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DETERMINATION OF ACIDITY AND BASICITY CONSTANTS OF N-ACETYL-PEPTIDE COMPOUNDS

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ABSTRACT

Acidity and basicity constants of N-acetyl-l-alanine, N-acetyl-l-histidine, N-acetyl-l-lysine, and N-acetyl-l-valine have been determined by means of potentiometric titration. The titrations have been observed at 25°C and ionic strength of 0.5 M potassium nitrate. The equilibrium constant of these N-acetyl-pepetide compounds were obtained by computer refinements.

INTRODUCTION

N-acetyl-peptide compound is a peptide derivative of which N-terminal group is acetylated.[1] N-acetyl-peptide is an essential compound as well as amino acid for human body.[2] Both N-acetyl-peptide and amino acid can form complex with many divalent metal ions.[3] Dipeptides and their complexes with divalent metals have been widely studied by various methods.[4-12] These complexes are of interest because most dipeptide complexes with divalent metal ions are unstable. Only one proton of dipeptide can be ionized from the compound. Dipeptide or polypeptide is usually present in form of zwitterionic species in aqueous solution. Monodeprotonation and its equilibria[5, 10] therefore exist in the solution; N-terminal group of the compound can be protonated and its acid proton can also be dissociated. However, the nitrogen peptide bond of dipeptide or polypeptide compounds[1, 10, 11] is usually too weak to be protonated.

As N-acetyl-peptide is a biological compound, its formation constant is therefore of use for further work on chemical reactions in a biological system. A study of acidity and basicity of N-acetyl-l-alanine, N-acetyl-l-histidine, N-acetyl-l-lysine, and N-acetyl-l-valine were performed.

MATERIALS AND METHODS

N-acetyl-l-alanine, N-acetyl-l-histidine, N-acetyl-l-lysine and N-acetyl-l-valine of Sigma grade were used. Potassium nitrate (AR grade) was used as the inert background solution. In the titration, the standard solutions of 0.1 M HC1 and NaOH (Merck Chemicals) were used to adjust the pH of solution.

An automatic potentiometer (ORION model EA 906) including combination pH electrode, model 81-02, was used. The pH electrode was calibrated by three standard pH buffers; pH of 4.01, 7.00 and 10.00 were used. Accuracy of the instrument was indicated by the

slope value based on the isopotential point of pH 7.00=0.0 mV, exceeding 99%. Dispenser of the automatic potentiometer was calibrated regularly.

All measurements of potentiometric titrations were carried out at 25.0±0.1°C and ionic strength of 0.5 M KNO_3. The acidity of the solution was adjusted by hydrochloric acid and the pH of the system solution was increased by adding 0.1 M sodium hydroxide (in 0.5 M KNO_3). The initial volume of each titration was 50 ml. The titrations were performed under nitrogen atmosphere saturated by potassium nitrate vapour, flowing through the titration chamber. Three titrations on each ligand were observed within pH range 3 to 11.

The pH range and the initial concentration of ligands used in refinements for determinination of acidity and basicity constants of N-acetyl-l-alanine, N-acetyl-l-histidine, N-acetyl-l-lysine and N-acetyl-l-valine are shown in Table 1, 2, 3 and 4, respectively.

Acidity and basicity constants of N-acetyl-l-alanine, N-acetyl-l-histidine, N-acetyl-l-valine and N-acetyl-l-lysine were obtained from computer refinement using MINIQUAD programme[12] performed on the IBM 3031/08 computer of Chulalongkorn University and SCANET 386SX Exclusive AT computer. The formation constants of these N-acetyl-peptides obtained from the computer refinements are shown in Table 5 and were simultaneously optimized together with dissociation constant of water (K_W); the value of log K_W was within −13.75 to −13.68.[13]

RESULTS AND DISCUSSION

Equilibrium constants for the N-acetyl-peptide (LH) are assigned as follow.

$$LH \xrightarrow{K_1} L^- + H^+$$

$$LH + H+ \xrightarrow{K_2} LH_2+$$

$$LH_2^+ + H^+ \xrightarrow{K_3} LH_3^{2+}$$

The detected species of N-acetyl-peptides, obtained from the optimizing process of the computer refinement are shown below.

