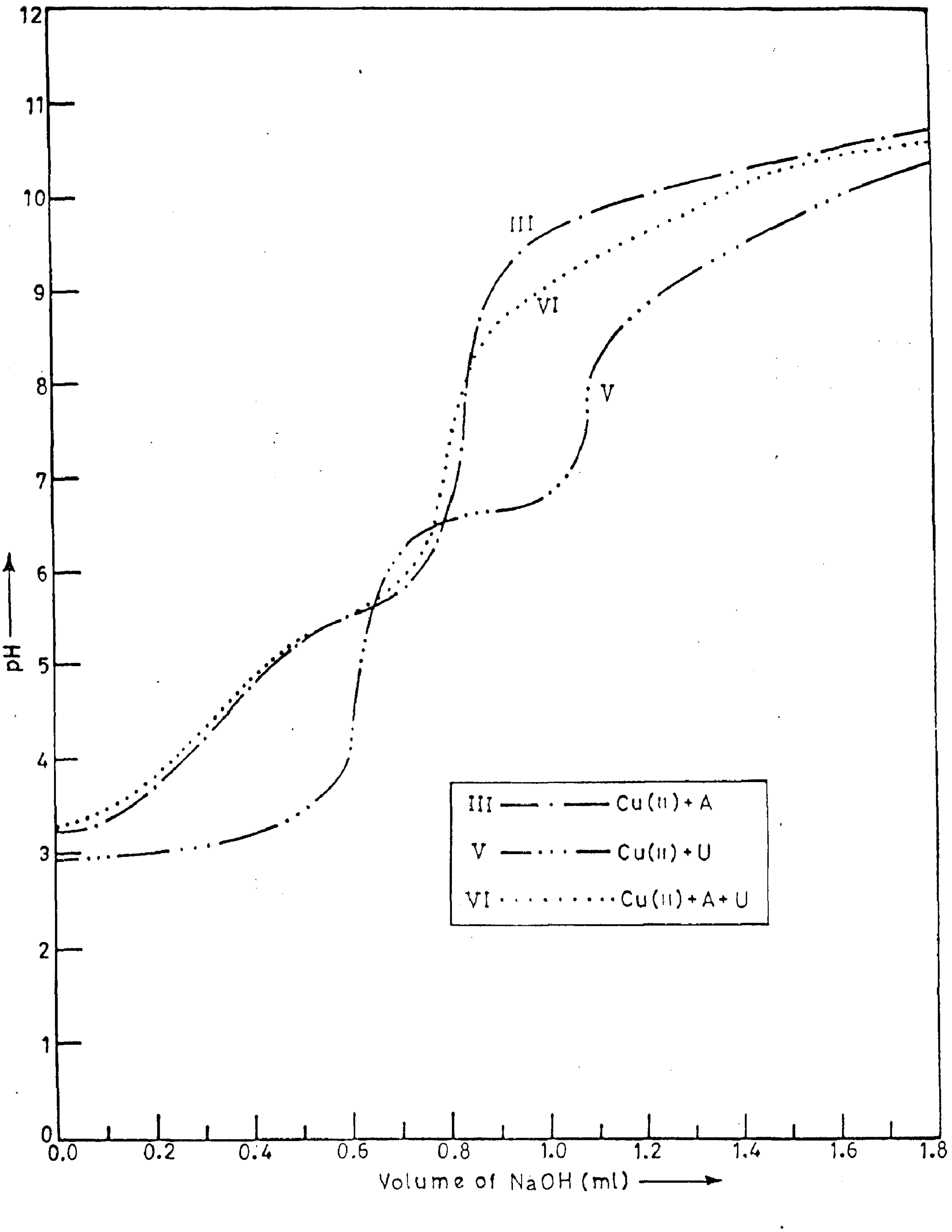


Fig. 4 : Potentiometric titration curves of Ternary (1:1:1) Cu(II)-A-U system (I, II and IV are omitted for clarity) in aqueous medium at T = 25 ± 0.1°C and μ = 0.1 M $NaNO_3$. The reading for curves I, II and IV are taken from Fig. 2 for calculation.

