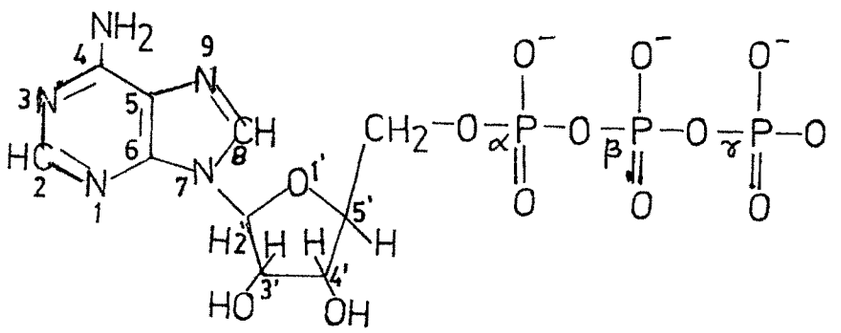
CHAPTER - V

Solvent Effect on Ternary Complexes Containing Tertiary amines and Adenosine-triphosphate

As many enzymes require activation by metal ions and as this often involves an enzyme substrate metal ion complex, ternary metal ion complexes have received considerable attention in recent years.[11,199,200] It appears that all enzymatic reactions involving nucleotides in particular ATP, are metal ion dependent.[201-204] Hence, the wide interest, that ATP and other nucleotide complexes are receiving, is certainly justified.[205-208]

Structure of ATP:



Adenosine 5 triphosphate (ATP)

The nucleotide consists of three structural units, the purine or pyrimidine base, the ribose unit and a mono-, di- or triphosphate group. Out of these, the ribose unit is least coordinating. In the binary complexes, nucleotides co-ordinate from the phosphate end [209-217] while the base moieties may or may not be co-ordinated depending on the base and the metal ion.[213] In case of Copper(II) complexes, weak co-ordination

of the base moieties of the nucleotides have been suggested.

Taquikhan and coworkers have determined the formation constants of the complexes of adenine nucleotide with variety of metal ions.[216-223] They have given the relative order of stabilities of metal adenine nucleotide complexes as ATP > ADP > AMP in accordance with the length of the phosphate chain.[216,218,219]

The nucleotides co-ordinate from the phosphate end and therefore the stability of the complexes of Cu-2,2'-dipyridyl and nucleotide phosphate[224] should be nearly same as other O^--O^- co-ordinating ligands like malonate. However, the Δ log K value was found to be more positive in cases of metal complexes [CudipyATP]. In the case of [M.dipy.ATP] complexes containing non-transition metal ions also, with less significant or no M ---> A π interaction Δ log K has been found to be positive.[213,225,226]

The extra stabilization in [MAL] where M = Co(II), Ni(II), Zn(II), A = 2,2'-dipyridyl and L = 5'-triphosphate of guanosine, uridine, thymidine or adenine has been explained by Sigel et al.,[213,227,228] by considering that in the ternary complex there is a charge transfer between bipyridyl and the purine or pyrimidine moiety of the 5'-triphosphates. They showed that in the ternary metal-bipyridyl-nucleotide complexes the metal ion is co-ordinated to the phosphate chain of the

nucleotide and the nitrogen of bipyridyl. The base part of ATP remains free and comes over the bipyridyl molecule giving rise to an intramolecular interaction between these two molecules.

Orioli and coworkers[229] have carried out x-ray study of [M.bipy.ATP] complex. According to them there are two types of stacking interaction a) Intramolecular and b) Intermolecular.

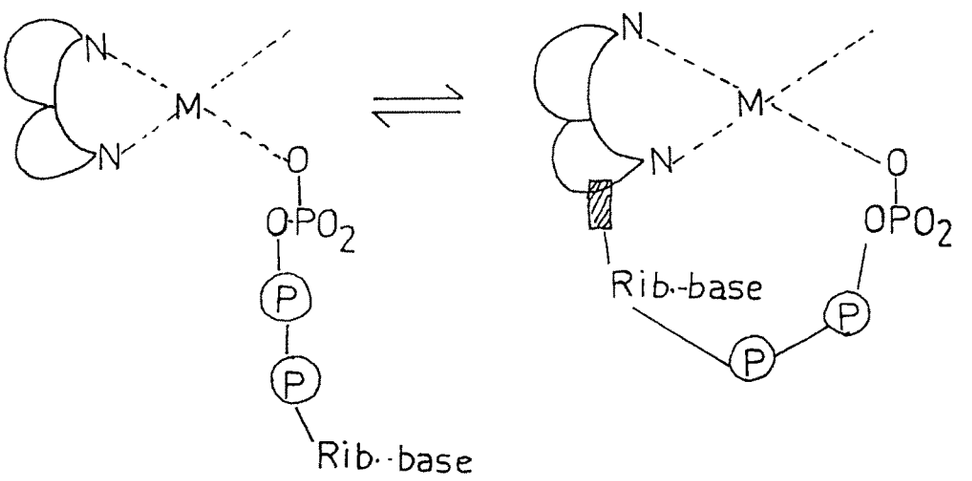
Intramolecular interaction is of purine-bipyridyl type via the metal-phosphate bridge. There are also two types of intermolecular stacking interaction i.e. bipyridyl-purine interaction and bipyridyl-bipyridyl self stacking. Thus, each bipyridyl ligand forms with one pyridine ring intramolecular stacking with imidazole of the purine base and with the other ring, intermolecular stacking with the pyrimidine ring of the purine base, belonging to a different molecule. However, the intramolecular interaction is by far the strongest one.

Sigel and coworkers[230] have also observed the intramolecular stacking interaction between covalently linked suitable groups like nicotinamide adenine dinucleotide $(NAD)^+$ and dihydronicotinamide (NADH). Rizzareli et al [227] have determined the stacking interaction in $[Zn(ATP)(hm)]^{2+}$ complexes by the help of thermodynamic parameters. They have shown that due to stacking interaction there is favourable enthalpic and unfavourable entropy contribution.

The UV Spectrophotometric studies[227] indicate that stacking interaction is charge transfer in nature. The charge transfer adduct is formed in which bipyridyl is the electron donor and purine moiety is the acceptor. Such an interaction exists between free bipyridyl and ATP also, the band occuring at 293-295 nm. It is observed that interaction is much higher in [Cu-bipy-ATP] and the charge transfer shifts to lower energy region. The co-ordination from the phosphate end with the metal ion makes the interaction of purine with the co-ordinated bipyridyl more probable.

NMR Spectral studies[226] indicate that the ^1H NMR resonance of those protons lying above or below a π system are shifted to a higher field compared to the signals of free molecule. In Cu(II) complex, this fact cannot be observed clearly because of the NMR line broadening due to unpaired electron on Cu(II). This has been observed clearly in diamagnetic Zn(II) complexes. In the absence of Zn^{+2}, a small shift in the resonance of $H_{(2)}$ (τ = 1.8) and H_8 (τ = 1.5) of ATP to higher field is observed confirming stacking between the aromatic part of the two ligands. When increasing concentration of Zn(II) are added to the solutions containing equimolar amount of ATP and bipy, the upfield shifts of $H_{(2)}$ or $H_{(8)}$ protons increase tremendously indicating considerable increase in stacking as a result of the stabilizing influence of Zn(II). The larger upfield shift of $H_{(8)}$ than $H_{(2)}$ may possibly imply that the imidazole part of the purine moiety is more involved in the formation of stacking adduct.

The intramolecular stacking has been observed in solution[227,228,230,231] and also in solid.[225,232,233] In the solution it is considered[236] that there exists an intramolecular equilibrium for these ternary complexes



and it has been shown that about 55% to 75% exist in the stacked isomeric form.

The intramolecular aromatic ring stacking interaction in mixed ligand complexes may be of hydrophobic nature. Furthermore, with an increasing size of the involved aromatic ring system, the extent of stacking interaction also increases.

The formation constants of complexes of the type [MAL] where M = Cu(II) or Ni(II), A = 2,2'-dipyridyl or 1,10-phenanthroline and L = ATP have been determined in different percentages of dioxan[235] and the effect of the less polar solvent on the hydrophobic stacking interaction has been studied. In the present chapter

ternary complexes of the type [M.A(ATP)] where M = Cu(II) or Ni(II) and A = 5-nitro-1,10-phenanthroline (A^1), 2,2'-dipyridylketone (A^2) or 2,2'-dipyridylamine (A^3), have been studied in different percentages of dioxan in order to see the effect of substitution on the ligand A on the stability of the ternary complexes and to see that the interligand interaction is hydrophobic in nature.

Experimental

Standardization of all the required solutions of metal perchlorates, sodium hydroxide, perchloric acid were done in the same way as detailed in chapter II A. The primary ligands (A^1 to A^3) were of the same quality as discussed earlier. The disodium salt of adenosine triphosphate, supplied by Kotch Light Laboratories Ltd., was used. The caliberation of microburette, pipette, pH meter was done as described in chapter II A. In each individual measurement, the pH values were corrected for 25% and 50% dioxan-water medium using the method suggested by Van Uitert and Haas.

