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Research Report

A Study of Protonation and Deprotonation of the Novel Amino Acids : The Compounds of Aspartic Acids

by

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October 1997

รายงานความก้าวหน้าของโครงการวิจัย

เรื่อง

การศึกษาการรับ-จ่ายโปรตอนของสารประกอบกรดแอสปาร์ติก

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บทคัดย่อ

การหาค่าคงที่ของความเป็นกรด และ ค่าคงที่ของความเป็นเบสของสารประกอบ acetylaspartic acid, aspartic acid, aspartylaspartic acid, Asp-Asp-Asp, Asp-Asp-Asp-Asp, Asp-Asp-Asp-Asp-Asp และ Asp-Asp-Asp-Asp-Asp-Asp ในสารละลาย 0.1 M โปแตสเซียมไนเทรต ณ อุณหภูมิ 25 °C โดยวิธีโพเทนชิโอเมทริกไทเทรชัน ค่าคงที่ของความเป็นกรดของสารประกอบ N-acetylaspartic acid คือ $\log K_1 = -3.41$ and $\log K_2 = -5.13$ ค่าคงที่ของความเป็นเบสโดยค่า log K ของสารประกอบ aspartic acid (asp) และ polyaspartic acids $(asp)_n$ (เมื่อ n = 2 ถึง 6) มีค่าเท่ากับ 9.80 และ 8.79, 8.34, 8.50, 8.56 และ 8.99 ตามลำดับ ค่าคงที่ของความเป็นกรดของสารประกอบ polyaspartic acids $(asp)_n$ เท่ากับ n+1 โดยเป็นจำนวนเดียวกับจำนวนกรดคาร์บอกซิลิกในโมเลกุล ค่า pI ของสารประกอบ aspartic acid, aspartylaspartic acid, Asp-Asp-Asp, Asp-Asp-Asp-Asp, Asp-Asp-Asp-Asp-Asp และ Asp-Asp-Asp-Asp-Asp-Asp ในสารละลาย 0.1 M โปแตสเซียมไนเทรต ณ อุณหภูมิ 25 °C มีค่าเท่ากับ 3.1, 3.3, 3.1, 2.8, 2.7 and 2.4 ตามลำดับ

Project Title	A Study of Protonation and Deprotonation of the Novel Amino Acids : The Compounds of Aspartic Acids
Name of investigator	Assoc.Prof. Dr.Vithaya Ruangpornvisuti
Year	October 1997

Abstract

Acidity and basicity constants of N-acetylaspartic acid, aspartic acid, aspartylaspartic acid , Asp-Asp-Asp, Asp-Asp-Asp-Asp, Asp-Asp-Asp-Asp-Asp and Asp-Asp-Asp-Asp-Asp-Asp were determined in 0.1 M potassium nitrate at 25 °C, by means of potentiometric titration. Acidity constants of N-acetylaspartic acid are log K_1 = -3.41 and log K_2 =-5.13. Basicity constants, expressed as log K, of aspartic acid (asp) and polyaspartic acids $(asp)_n$; when n=2 to 6, are 9.80 and 8.79, 8.34, 8.50, 8.56 and 8.99 respectively. The number of acidity constant of polyaspartic acids $(asp)_n$ is equal to n+1 that is equivalent to the number of carboxylic acid in molecule. The pI's of aspartic acid, aspartylaspartic acid, Asp-Asp-Asp, Asp-Asp-Asp-Asp, Asp-Asp-Asp-Asp-Asp and Asp-Asp-Asp-Asp-Asp-Asp in aqueous solution of 0.1 M potassium nitrate at 25 °C are 3.1, 3.3, 3.1, 2.8, 2.7 and 2.4 respectively.

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CHAPTER I

INTRODUCTION

1.1 Nature of Compounds of Aspartic Acid

Natural amino acids are the essential acids for biological body [1]. Acidity and basicity constants of many essential amino acids have been reported in handbooks [2-3]. In order to get more understanding of their biological behavior and to be used for further study of their complexes with interesting metal cations, the determination of acidity and basicity constants of many dipeptides was carried out [4-11]. Furthermore, proteins or peptides and their complexes with transition metals have been widely studied for their role in biological systems [12-16]. Recently, novel amino acids which mostly are synthetic compounds have been never found in human body or biological body were investigated in aqueous and non-aqueous systems for their physico-chemical properties [17]. Generally, the terminal nitrogen of the amino acids can be easily protonated and their acid proton can also be loosen to form zwitter ions in aqueous solution and usually, the amino-nitrogen of N-acetyl amino acids can not be protonated in any solution. Aspartic acid is an essential amino acid that contains two acid protons, one at the end and the other at the side chain. For dipeptide such as aspartylaspartic acid which composes of three acid protons and one terminal amino-nitrogen, three acidity and basicity constants should be found. For polypeptides such as Asp-Asp-Asp and longer chain of aspartic unit up to 6 units like Asp-Asp-Asp-Asp-Asp-Asp, they should dissociate their carboxylic protons and be protonated at terminal amino-nitrogen to form many ionic species.