Compound	Species present
N-acetyl-l-histidine	L^-, LH, $LH2^+$ and $LH3^{2+}$
N-acetyl-l-alanine	L^- and LH
N-acetyl-l-lysine	L^-, LH and $LH2^+$
N-acetyl-l-valine	L^-, LH and $LH2^+$

TABLE 1. Experimental data used in computer refinements for determining dissociation constant of N-acetyl-l-alanine.

Titration	Initial concentration (mM)		pH Range	Data
	N-acetyl-l-alanine	HCl		points
1	0.990	–	3.48-11.51	30
2	1.381	–	3.12-11.52	31
3	1.961	–	3.24-11.50	44

TABLE 2. Experimental data used in computer refinements for determining dissociation and basicity constant of N-acetyl-l-histidine.

Titration	Initial concentration (mM)		pH Range	Data
	N-acetyl-l-histidine	HCl		points
1	1.739	3.662	3.05-11.03	54
2	1.773	1.773	5.21-11.05	36
3	2.608	3.569	3.49-11.02	53
4	3.478	3.477	5.17-11.01	43

TABLE 3. Experimental data used in computer refinements for determining acidity and basicity constant of N-acetyl-l-lysine.

Titration	Initial concentration (mM)		pH Range	Data
	N-acetyl-l-lysine	HCl		points
1	0.971	1.942	2.78-11.00	53
2	1.923	1.923	2.86-11.08	54
3	2.857	1.905	2.93-11.00	73

TABLE 4. Experimental data used in computer refinements for determining acidity and basicity constant of N-acetyl-l-valine.

Titration	Initial concentration (mM)		pH Range	Data
	N-acetyl-l-valine	HCl		points
1	0.970	1.941	2.75-11.02	45
2	1.923	1.923	2.70-11.00	55
3	2.857	1.905	2.67-11.02	55

TABLE 5. Equilibrium constants of various N-acetyl-peptide compounds at 25°C and ionic strength of 0.5 M potassium nitrate.

Ligand	log K_1	log K_2	log K_3
N-acetyl-l-alanine	-3.52 ± 0.05	–	–
N-acetyl-l-histidine	-7.19 ± 0.01	-2.96 ± 0.04	-2.47 ± 0.05
N-acetyl-l-lysine	-9.11 ± 0.03	-2.37 ± 0.01	–
N-acetyl-l-valine	-3.35 ± 0.02	-2.02 ± 0.06	–

Dissociation of N-acetyl-l-alanine took place at pH approximately less than 5. No protonated species can be formed in the solution (Fig. 1). Species distribution of N-acetyl-l-histidine, calculated according to its equilibrium constant, is shown in Fig. 2. Species domination of N-acetyl-l-histidine depending on the pH of solution is in the sequence of $LH_3^{2+} \rangle LH_2^{+} \rangle LH \rangle L^{-}$ at increasing acidity.[5, 6, 15, 16] Protonated species of N-acetyl-l-histidine (LH_3^{2+} and LH_2^{+}) correspond to the protonations on nitrogen atoms of pyridyl group.

Figure 3 shows that LH species of N-acetyl-l-lysine was present over a wide range of pH (pH 2 to 11) but LH_2^{+} exists in the acidic solution (pH below 5). All species of N-acetyl-l-valine exist in the solution at pH below 7.5 accept L^{-} ; LH_2^{+} and LH exist at pH below 4.5 and 6 respectively (see Fig. 4).[2]

Table 5 indicates that order of magnitude for acidity constant of N-acetyl-peptides is in the sequence of N-acetyl-l-valine $\rangle$ N-acetyl-l-alanine $\rangle\rangle$ N-acetyl-l-histidine $\rangle$ N-acetyl-l-lysine.