The titration data for aqueous, 25% and 50% dioxan-water medium are given in Figures V1 to V13, where the concentrations of various reagents taken have also been shown.

The formation constants of ternary complexes were refined using SCOGS computer program as detailed in chapter II A. The refined values of the ternary complex formation constants in aqueous, 25% (1 : 3 v/v) and 50% (1 : 1 v/v) dioxan-water medium have been tabulated in Table V1 and 2. This also includes standard deviation σp and Δ log K values.

Table V 1

Ternary complex stability constants of Copper(II) in aqueous and dioxan-water (3 : 1, 1 : 1, v/v) medium and 0.2M $NaClO_4$ at 30°C, with standard deviation $\sigma\beta$ in parentheses

Solvent	$\log K^{CuA}_{CuA(ATP)}$					
	A^1	$\Delta \log K$	A^2	$\Delta \log K$	A^3	$\Delta \log K$
Aqueous medium	-	-	5.93 (± 0.07)	- 0.45	-	-
Dioxan-water (3 : 1, v/v) medium	6.65 (± 0.06)	+ 0.35	6.00 (± 0.08)	- 0.25	6.1 (± 0.09)	- 0.15
Dioxan-water (1 : 1, v/v) medium	8.53 (± 0.06)	+ 0.31	8.8 (± 0.04)	+ 0. 62	7.90(± 0.08)	- 0.32

Table V 2

Ternary complex stability constants of Nickel(II) in dioxan-water (3 : 1, 1 : 1, v/v) medium and 0.2M $NaClO_4$ at 30°C, with standard deviation $\sigma\beta$ in parentheses

Ligands	$\log K^{NiA}_{NiAL}$					
	A^1	$\Delta \log K$	A^2	$\Delta \log K$	A^3	$\Delta \log K$
Dioxan-water (1 : 3, v/v) medium	4.35 (± 0.02)	- 1.54	3.91 (± 0.03)	- 1.90	4.36 (± 0.02)	- 1.52
Dioxan-Water (1 : 1, v/v) medium	5.86 (± 0.05)	- 2.71	6.56 (± 0.1)	- 1.87	5.62 (± 0.02)	- 2.95

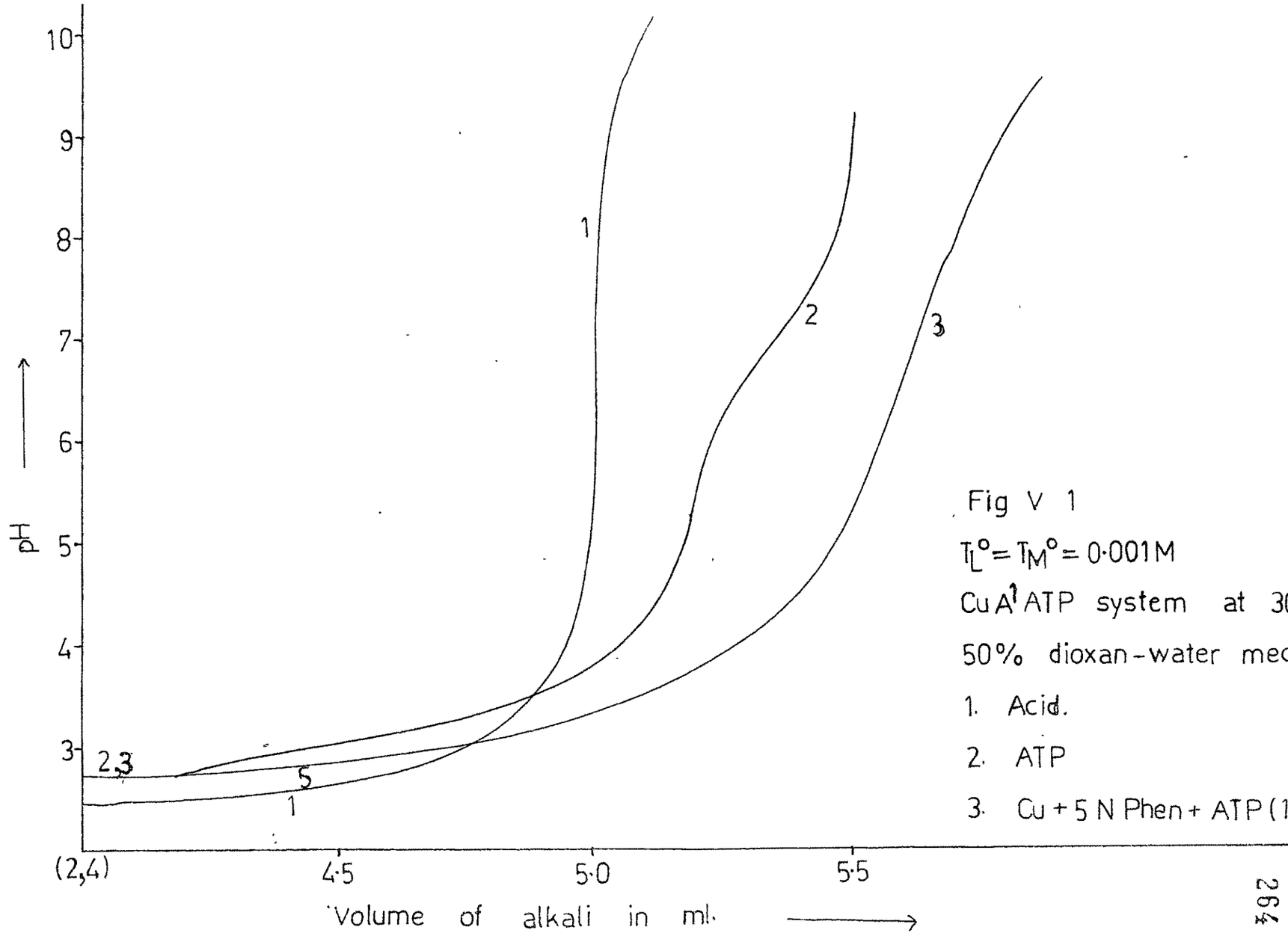


Fig V 1

$T_L^\circ = T_M^\circ = 0.001M$

Cu A^1 ATP system at 30°c

50% dioxan-water medium

1. Acid.
2. ATP
3. Cu + 5 N Phen + ATP (1:1:1)

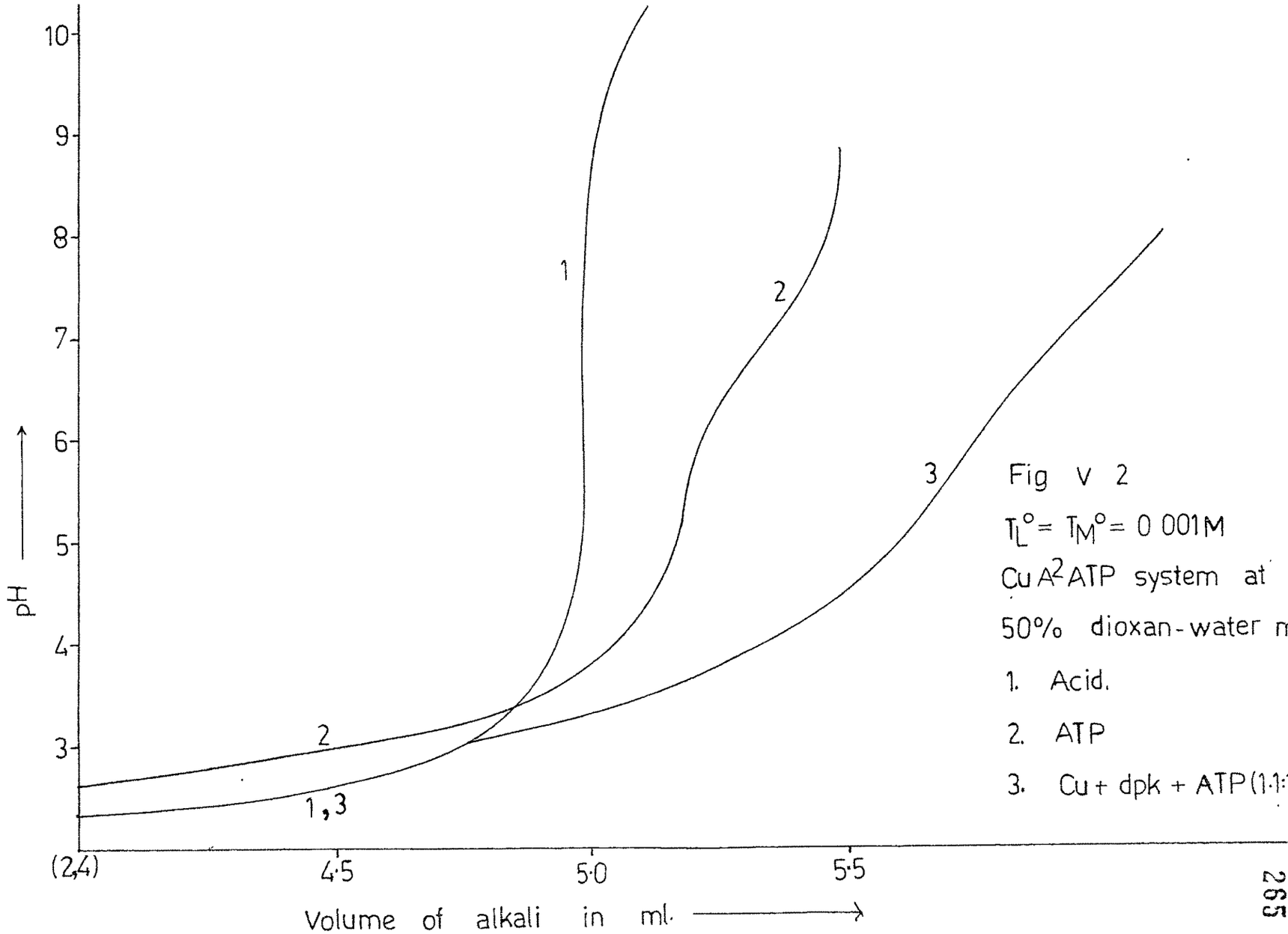


Fig V 2

$T_L^\circ = T_M^\circ = 0.001M$

$Cu\,A^2ATP$ system at 30°c

50% dioxan-water medium.