Therefore, equilibrium constants of aspartylaspartic acid polyaspartic acids and their ionic species which correspond to the protonation and deprotonation should be investigated in order to reveal their behavior and properties in aqueous solution.

The acidity and basicity of aspartic acid and N-acetylaspartic acid is very important information to complete the research work of other compounds of aspartic acid so that we can make conclusion on their common properties. The acidity and basicity constants of the polyaspatic acids is also very useful information for study of their complexation with metal cations for environmental purpose. Therefore, determination of the acidity and basicity constants of compounds of aspartic acids is the objective of this research.

1.2 pH Dependent Structure of Amino Acids

In aqueous solution, amino acids can be protonated and deprotonated to form stable species present in the solution. The population of existing species of amino acid depend upon the pH of aqueous solution. According to pH dependent of amino acids' structure in aqueous solution, the population of all related species of amino acids should be also observed in terms of species distribution. The existing species of aspartic acid (denoted as LH_2) in aqueous solution depending on pH can be written as shown in Figure 1.1

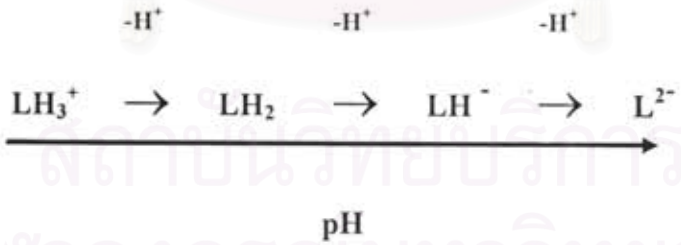


Figure 1.1 pH dependent structure of aspartic acid (LH_2)

In aqueous solution, the chemical equilibria of existing species of aspartic acid expressed in terms of equilibrium constants can be written as following equations

$$K_1 : LH_3^+ \rightleftharpoons LH_2 + H^+$$
$$K_2 : LH_2 \rightleftharpoons LH^- + H^+$$
$$K_b : L^{2-} + H^+ \rightleftharpoons LH^-$$

Where K_1 and K_2 are acidity constants and K_b is a basicity constant. The structure of each related species of aspartic acid in aqueous solution are shown in Figure 1.2.

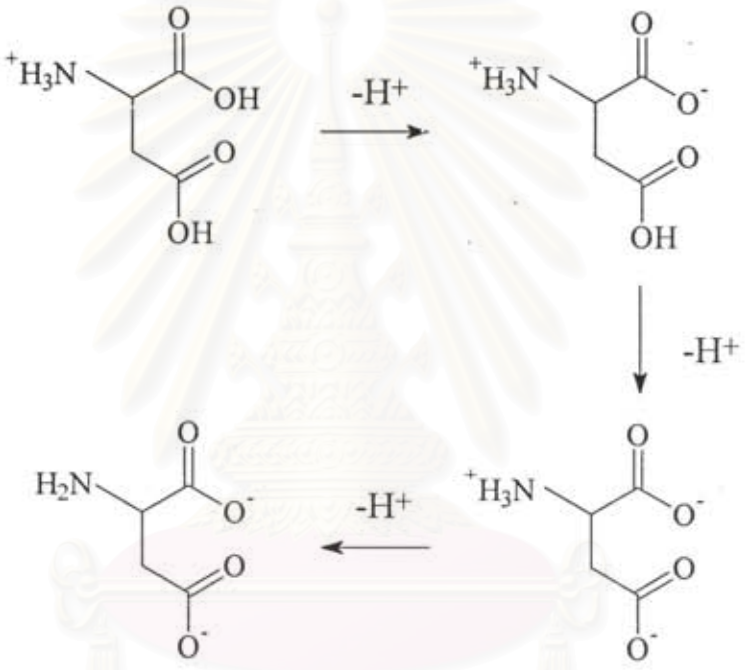
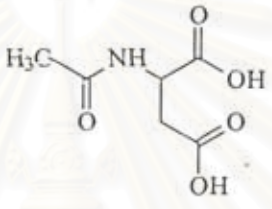


Figure 1.2 Diagram shows the proton related species of aspartic acid.

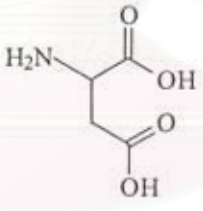
1.3 Structure of Compounds of Aspartic Acid

Compounds of aspartic acid being studied for this research are an aspartic acid and polyaspartic acids. The molecules of these compounds and their structures are listed and shown below.