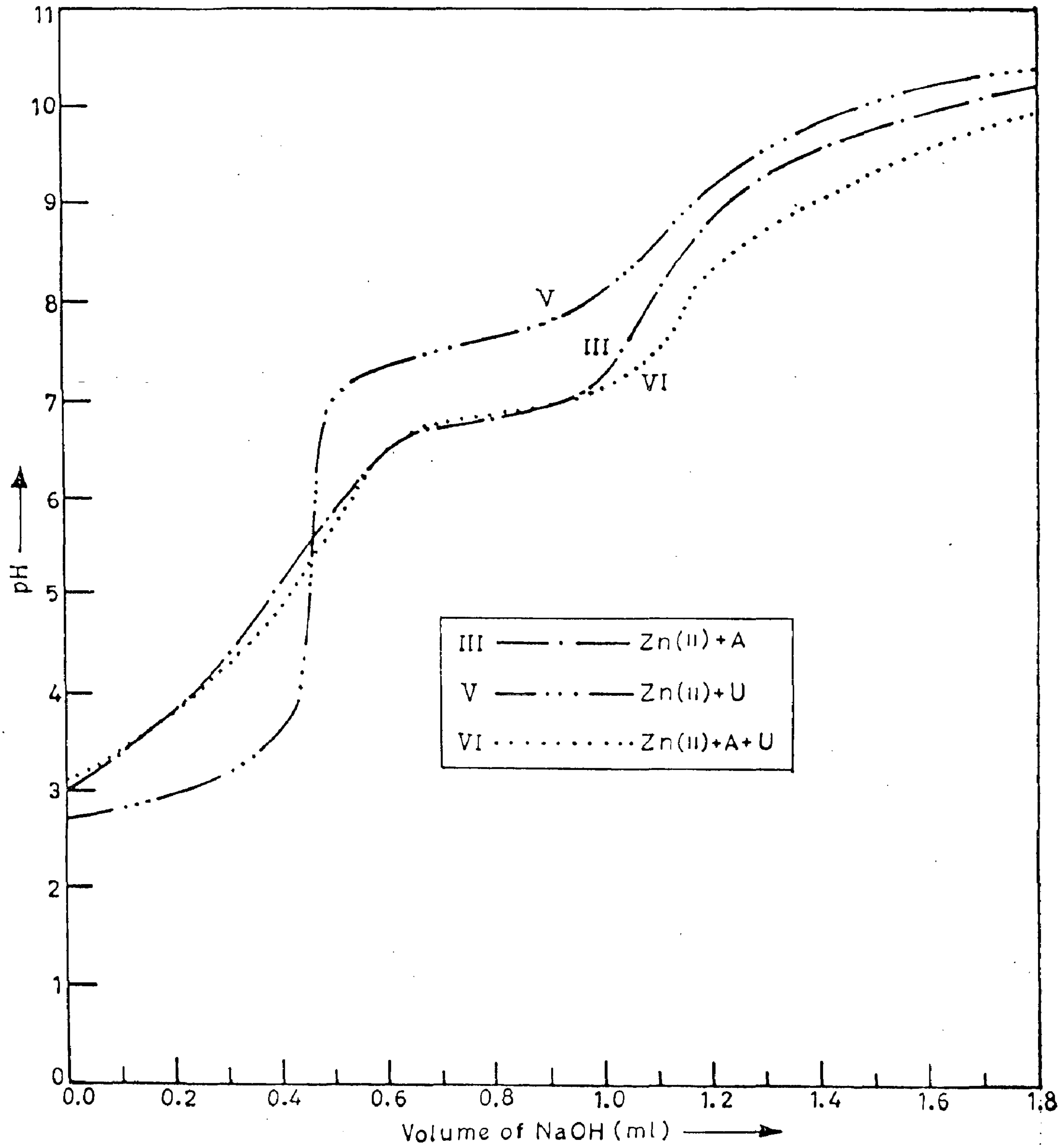


Fig. 5 : Potentiometric titration curves of Ternary (1:1:1) Zn(II)-A-U system (I, II and IV are omitted for clarity) in aqueous medium at T = 25 ± 0.1°C and μ = 0.1 M $NaNO_3$. The reading for curves I, II and IV are taken from Fig. 2 for calculation.

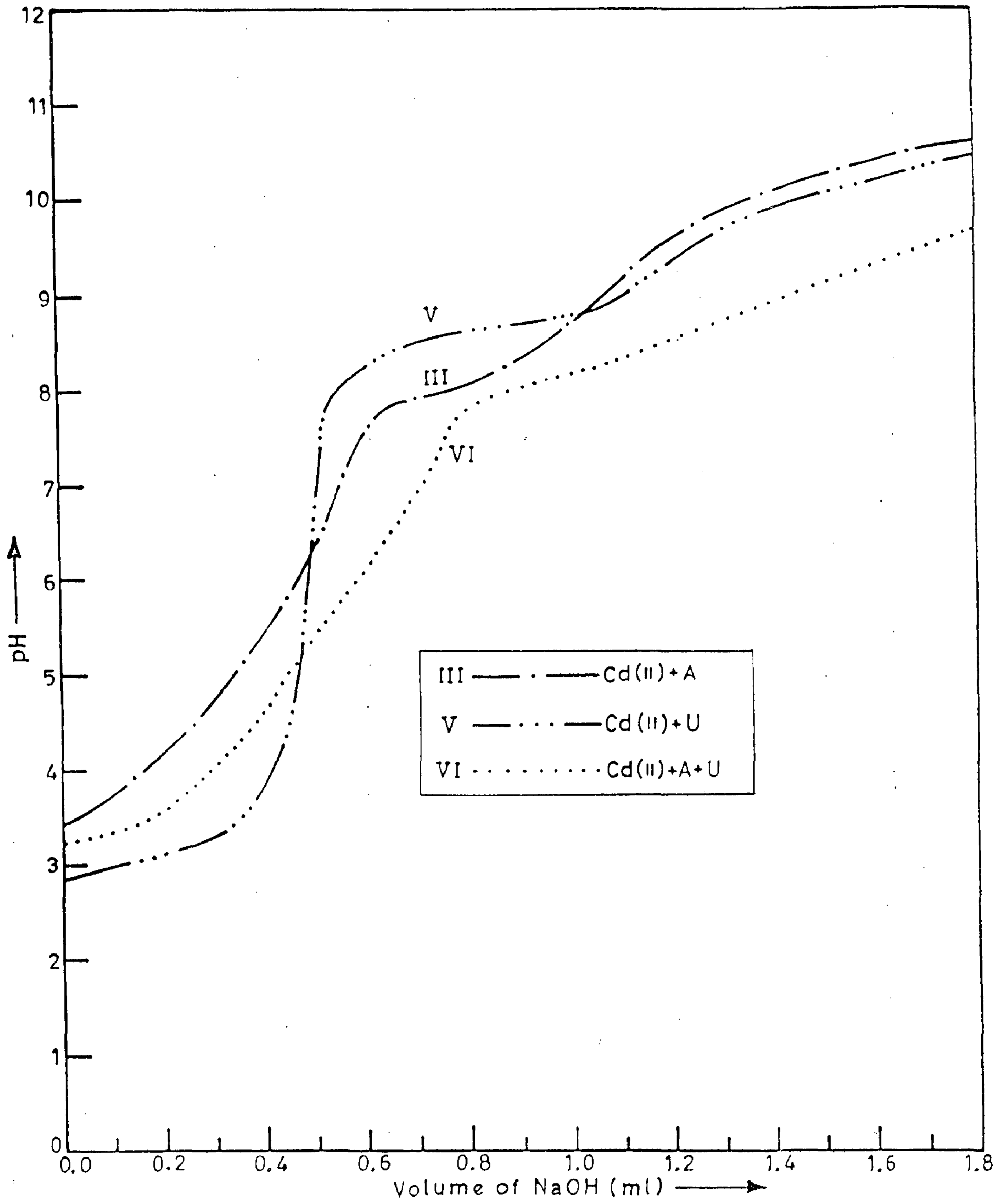


Fig. 6 : Potentiometric titration curves of Ternary (1:1:1) Cd(II)-A-U system (I, II and IV are omitted for clarity) in aqueous medium at T = 25 ± 0.1°C and μ = 0.1 M $NaNO_3$. The reading for curves I, II and IV are taken from Fig. 2 for calculation.