1. Acid.
2. ATP
3. Cu + dpk + ATP (1:1:1)

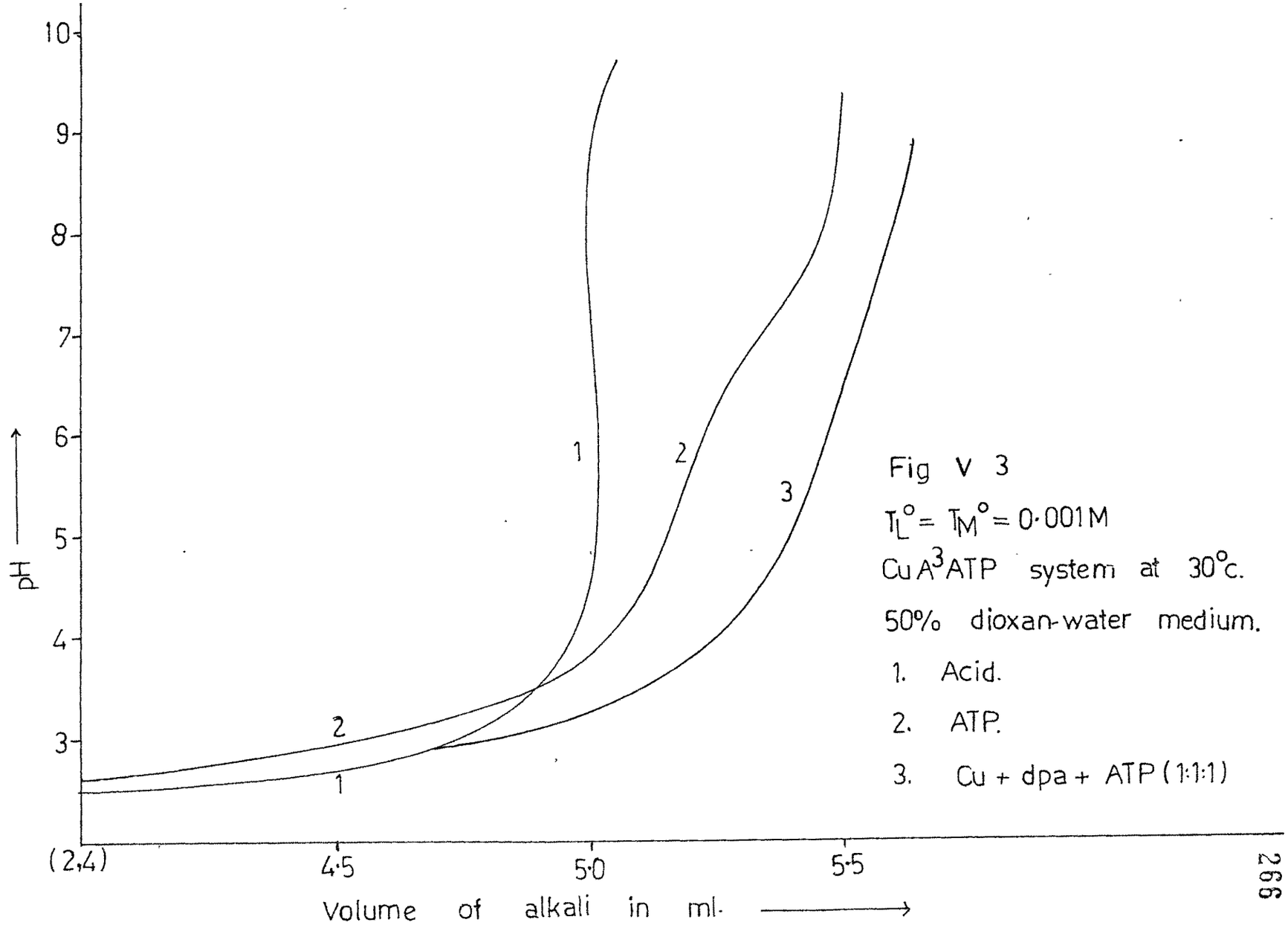


Fig V 3

$T_L^\circ = T_M^\circ = 0.001M$

CuA^3ATP system at 30°c.

50% dioxan-water medium.