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บทคัดย่อ

ได้ศึกษา acidity และ basicity constants ของสาร N-acetyl-l-alanine, N-acetyl-l-histidine, N-acetyl-l-lysine และ N-acetyl-l-valine โดย potentiometric titration ใน 0.5 M potassium nitrate ที่อุณหภูมิ 25°C พร้อมกับคำนวณ equilibrium constants โดย computer refinement

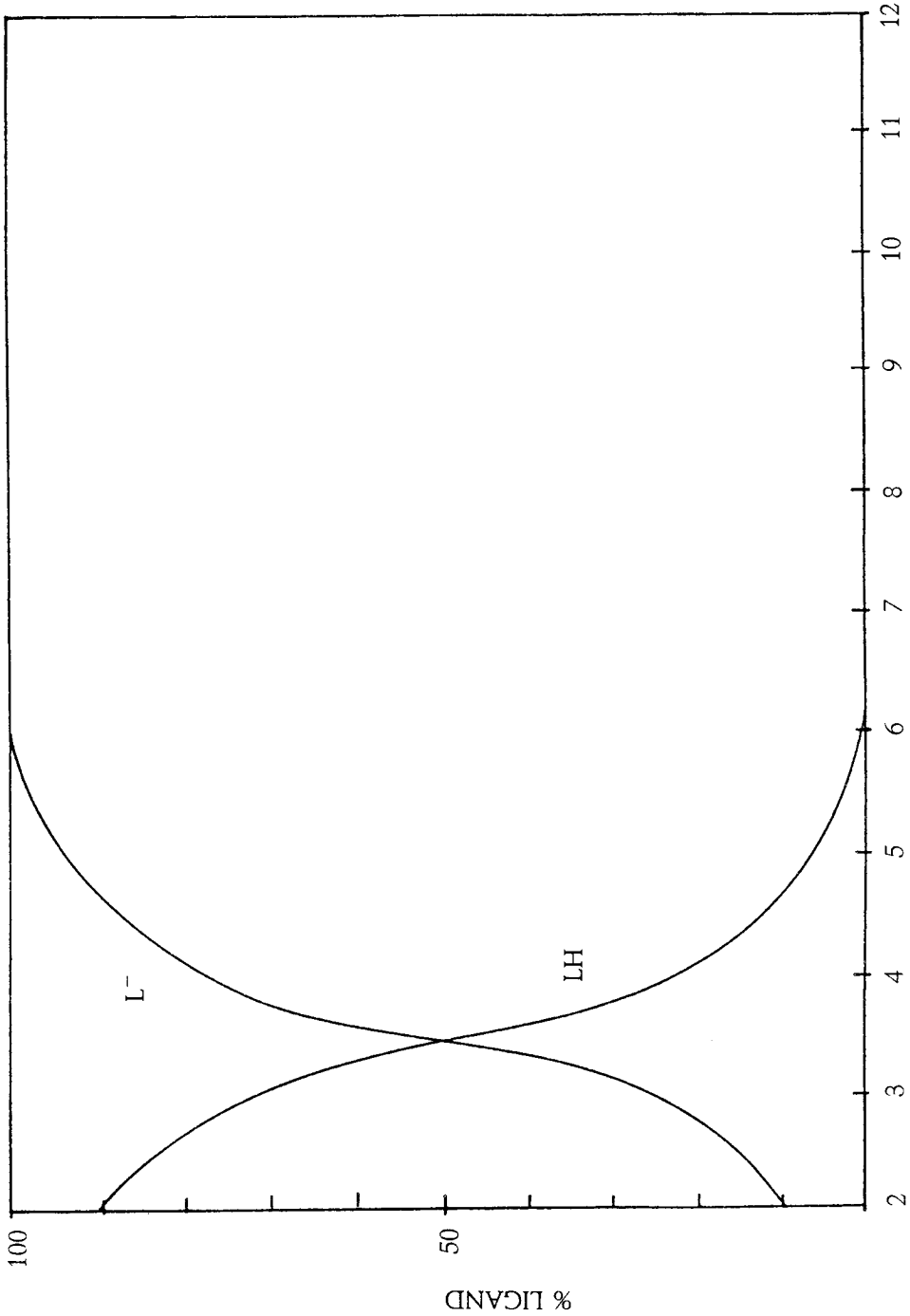

Fig. 1. Species distribution of N-acetyl-l-alanine at 0.00138 M.