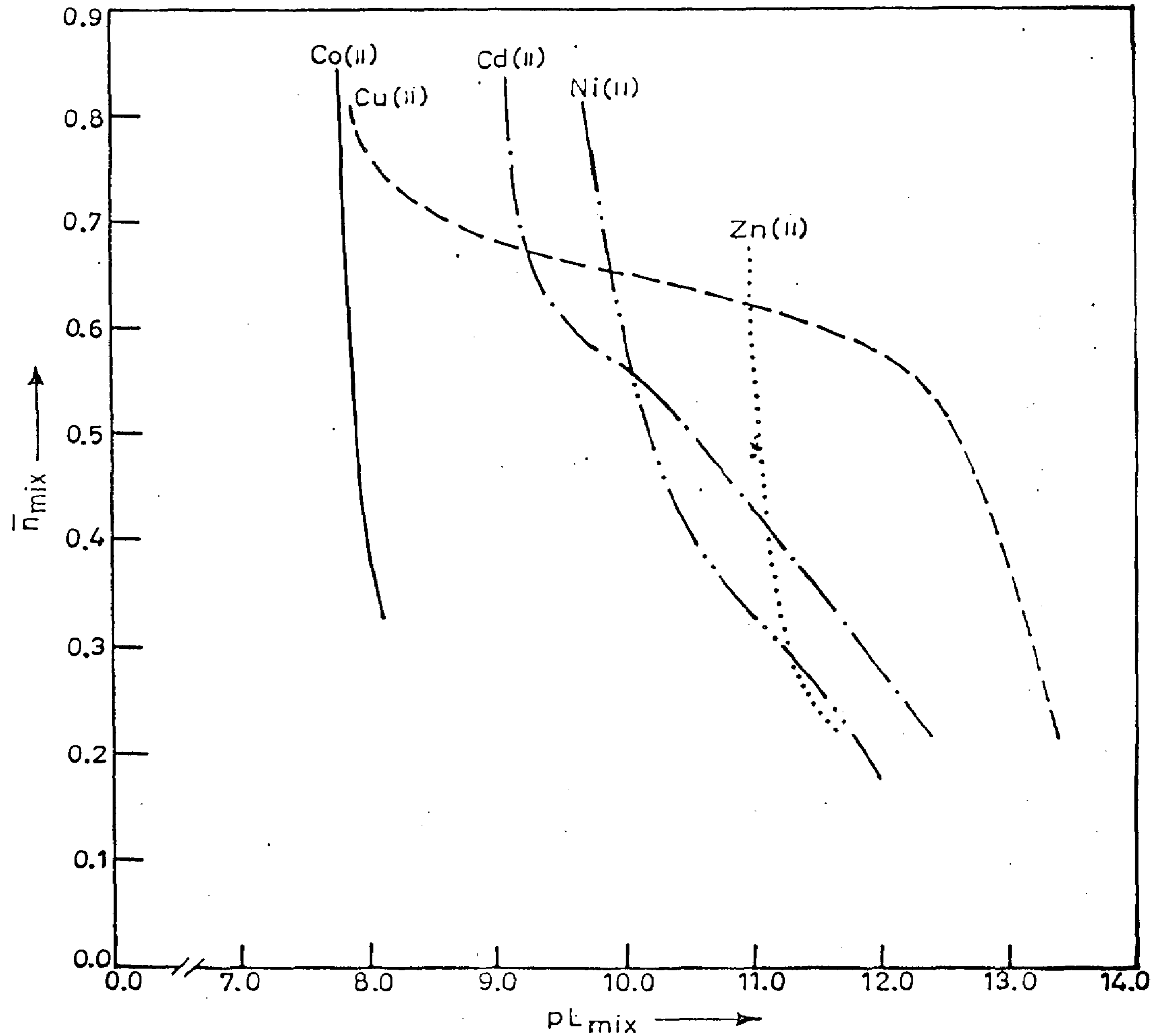


Fig. 7 : Formation curves of Ternary (1:1:1) M(II)-A-U systems in aqueous medium at T = 25 ± 0.1°C and μ = 0.1 M $NaNO_3$.