1. Acid.
2. ATP.
3. Cu + dpa + ATP (1:1:1)

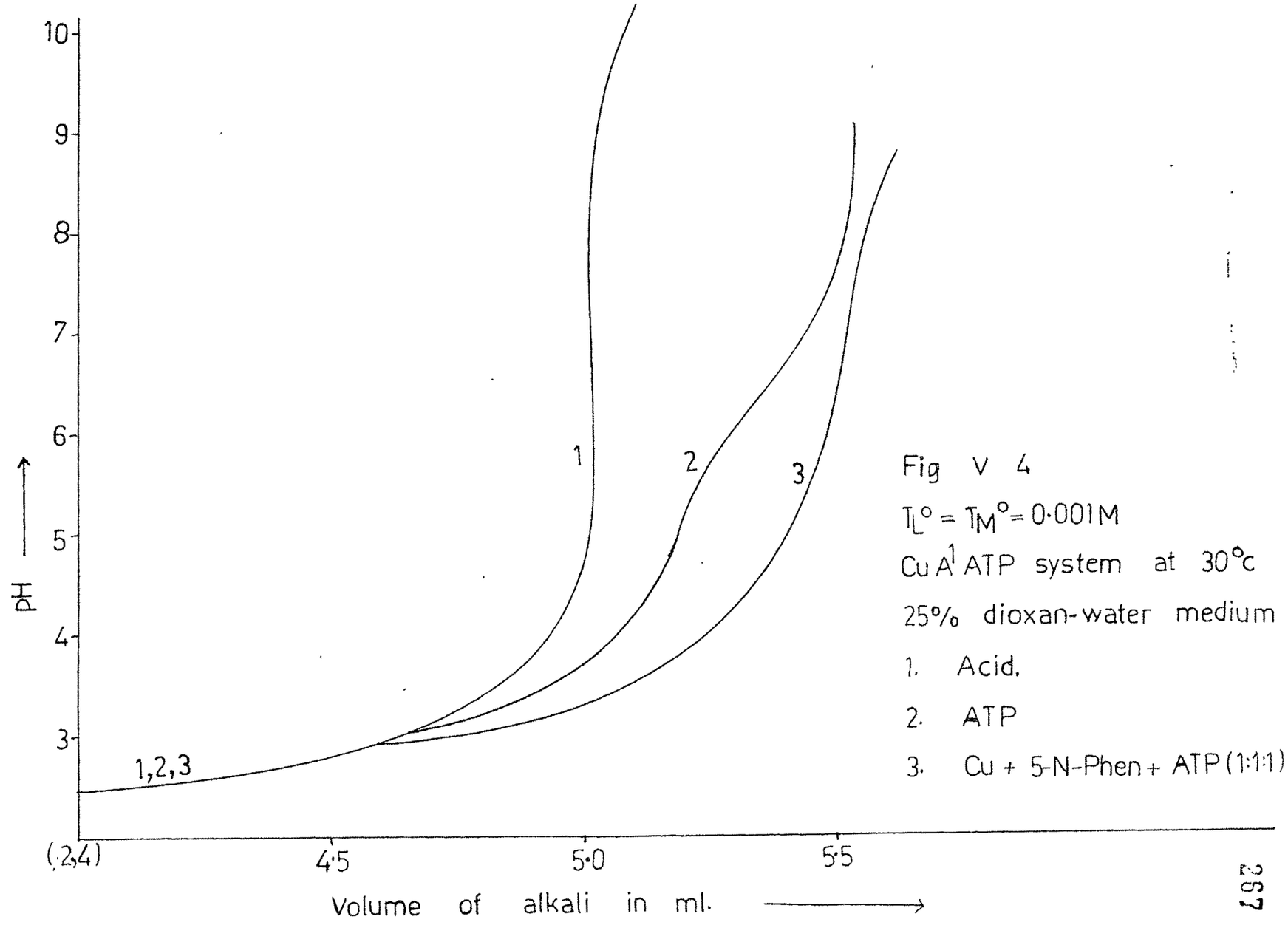


Fig V 4

$T_L^\circ = T_M^\circ = 0.001M$

Cu A^1 ATP system at 30°c

25% dioxan-water medium

1. Acid.
2. ATP
3. Cu + 5-N-Phen + ATP (1:1:1)

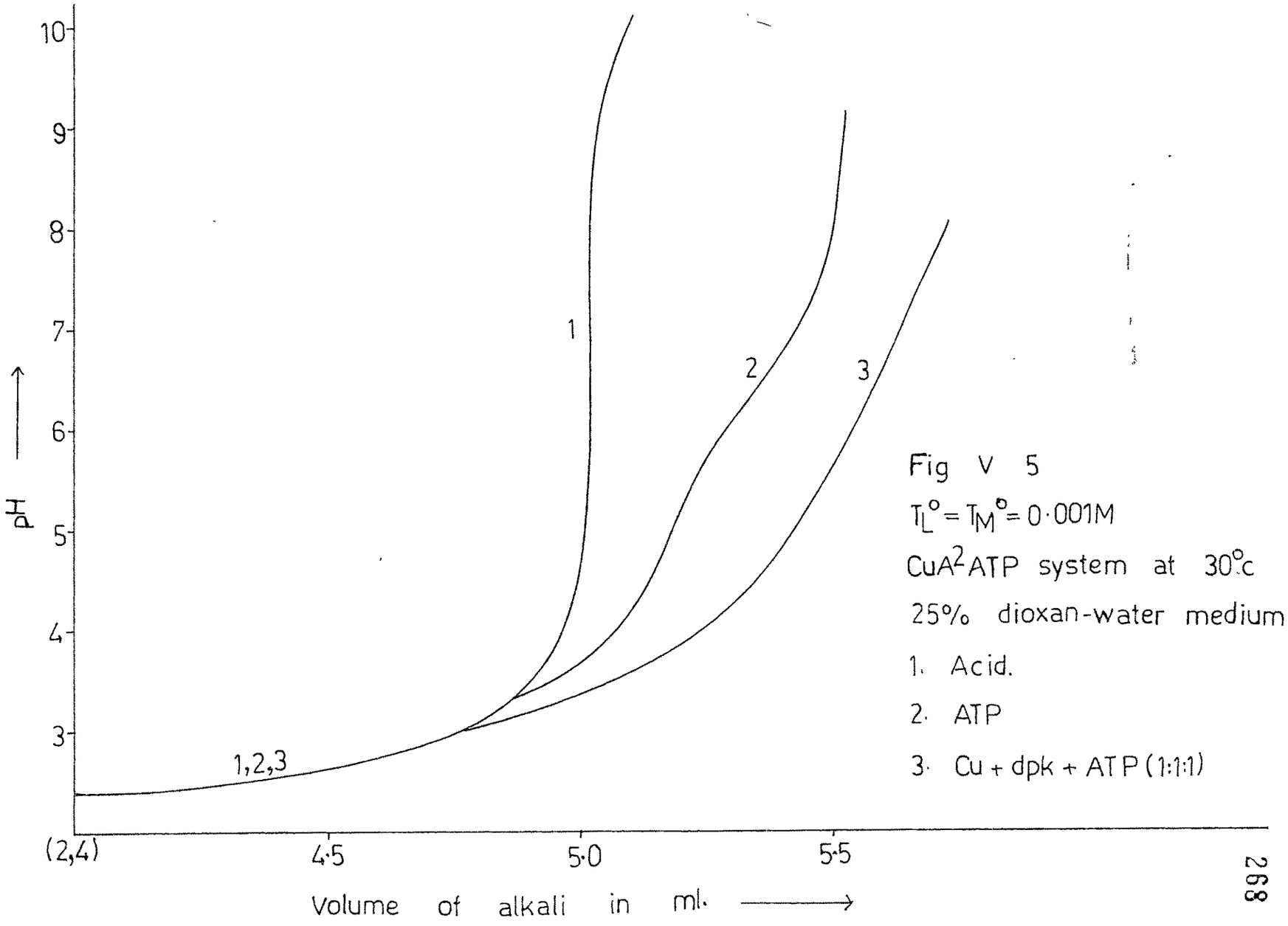


Fig V 5

$T_L^\circ = T_M^\circ = 0 \cdot 001M$

CuA^2ATP system at 30°c

25% dioxan-water medium

1. Acid.
2. ATP
3. Cu + dpk + ATP (1:1:1)

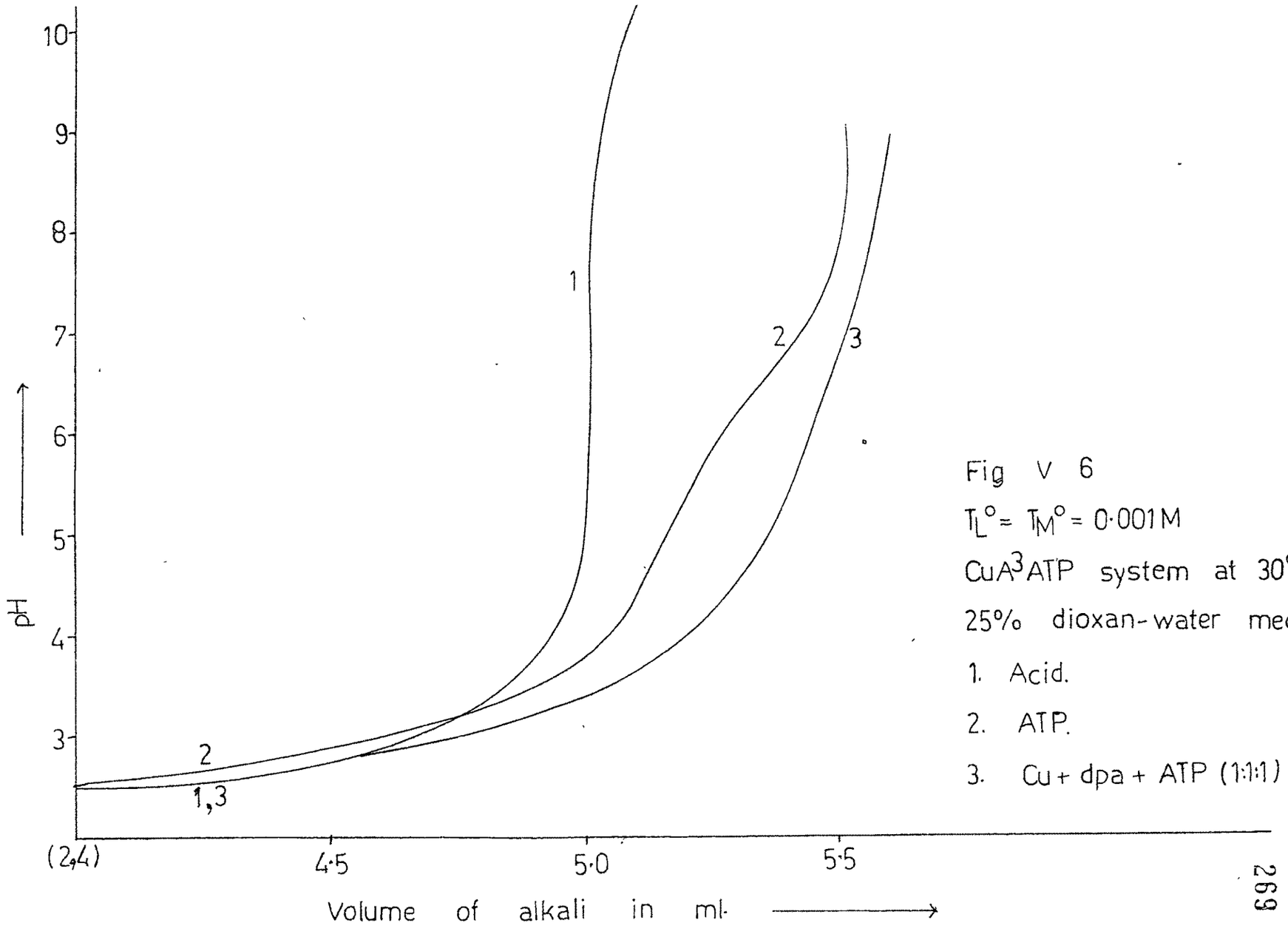


Fig V 6

$T_L^\circ = T_M^\circ = 0{\cdot}001\,M$

CuA^3ATP system at 30°c