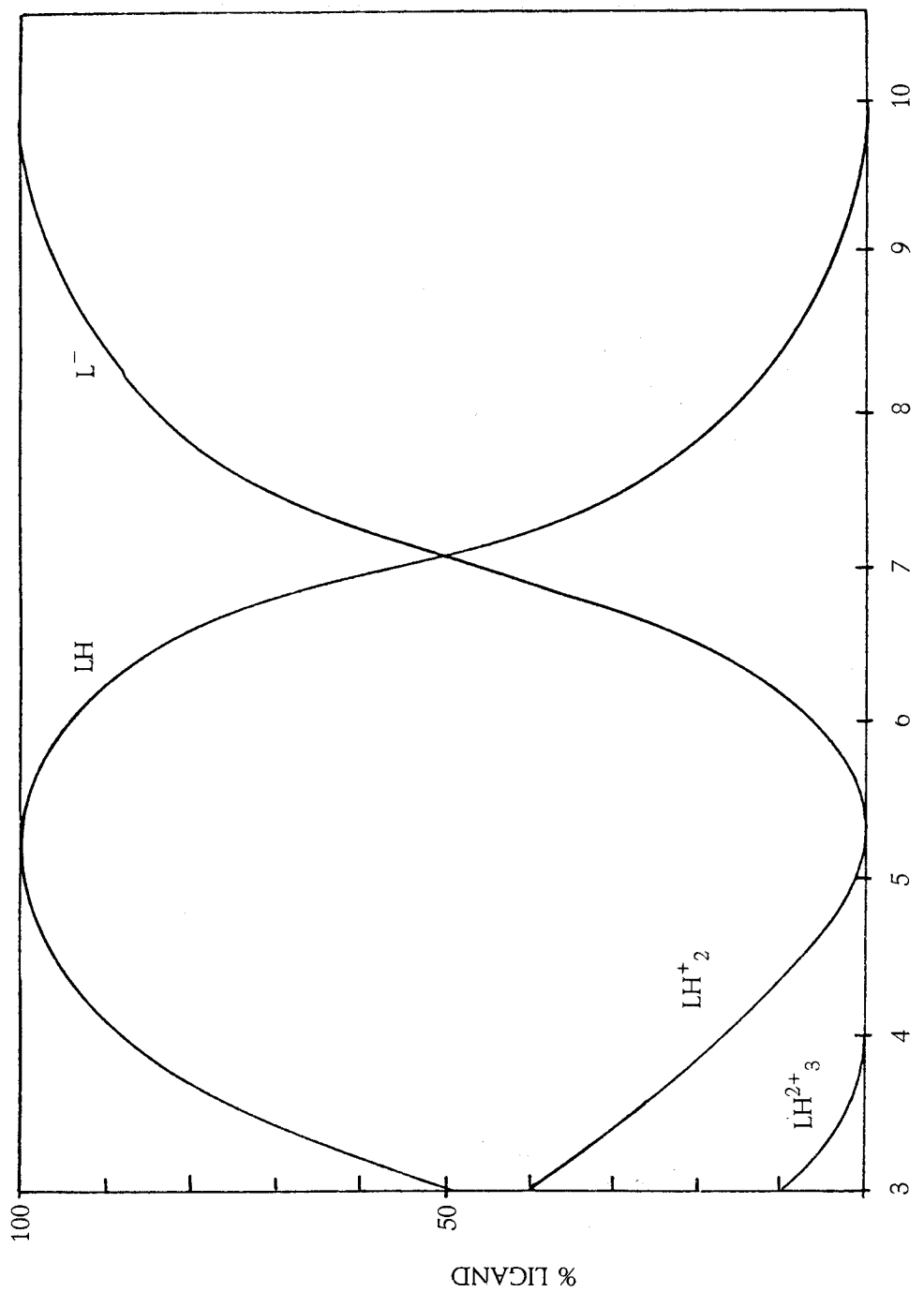

Fig. 2. Species distribution of N-acetyl-histidine at 0.00177 M.