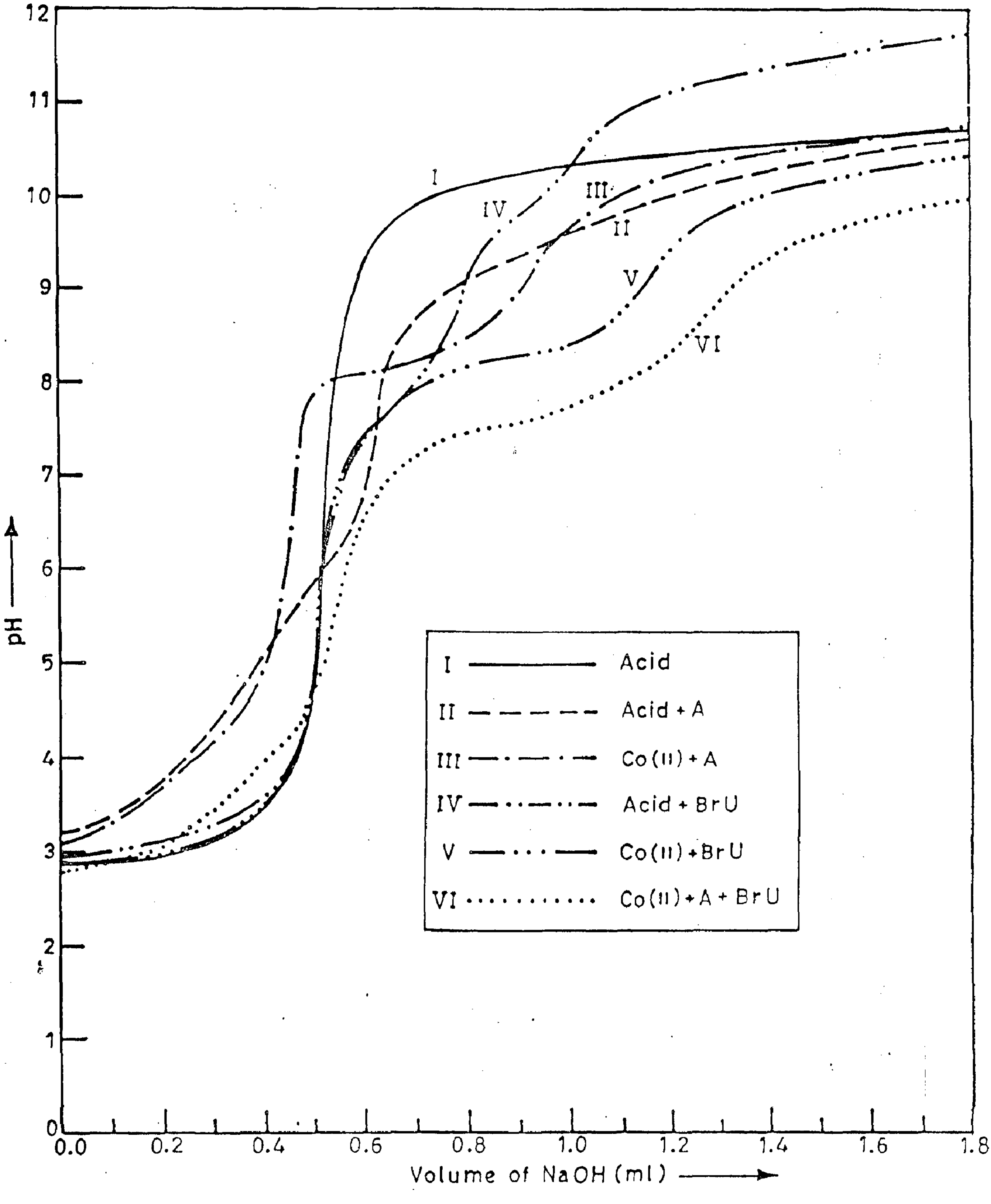


Fig. 8 : Potentiometric titration curves of Ternary (1:1:1) Co(II)-A-5BrU system in aqueous medium at T = 25 ± 0.1°C and μ = 0.1M $NaNO_3$.

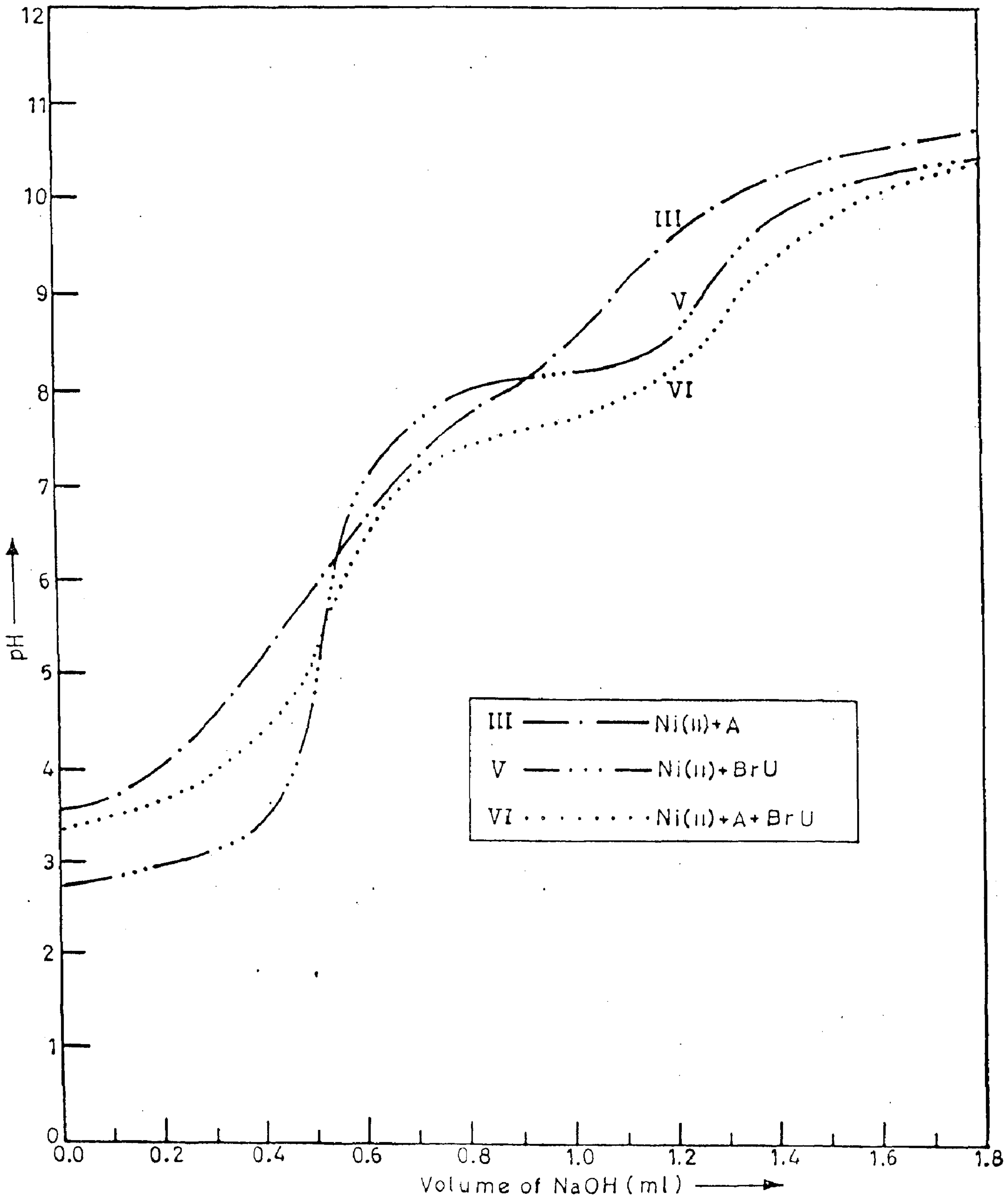


Fig. 9 : Potentiometric titration curves of Ternary (1:1:1) Ni(II)-A-5BrU system (I, II and IV are omitted for clarity) in aqueous medium at T = 25 ± 0.1°C and μ = 0.1M $NaNO_3$. The reading for curves I, II and IV are taken from Fig. 8 for calculation.