25% dioxan-water medium

1. Acid.
2. ATP.
3. Cu + dpa + ATP (1:1:1)

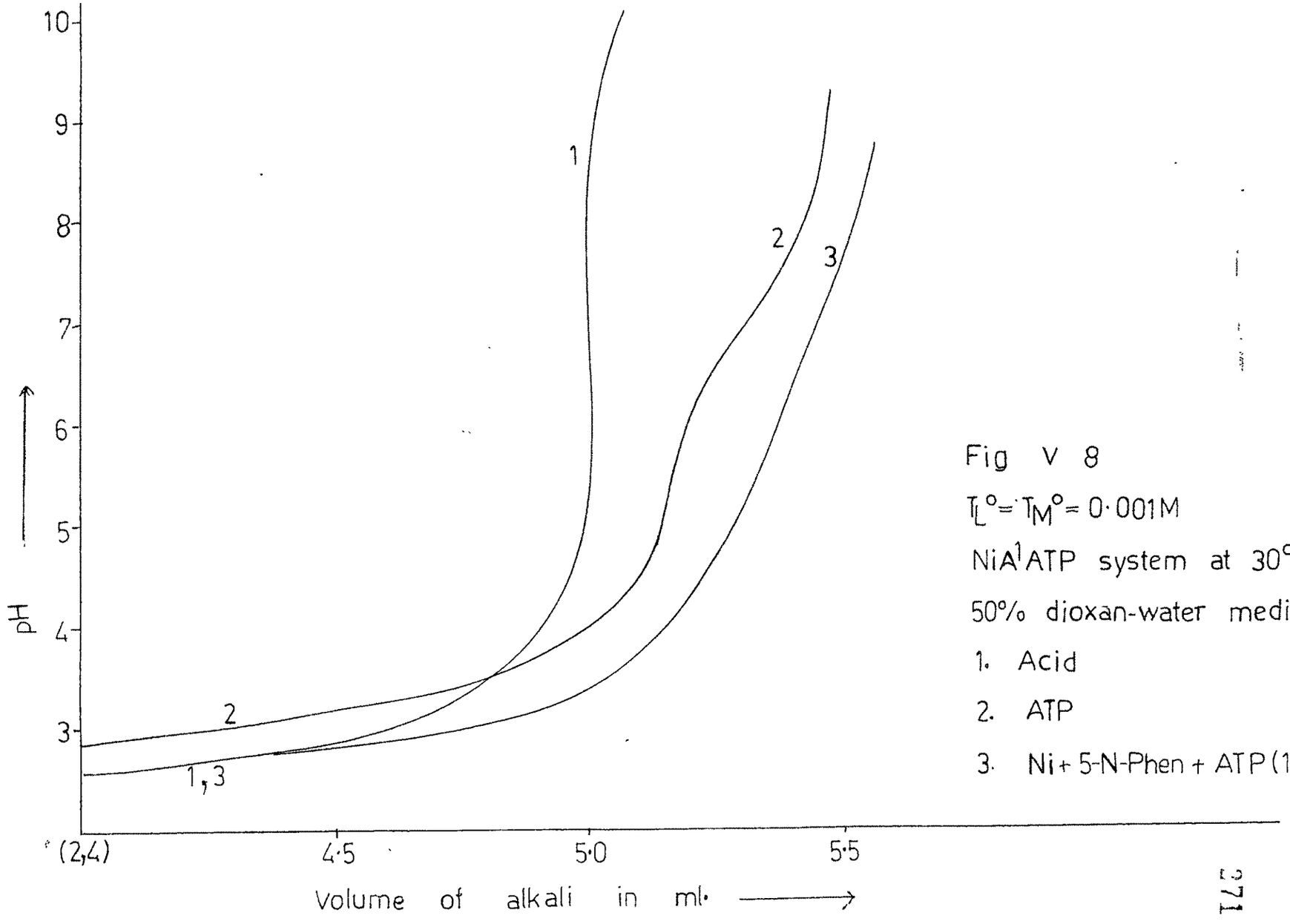


Fig V 8

$T_L^\circ = T_M^\circ = 0{\cdot}001M$

NiA^1ATP system at 30°c

50% dioxan-water medium

1. Acid
2. ATP
3. Ni + 5-N-Phen + ATP (1:1:1)

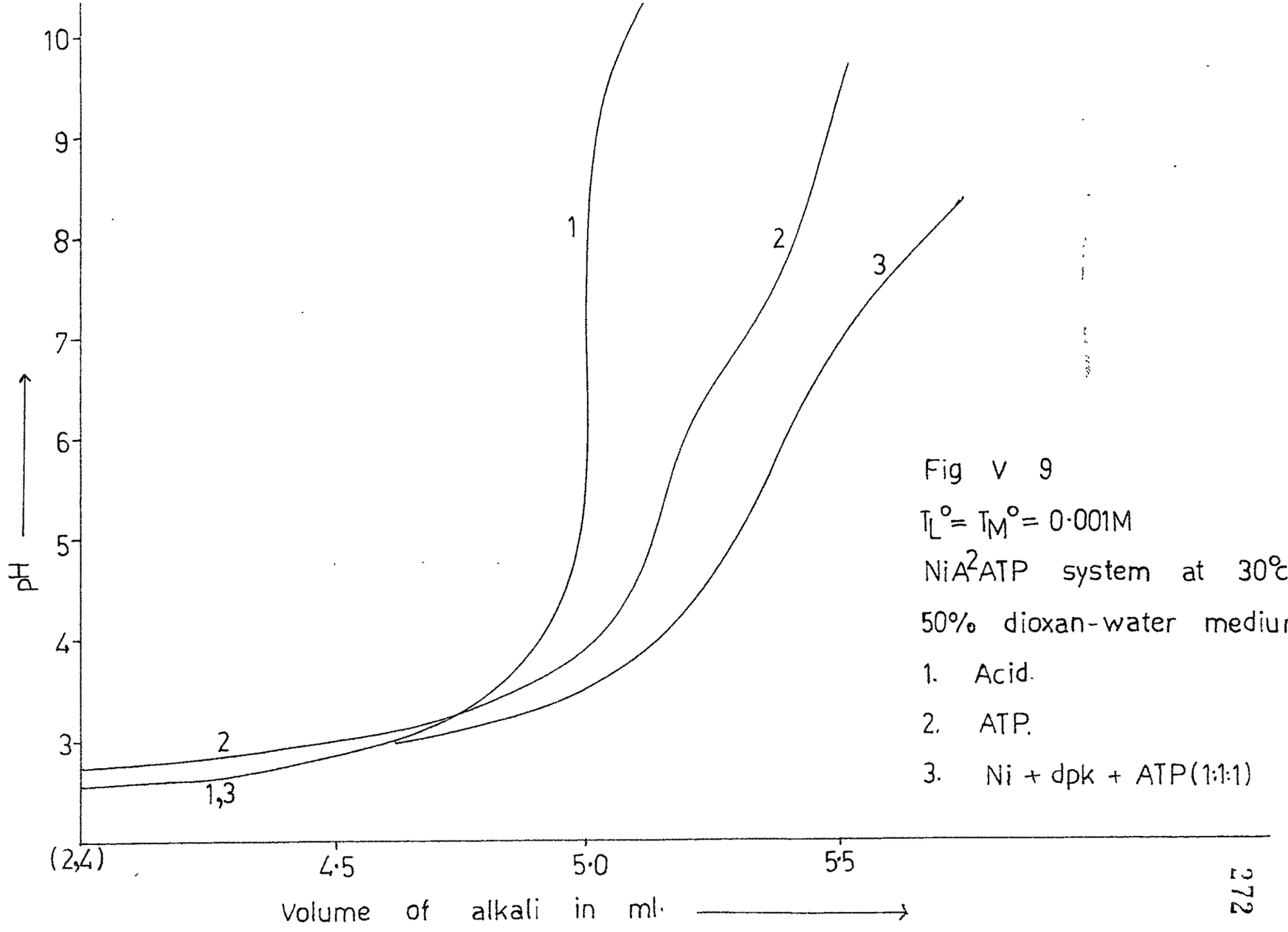


Fig V 9

$T_L^\circ = T_M^\circ = 0{\cdot}001M$

NiA^2ATP system at 30°c

50% dioxan-water medium

1. Acid.
2. ATP.
3. Ni + dpk + ATP(1:1:1)

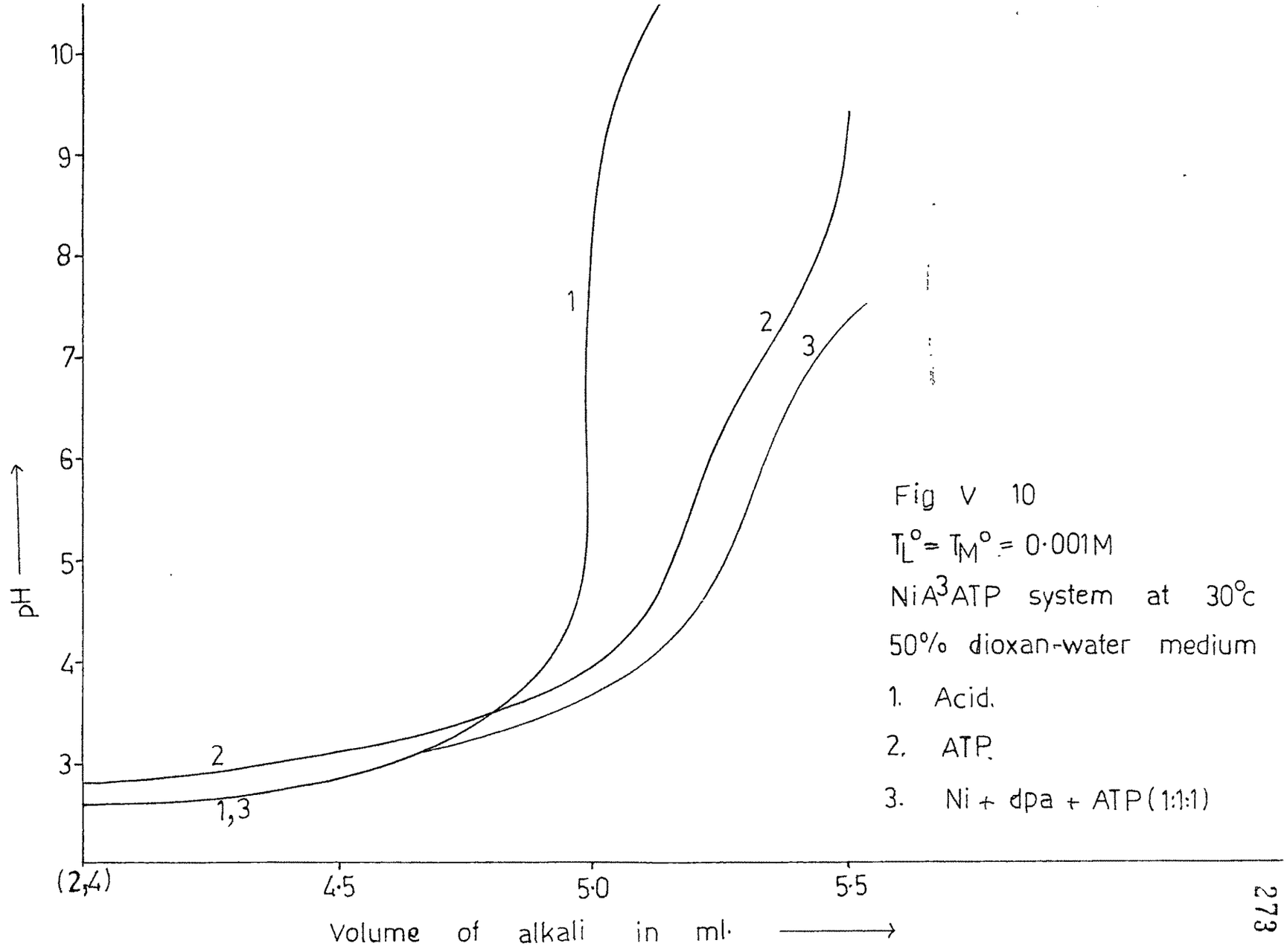


Fig V 10

$T_L^o = T_M^o = 0{\cdot}001M$