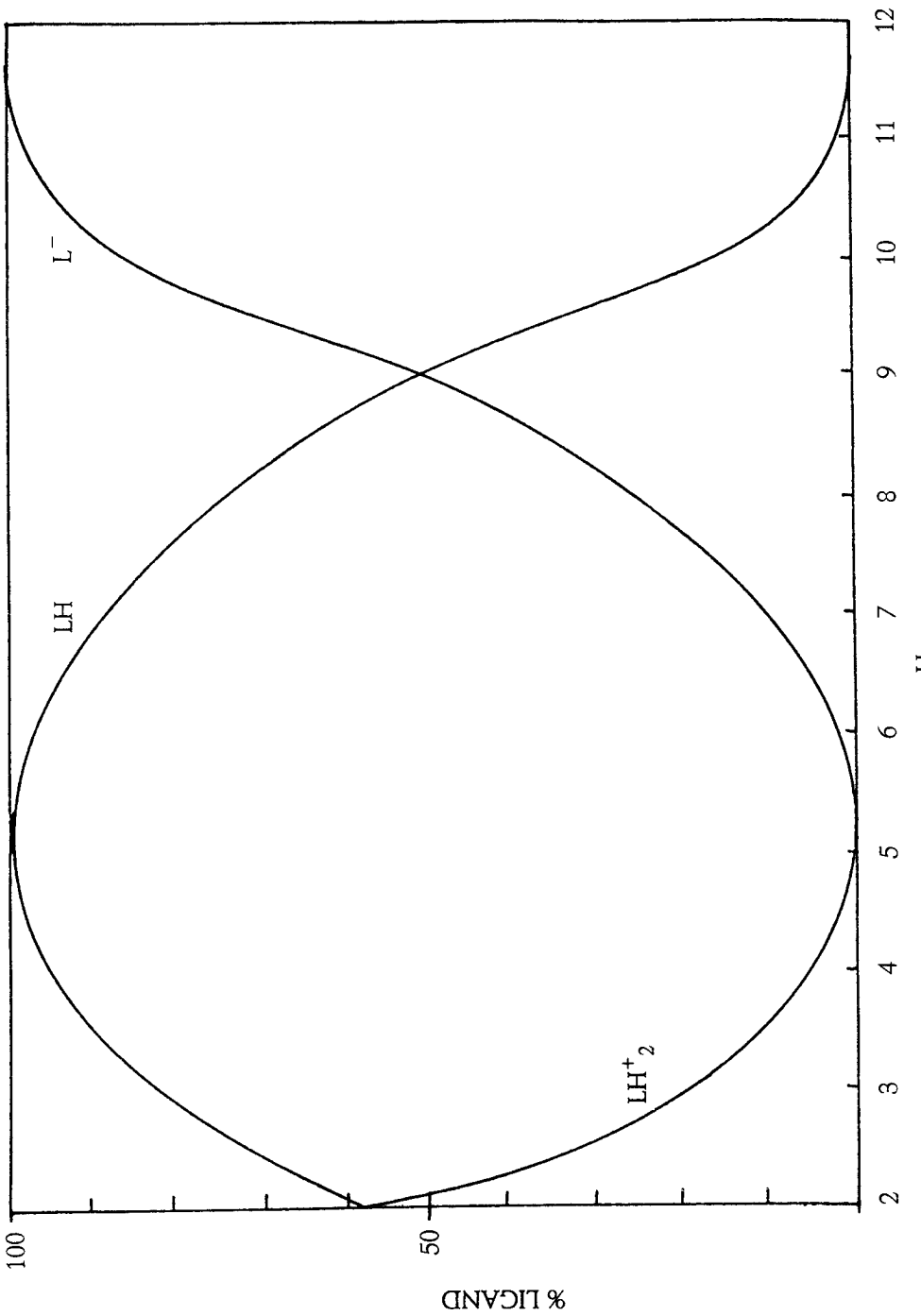

Fig. 3. Species distribution of N-acetyl-l-lysine at 0.00097 M.