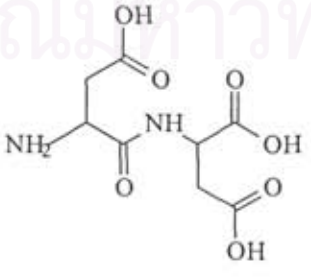
1.3.1 N-acetyl Aspartic acid



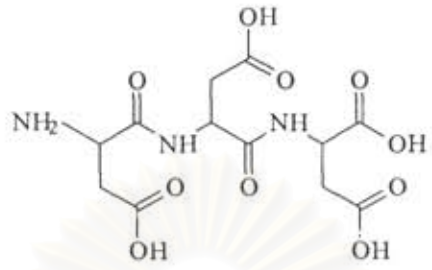
1.3.2 Aspartic acid



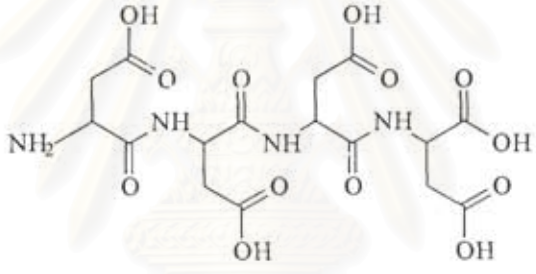
1.3.3 Aspartylspartic acid



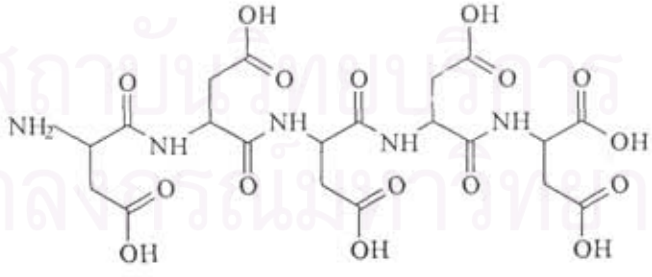
1.3.4 Asp-Asp-Asp



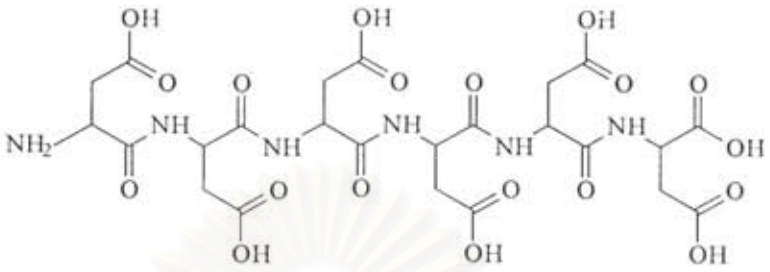
1.3.5 Asp-Asp-Asp-Asp



1.3.6 Asp-Asp-Asp-Asp-Asp



1.3.7 Asp-Asp-Asp-Asp-Asp-Asp



1.4 Systematic Investigation of Solution Equilibria

The investigation on nature of the species which present in a solution at equilibrium can be systematically studied by the following steps :

(a) The nature of each of the species present in solution is identified by using a mixture of chemical intuition and a number of physicochemical techniques which determine either the stoichiometric numbers representing the existing species.

(b) Expression relating the concentrations of the initial reactants and final products such as the acidity and basicity constants are set up.

(c) Equilibrium constants of possible model in solution are evaluated by suitable means such as potentiometric, ultraviolet-visible spectroscopic, nuclear magnetic resonance spectroscopic and calorimetric methods.

(d) The errors inherent in these measurements such as systematic and random errors are discussed.

(e) The concentrations of all the species present in solution at equilibrium are calculated.

1.5 Objective of the Research

This work is for determination of acidity and basicity constants of the N-acetyl aspartic acid, aspartic acid, aspartylaspartic acid, Asp-Asp-Asp, Asp-Asp-Asp-Asp, Asp-Asp-Asp-Asp-Asp and Asp-Asp-Asp-Asp-Asp-Asp in aqueous solution of 0.1 M potassium nitrate at standard temperature by potentiometric titration. Species distribution over the varied pH of the solution of each compounds will be calculated from their equilibrium constants which obtain from the process of computer refinement. The pI of these compounds of aspartic acid will be estimated from the results.