NiA^3ATP system at 30°c

50% dioxan-water medium

1. Acid.
2. ATP.
3. Ni + dpa + ATP (1:1:1)

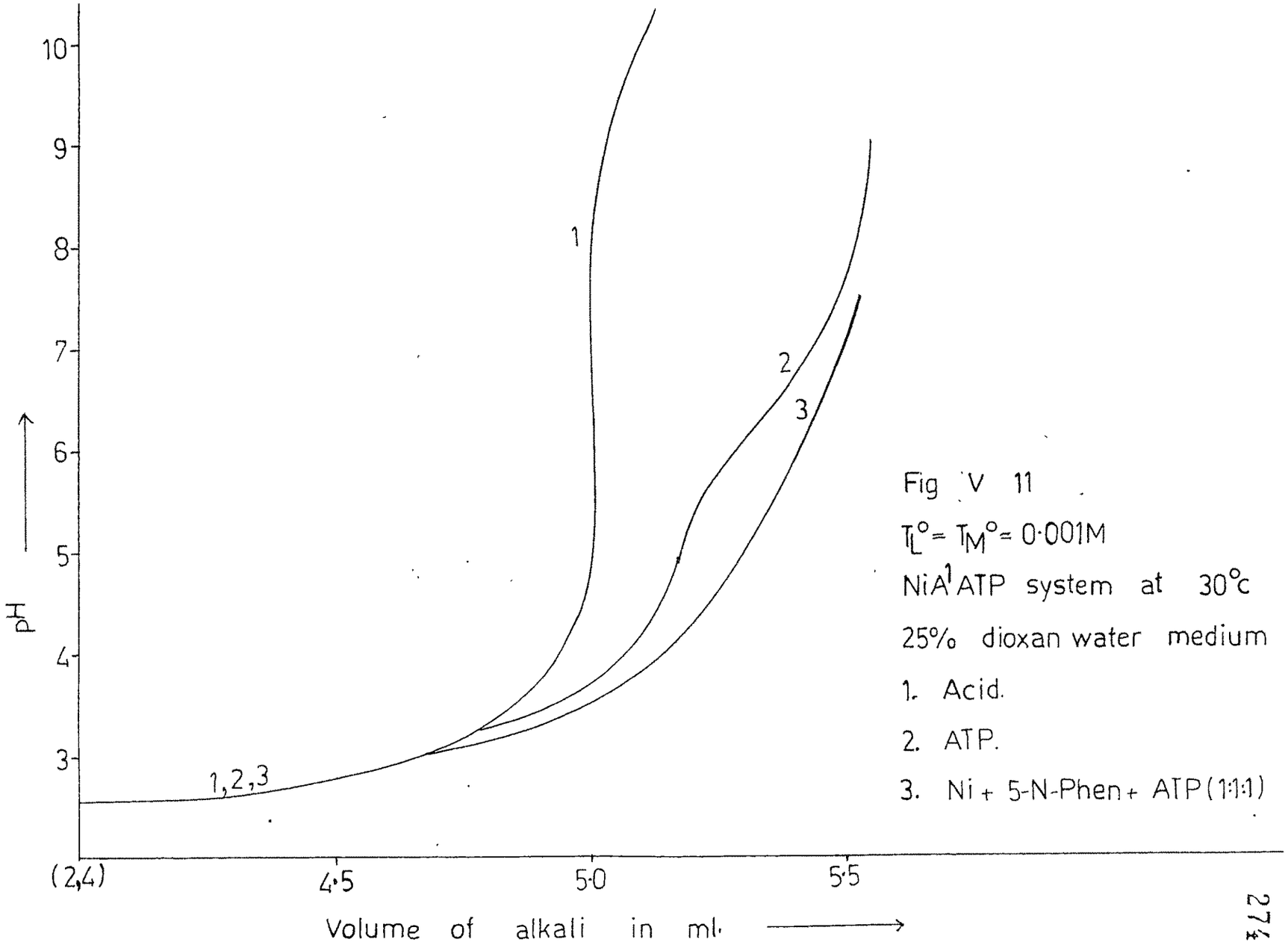


Fig V 11

$T_L^\circ = T_M^\circ = 0{\cdot}001M$

NiA^1ATP system at 30°c

25% dioxan water medium

1. Acid.
2. ATP.
3. Ni + 5-N-Phen + ATP (1:1:1)

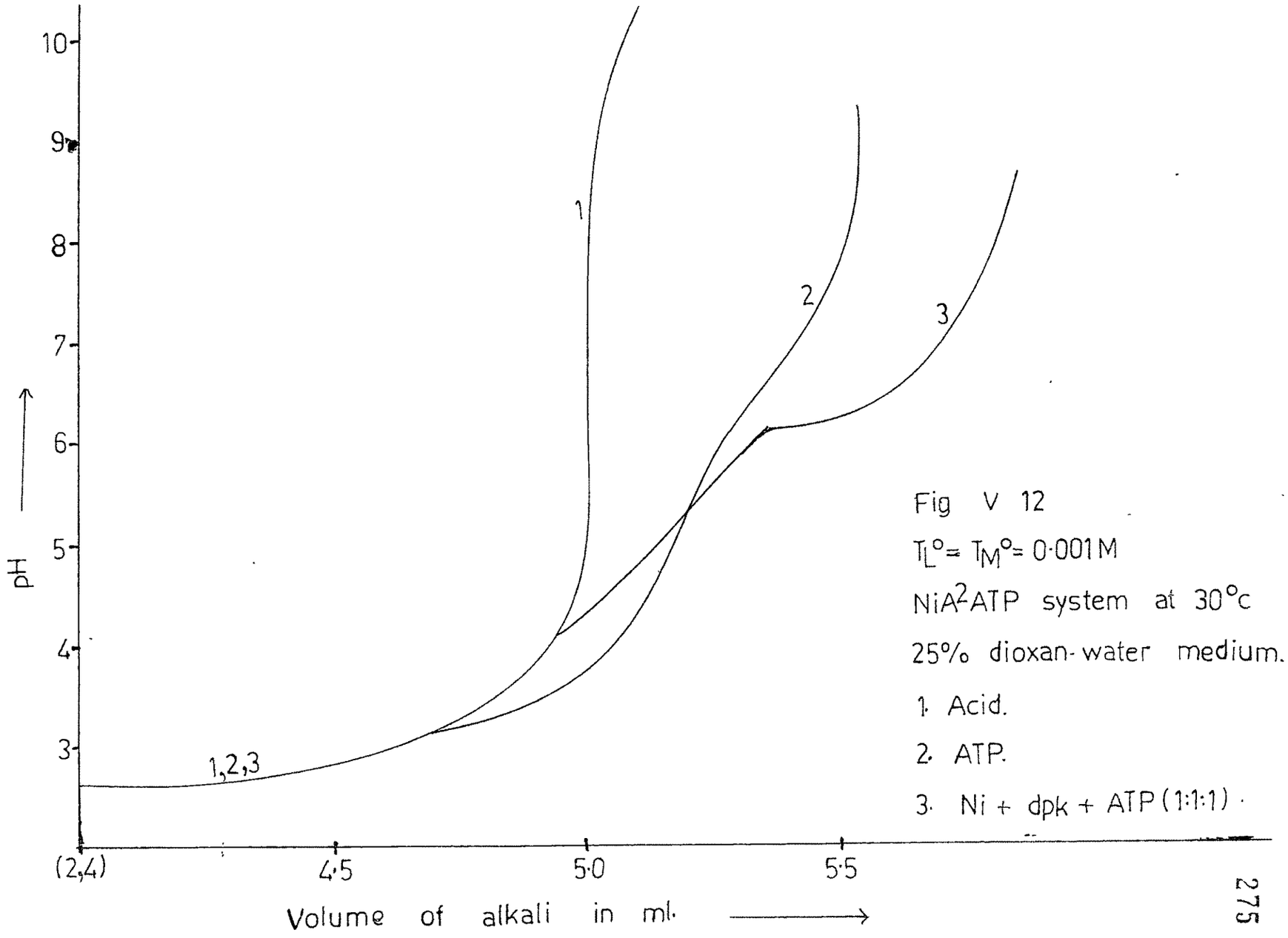


Fig V 12

$T_L^\circ = T_M^\circ = 0.001M$

NiA^2ATP system at 30°c

25% dioxan-water medium.

1. Acid.
2. ATP.
3. Ni + dpk + ATP (1:1:1).

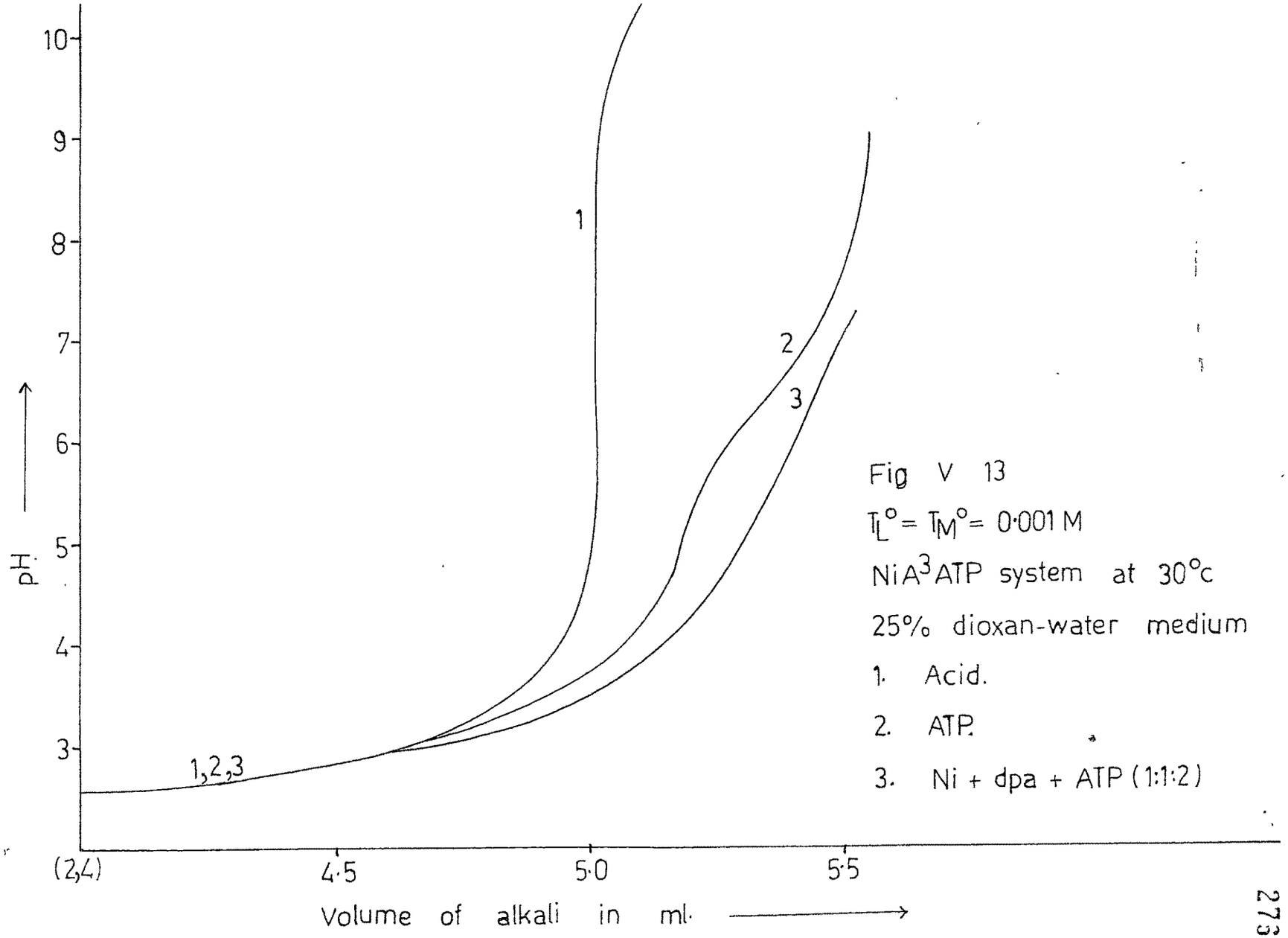