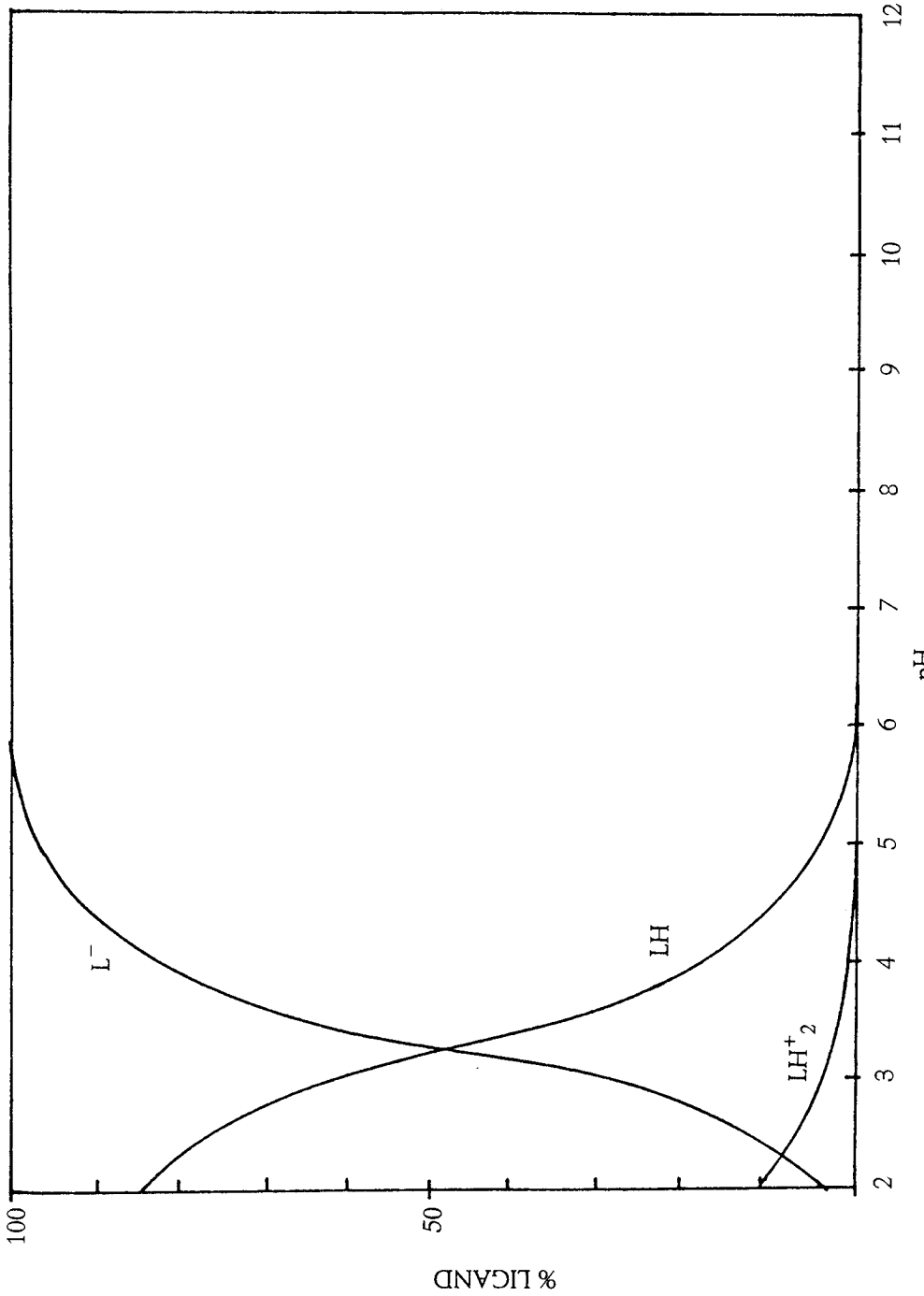

Fig. 4. Species distribution of N-acetyl-l-valine at 0.00097 M.