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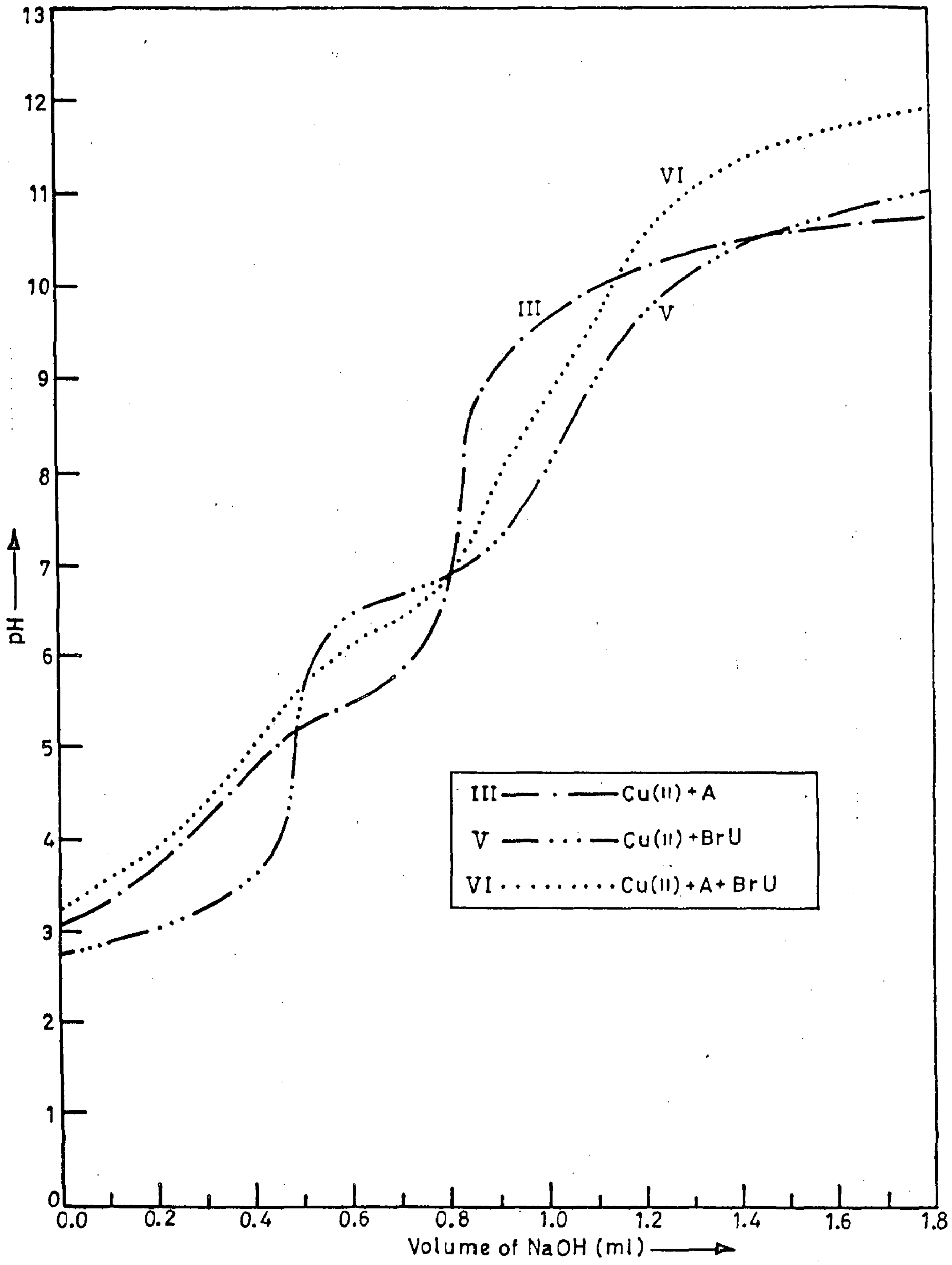

Fig. 10 : Potentiometric titration curves of Ternary (1:1:1) Cu(II)-A-5BrU system (I, II and IV are omitted for clarity) in aqueous medium at T = 25 ± 0.1°C and μ = 0.1M $NaNO_3$. The reading for curves I, II and IV are taken from Fig. 8 for calculation.