Fig V 13

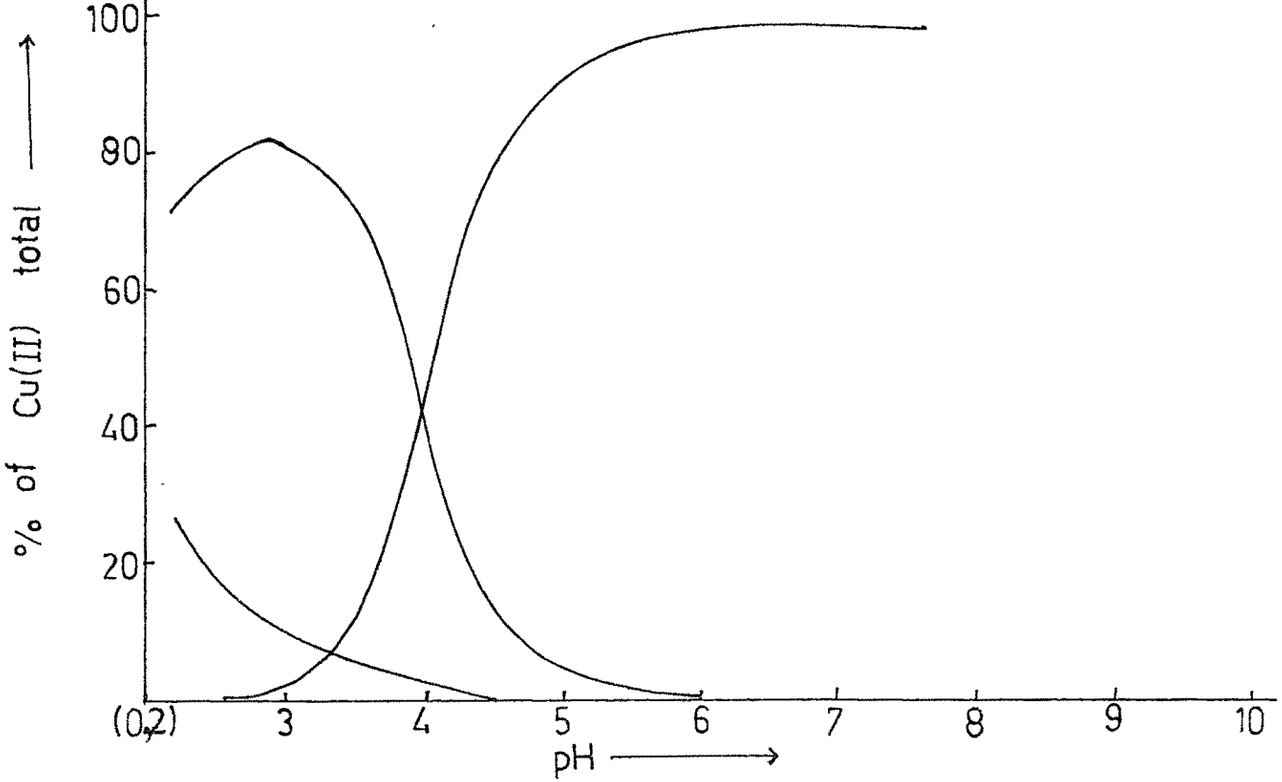
$T_L^\circ = T_M^\circ = 0{\cdot}001$ M

NiA^3ATP system at 30°c

25% dioxan-water medium

1. Acid.
2. ATP.
3. Ni + dpa + ATP (1:1:2)

Fig V 14 (CuA^1ATP) system



Variation of concentrations of different species with pH

Results and Discussion

The formation constants of the mixed ligand complexes obtained by the computer calculation are nearly in agreement with the preliminary values fed into the computer, considering the reaction to be taking place in steps.