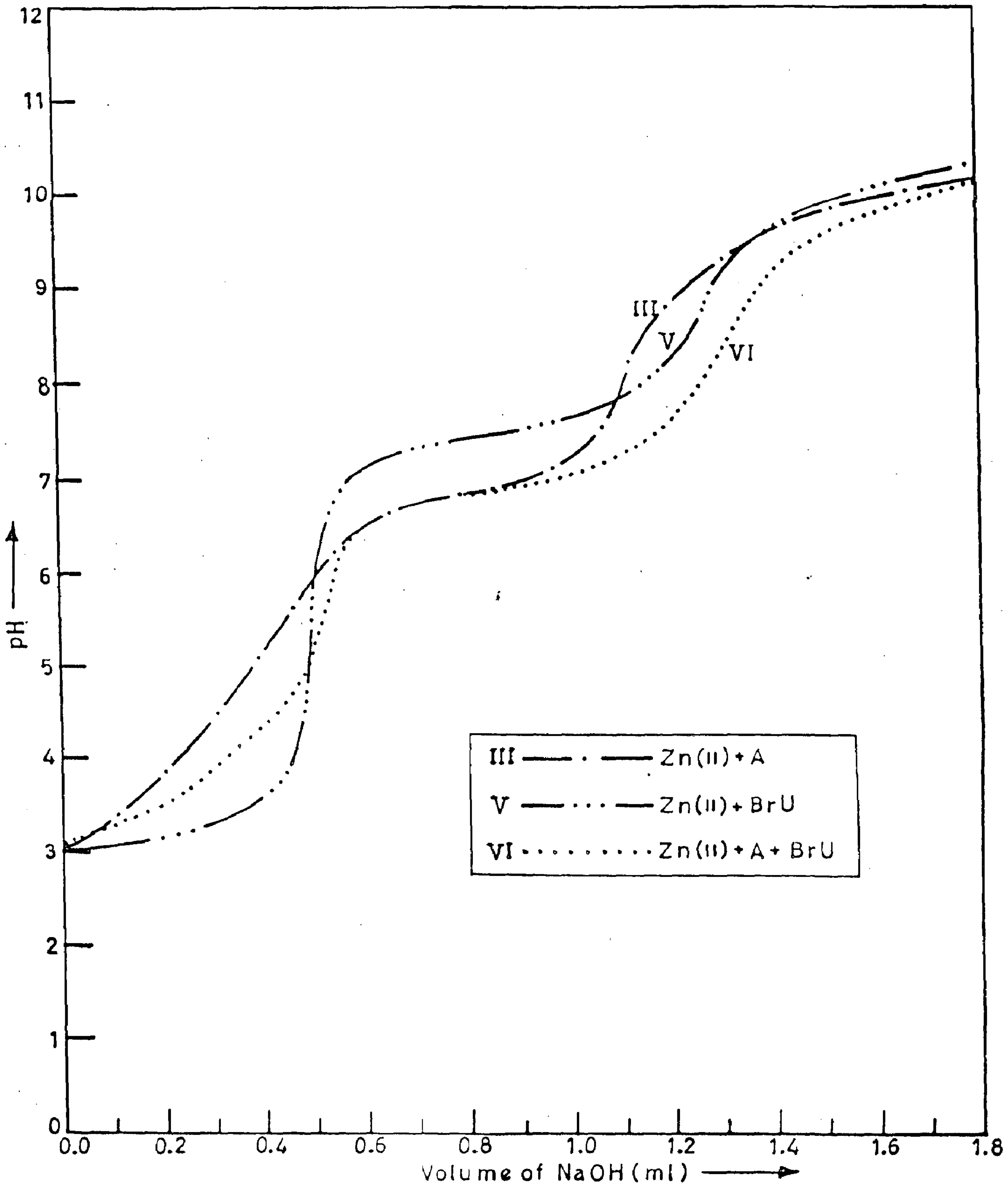

Fig. 11 : Potentiometric titration curves of Ternary (1:1:1) Zn(II)-A-5BrU system (I, II and IV are omitted for clarity) in aqueous medium at T = 25 ± 0.1°C and μ = 0.1M $NaNO_3$. The reading for curves I, II and IV are taken from Fig. 8 for calculation.