The analysis of representative species distribution curves (fig. 14), shows that in the pH range 1 to 3, Cu(II) and [CuA] are the major species and in the pH range 4 to 7 the species [CuA] and [CuAL] are predominant. Formation of $[CuA_2]$, [CuL] and $[CuL_2]$ is very less. This shows that the reaction takes place in steps.

The stability of $[CuA^1L]$ is found to be much greater than that of [M 1,10-phenanthroline L] because of the presence of the electron withdrawing group over the ligand A^1. This depletes the electron density over the ring, making it a stronger π acid than 1,10-phenanthroline and hence stabilizes the ternary complex.

The ligand, 2,2'-dipyridylamine (dpa) forms a six membered ring on co-ordination to the metal ion. Moreover, an electron donating imino group (-NH group) in between two pyridine moieties enhances the electron density on the ring due to the presence of a lone pair of electrons on the nitrogen atom. This should reduce M -----→ L π interaction resulting in lower value of

the mixed ligand formation constant. However, delocalization of electron density in dpa is over a larger area than in 2,2'-dipyridyl. Hence the formation constant of ternary complexes are close to that for [Cu 2,2'-dipyridyl L] and [CuA^3L].

In case of [CuA^2ATP] the stability is not that much as expected. This is due to the equilibrium between the keto and gem-diol form as discussed in chapter III. In 50% dioxan medium the equilibrium is shifted more towards the keto form. The stabilities of the complexes [$CuA^2(ATP)$] are found to be much greater than [Cu 2,2'-dipyridyl ATP], due to the presence of electron withdrawing group $>C=O$ over A^2. The keto form of dpk is a stronger π acid due to π delocalization over $>C=O$ part. This depletes the electron density over the ring making it a stronger π acid than 2,2'-dipyridyl and hence stabilizes the ternary complexes.

As in the case of [Cu 2,2'-dipyridyl ATP] complexes, in the complexes of [$CuA(ATP)$] where A = A^1, A^2 or A^3, the value of Δ log K is positive. This is evidently due to stacking interaction between the adenine base of ATP molecule and the tertiary amine. Further, Δ log K value in case of [$CuA^2(ATP)$] is as much as in case of [Cu 2,2'-dipyridyl ATP] indicating that the extent of stacking interaction is same in both the cases.

In the case of [$CuA^1(ATP)$] and [$CuA^2(ATP)$], Δ log K is more positive than that for [Cu 1,10-

phenanthroline(ATP)] or [Cu 2,2'-dipyridyl ATP] complexes. The higher stability of these ternary complexes may be attributed to two different co-operative effects : (i) π accepting heteroaromatic N bases co-ordinated to metal ion (ii) Intramolecular aromatic ring stacking enhances the stability of ternary complexes. However, the value of Δ log K is equally more positive in case of [CuAcatecholate] complexes where A = A^1 or A^2 than that for [CuAcatecholate] where A = 1,10-phenanthroline as 2,2'-dipyridyl. It is due to greater M ----→ A π interaction. This shows that more positive values of Δ log K for [CuA^1(ATP)] and [CuA^2(ATP)] complexes are because of increase in M ----→ A π interaction. The extent of stacking interaction in [CuA^1(ATP)] or [CuA^2(ATP)] is of the same magnitude as in case of [Cu 1,10-phenanthroline ATP] or [Cu 2,2'-dipyridyl ATP], respectively. There is no significant effect of nitro or carbonyl group on the stacking interaction. This may be because the intramolecular stacking is due to interaction between one of the pyridine rings and purine base of ATP. Hence probably the presence of nitro or carbonyl group over A^1 or A^2, respectively, may not hinder the stacking interaction.

It is interesting to compare the values of Δ log K in the different percentages of solvent. It is known that the organic solvents affect log K^H values of the ligands. With increase in the solvent dielectric constants log K^H of the ligand decreases and it becomes more acidic. With

decreasing dielectric constant of the solvent content in a mixture of the two solvents, log K^H increases and ligand becomes more basic.

The increasing or decreasing values of protonation constants of the ligands in turn, increase or decrease the value of [ML] formation constant. Hence, the formation constants of the metal complex of a ligand should be higher in a solvent with lower dielectric constant. Further, the stability constants of metal complexes are strongly affected by the dielectric constants of the medium because of the fact that the metal ion is charged and the ligand is either charged or has a dipole moment. Hence the formation constant of the resulting complex is dependent on the dielectric constant of the solvent.

However, besides the above effects, the solvent may itself get protonated and also increase the proton accepting property of water by breaking the hydrogen bond. The latter two effects should decrease log K^H value of the ligand and work against the former dielectric effect.

Steger and Corsini[236] measured the protonation constants and stability constants of metal complexes of some 5-substituted derivatives of oxine in 40%, 60% and 75% dioxan. They observed that variation of log K^H and log K^M_{ML} increased linearly with increasing mole fraction of dioxan. Van Uitert[237] has shown that the value of log K^M_{ML} for several acetylacetonates are linear functions of the molar fraction of dioxan in the solvent.

It can, therefore, be presumed that the factor affecting the formation constants of the complexes is only the dielectric constant of the medium and protonation of solvent is not significant. Hence, in the present study of the formation constant of [M.bipy.ATP] complexes, using computer program, protonation of the solvent has not been considered.

It can be expected that the dielectric constant of the solvent should also affect the stability of the ternary complex in the same way as the binary complex. In the case of all O^{-}-O^{-} co-ordinating ligands it has been observed earlier that the formation constants of both binary and ternary complexes in 50% dioxan-water medium are higher than in aqueous medium. Hence Δ log K is found to be of same magnitude in aqueous and 50% dioxan-water medium.

As stated earlier [CuA(ATP)] shows positive Δ log K value in aqueous solution. However, in the present study it is observed (Table V 1,2) that Δ log K becomes less positive with increasing dioxan percentages in the solvent. In 25% and 50% dioxan-water medium, Δ log K value is negative in $[M\ A^{2}(ATP)]$ complexes. The value of Δ log K is positive in case of $[Cu\ A^{1}(ATP)]$ complexes.

Destabilization of the ternary complexes in dioxan-water medium is also evident from the titration curves of $[CuA^{3}(ATP)]$ in aqueous and 50% dioxan-water medium as shown in Figures V 2 to V 8, respectively. In 25% dioxan-

water medium Cu(II) + ATP curve is below Cu(II) + A^3 + ATP curve but above Cu(II) + A^1 + ATP curve. This shows that in 50% dioxan-water medium in case of $[CuA^3ATP]$ there is less stabilization leading to less positive Δ log K, whereas $[CuA^1(ATP)]$ is more stable giving more positive Δ log K. The position of the curves have to be considered only upto 5.0 pH, because after that [Cu.ATP] undergoes hydroxocomplex formation and goes below $[CuA^1(ATP)]$ curve in every case.

In case of $[CuA^2(ATP)]$ the stability of the ternary complex increaseswith increasing percentage of dioxan. The value of Δ log K is more positive in 50% aqueous-dioxan solvents compared to the value in aqueous solution. This can be explained by considering the equilibrium between keto and geminal diol form. In aqueous-dioxan solution of the complex, geminal diol formation may be less (equation 4 shifting more to left) and dpk is more in keto form, with greater π accepting ability than the geminal-diol form. This results in greater stability of log K^{Cudpk}_{CudpkL} complexes compared to log K^{Cu}_{CuL} in aqueous-dioxan solution and hence Δ log K is more positive. However, in case of $[CuA^2(ATP)]$ the value of Δ log K does not increase as much as in case $[CuA^2catecholate]$.

This shows that the solvent molecules play an important role in the destabilization of the ternary complex. It can be expected that, in case of [CuA(ATP)], intramolecular stacking interaction between tertiary

amines and adenine base of ATP may be partly hydrophobic in nature. This interaction is greater in aqueous medium, but probably in dioxan-water medium the solvent molecules interfere with the intramolecular stacking interaction between bipyridyl and adenosine ring and thus, the ternary complex is destabilized. This leads to negative Δ log K values. In case of $[CuA^1(ATP)]$ and $[CuA^2(ATP)]$, however, even in 50% dioxan-water medium, Δ log K is found to be positive. This may be because there is increase in M ----→ A π interaction and hence stacking interaction is more in $[CuA^1(ATP)]$ and $[CuA^2(ATP)]$ and holds even in 50% dioxan-water medium and results in positive Δ log K values.

In the case of $[CudpkATP]$ the stability of ternary complexes increases with increasing percentage of dioxan. This is because in aqueous solution dpk is in gem-diol form with sp^3 hybridized carbon atom. Hence, the planarity of the complex and the extent of π delocalization in dpk.H_2O is less and hence hydrophobic stacking interaction is less. With increasing dioxan content there is an increase in the keto form of dpk. The keto form is more planar with greater π delocalization and hence hydrophobic stacking interaction is more with base part of ATP and Δ log K is more positive.