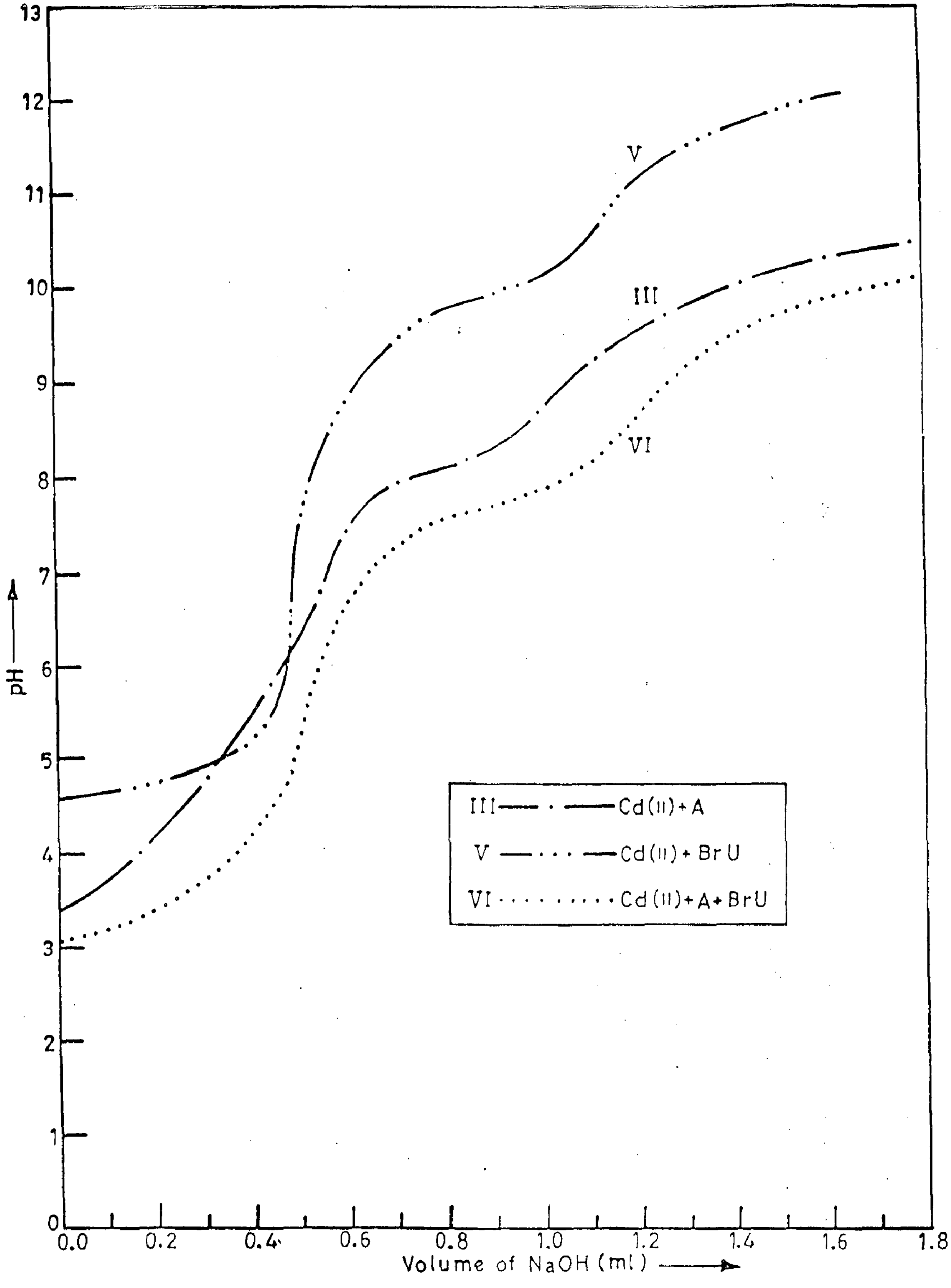

Fig. 12 : Potentiometric titration curves of Ternary (1:1:1) Cd(II)-A-5BrU system (I, II and IV are omitted for clarity) in aqueous medium at T = 25 ± 0.1°C and μ = 0.1M $NaNO_3$. The reading for curves I, II and IV are taken from Fig. 8 for calculation.

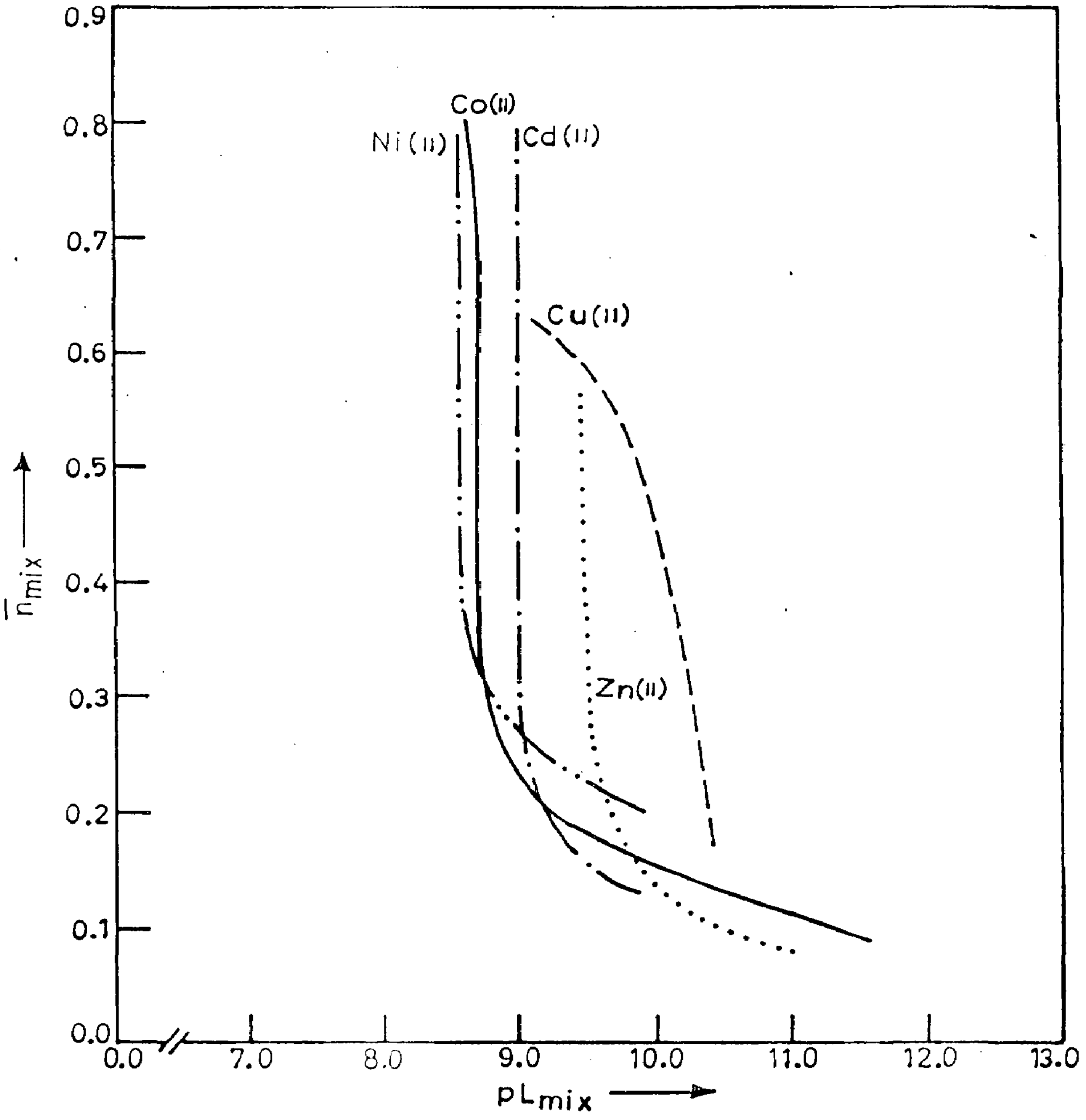

Fig. 13 : Formation curves of Ternary (1:1:1) M(II)-A-5BrU systems in aqueous medium at T = 25 ± 0.1°C and $\mu = 0.1$ M $NaNO_3$.

Table 1 : Formation constants (logK) of binary (1:1) and ternary (1:1:1) metal ligand complexes and comparison of logK* values in aqueous medium at T = 25 ± 0.1°C and $\mu = 0.1$ M $NaNO_3$.

Metal ion	Metal : A ($\log K^{M}_{MA}$)		Metal : U ($\log K^{M}_{ML}$)		Metal : A : U ($\log K^{M}_{MAL}$)		$\Delta\log K^{M}_{MAL}$		$\Delta\log K^{ML}_{MAL}$	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Co(II)	6.08	5.07	8.50	8.35	7.82	7.90	1.74	2.83	– 0.68	– 0.45
Ni(II)	6.71	6.75	11.41	10.95	10.76	10.15	4.05	3.40	– 0.65	– 0.80
Cu(II)	8.03	7.85	13.23	12.88	10.59	12.50	2.56	4.65	– 2.64	– 0.38
Zn(II)	6.62	6.85	11.98	11.40	11.35	11.05	4.73	4.20	– 0.63	– 0.35
Cd(II)	5.80	5.60	11.68	10.65	10.92	10.50	5.12	4.90	– 0.76	– 0.15

* $\Delta\log K^{M}_{MAL} = \log K^{M}_{MAL} - \log K^{M}_{MA}$ & $\Delta\log K^{ML}_{MAL} = \log K^{M}_{MAL} - \log K^{M}_{ML}$

The $\log K^{M}_{MA}$ and $\log K^{M}_{ML}$ represent the formation constants of binary metal-A and metal-U complexes respectively and $\log K^{M}_{MAL}$ represents the ternary formation constants.

Method (a) : Average Value Method and

Method (b) : Graphical Value

Table 2 : Formation constants (logK) of binary (1:1) and ternary (1:1:1) metal ligand complexes and comparison of logK* values in aqueous medium at T = 25 ± 0.1°C and $\mu = 0.1$ M $NaNO_3$.

Metal ion	Metal : A ($\log K^{M}_{MA}$)		Metal : 5BrU ($\log K^{M}_{ML}$)		Metal : A : 5BrU ($\log K^{M}_{MAL}$)		$\Delta \log K^{M}_{MAL}$		$\Delta \log K^{ML}_{MAL}$	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Co(II)	6.08	5.07	10.08	9.6	9.63	8.70	3.55	3.63	– 0.45	– 0.90
Ni(II)	6.71	6.75	9.73	9.40	9.36	8.55	2.65	1.80	– 0.37	– 0.85
Cu(II)	8.03	7.85	9.59	9.45	9.18	9.87	1.15	2.02	– 0.41	0.42
Zn(II)	6.62	6.85	10.15	10.00	9.87	9.45	3.25	2.60	– 0.28	– 0.55
Cd(II)	5.80	5.60	9.64	9.35	9.07	9.00	3.27	3.40	– 0.57	– 0.35

* $\Delta \log K^{M}_{MAL} = \log K^{M}_{MAL} - \log K^{M}_{MA}$ & $\Delta \log K^{ML}_{MAL} = \log K^{M}_{MAL} - \log K^{M}_{ML}$

The $\log K^{M}_{MA}$ and $\log K^{M}_{ML}$ represent the formation constants of binary metal-A and metal-5BrU complexes respectively and $\log K^{M}_{MAL}$ represents the ternary formation constants.

Method (a) : Average Value Method and

Method (b) : Graphical Value

$$MA + HL \rightleftharpoons MAL$$

$$K_{MAL}^{MA} = \frac{[MAL]}{[MA][HL]}$$

where K_{MAL}^{MA} represents the formation constant of the ternary complex.

In ternary systems where the solution contains a metal ion and two ligands as in the present case, the formation of ternary complex in solution is influenced by mainly two factors.

(i) inter ligand interactions, viz., electrostatic, hydrophobic and stacking, and

(ii) where there is no such interactions.

The stability of the ternary complexes is mainly affected by the interligand interactions along with several other factors, like the nature of the metal ion, geometry of the metal complex and the solvent effects, etc.. The interligand interactions are highly specific and selective depending on the reactants in solution.

The effect of secondary ligand on the stability of 1:1 metal ligand complexes is quantified in terms Δlog K. The effect of charge neutralization, the π-accepting capacity of the secondary ligand and the stacking interactions are usually represented by Δlog K, since Δlog K is derived from two different constants, it is also a constant which varies with temperature like any other constant. The positive value of Δlog K indicates that the ternary complexes are more stable than the binary complexes and involves interligand interactions (59) and its negative values implies that binary complexes are more stable than the ternary complexes. But it does not mean that the negative values of Δlog K preclude the formation of ternary complexes in solution (48, 60).

Δlog K values are also influenced by the stability constants of the simple binary complexes (31). The extent to which the two ligands influence the metal ion in the presence of each other as compared to simple 1:1 parent complexes, affecting the coordination modes of the ligand and the geometry of the complexes as a whole can also be assessed by the value of Δlog K.

It is well evident from the Table 1 and 2 that the Δlog K values of the ternary systems are positive in comparison to the binary metal-A systems and these with respect to binary metal-U or 5 BrU systems are mostly negative. This shows that the ternary complexes of U or 5 BrU are more stable than metal-A binary systems but are less stable than corresponding metal-U or 5 BrU binary complexes. The negative Δlog K values support the view that the mode of binding is different in binary and ternary systems. In the ternary complexes U or 5 BrU probably acts as a monodentate ligand involving only N_3 in metal coordination (61). However, in the case of A, metallation occurs indirectly at N_7 (62) involving the possible contribution from C_6-NH_2 in binary systems while in ternary complexes, A coordinates only through N_7 with metal ions (32, 63, 64) as it is evident from the higher values of equilibrium constants of the mixed ligand complexes which is probably favoured by electrostatic interaction between amino-hydrogen of the A and the oxygen ($C = O$) of U or 5 BrU (59).

The stabilization of the ternary complexes may be due to the fact that A and U or 5 BrU may take part in the stacking interactions. The stacking interactions are supposed to occur between the pyrimidine moiety of U or 5 BrU with A. It is well established fact that purine and pyrimidine bases, nucleosides and nucleotides associate extensively in aqueous solution by a mechanism involving vertical stacking of the bases (48, 65). The divalent metal cations like Mg (II) and Zn (II) have been found to promote the stacking by a factor of about 4 in nucleoside 5' - tri - phosphates (48).

Therefore in the ternary systems in the present case the stacking is expected to be promoted by divalent metal ions though to a lesser extend as compared to the nucleotides. On the basis of the observations made earlier in the case of metal - ATP - histidine (31), metal - cytidine – histidine and metal – cytidine - histamine it is likely that in metal - A - U or 5 BrU complexes, the A involves imidazole group (N_7) for metal bonding.

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