CHAPTER II

THEORY

2.1 Equilibrium Constant

2.1.1 Equilibrium Concentration Constant

An equilibrium constant is a quotient involving the concentrations or activities of reacting species in solution at equilibrium. Generally it is defined as the ratio of the product of the activities *a* of the reaction products, raised to appropriate power, to the products of the activities of the reactants, raised to appropriate power, illustrated by equation (2.1) where a, b, c and d are the stoichiometric coefficients of the solution species A, B, C and D respectively.

$$aA + bB \rightleftharpoons cC + dD \qquad K_{eq} = \frac{a_C^c \; a_D^d}{a_A^a \; a_B^b} \qquad (2.1)$$

The determination of the activities of complex ionic species at both infinite solution and in real solution is a complicated and time-consuming task. However concentrations are related to activities by the expression

$$a_X = [X]\,\gamma_X \qquad (2.2)$$

where a_X, [X] and γ_X are activity, concentration and activity coefficient of X respectively. Activity coefficients of reacting species are in general tedious and difficult to measure. They also depend very significantly on the nature and concentrations of other species present in solution so that it is not possible to build universal tables of activity coefficients. Theoretical attempts at calculating activity coefficients, based on the Debye-Huckel approach and its extensions, are at best of only limited accuracy. Substituting the activities from equation (2.2) in (2.1), then the equilibrium constant can be rewritten as follow.

$$K_{eq} = \frac{a_C^c \; a_D^d}{a_A^a \; a_B^b} = \frac{[C]^c \; [D]^d}{[A]^a \; [B]^b} \cdot \frac{\gamma_C^c \; \gamma_D^d}{\gamma_A^a \; \gamma_B^b} \qquad (2.3)$$

where[A] indicates molar concentrations. If now it is possible to ensure that the term $\frac{\gamma_C^c \gamma_D^d}{\gamma_A^a \gamma_B^b}$ remains constant then the term $\frac{\gamma_C^c \gamma_D^d}{\gamma_A^a \gamma_B^b} K_{eq}$ is also a constant. Therefore, the equilibrium constant expressed in terms of the reacting species, called equilibrium concentration constant, K_c can be written as indicated by equation (2.4).

$$aA + bB \rightleftharpoons cC + dD \qquad K_c = \frac{[C]^c [D]^d}{[A]^a [B]^b} \qquad (2.4)$$

Equilibrium concentration constant, K_c is also known as the stoichiometric equilibrium constant which determined at constant ionic strength where as K_{eq} is indicated by equation (2.1) which is known as an equilibrium activity constant or thermodynamic equilibrium constant.

The term $\frac{\gamma_C^c \gamma_D^d}{\gamma_A^a \gamma_B^b}$ in equation (2.3) may be maintained effectively constant by having a large excess of an inert background electrolyte present and using only low concentrations of the reacting ionic species so that any change in their concentrations as a result of their reaction together has an insignificant change on the overall ionic strength of the medium. It is generally possible to replace about 5% of the ions in the inert background electrolyte without appreciably altering the activity coefficients of the minor species present. However, in recording a stoichiometric equilibrium constant it is essential to record not only the concentration of the inert background electrolyte, but also its nature, since the activity coefficients depend on the electrolyte. Consequently, of course, in comparing stoichiometric equilibrium constants, only data obtained under very similar conditions should be used unless the differences between the equilibrium constants are large.

2.1.2 Acidity and Basicity Constants

The acid-base equilibria of the ligands can be treated by protonation and deprotonation constant. Protonation constant is the equilibrium constant for the addition the n^{th} proton to a charged or uncharged ligand. Protonation constant is known as basicity constant. The reciprocal of protonation constant is called deprotonation constant and defined as the equilibrium constant for the splitting off n^{th} proton from a charged or uncharged ligand. Deprotonation constant is also known as acidity constant. The following equations define these constants and show their interrelation.

$$L + H \rightleftharpoons LH : K_1 = \frac{[LH]}{[L][H]} \quad (2.5)$$

$$LH + H \rightleftharpoons LH_2 : K_2 = \frac{[LH_2]}{[LH][H]} \quad (2.6)$$

$$LH_2 + H \rightleftharpoons LH_3 : K_3 = \frac{[LH_3]}{[LH_2][H]} \quad (2.7)$$

$$\vdots \qquad\qquad \vdots \quad \vdots$$

$$LH_{n-1} + H \rightleftharpoons LH_n : K_n = \frac{[LH_n]}{[LH_{n-1}][H]} \quad (2.8)$$

Another way of expressing the equilibria relations can be shown as follow:

$$L + H \rightleftharpoons LH : \beta_1 = \frac{[LH]}{[L][H]} \quad (2.9)$$

$$L + 2H \rightleftharpoons LH_2 : \beta_2 = \frac{[LH_2]}{[L][H]^2} \quad (2.10)$$

$$L + 3H \rightleftharpoons LH_3 : \beta_3 = \frac{[LH_3]}{[L][H]^3} \quad (2.11)$$

$$\vdots \qquad\qquad \vdots \quad \vdots$$

$$L + nH \rightleftharpoons LH_n : \beta_n = \frac{[LH_n]}{[L][H]^n} \quad (2.12)$$

The K_i's are called the stepwise protonation constants and the β_i's are called the overall or cumulative protonation constants.

2.2 Method of Calculations

2.2.1 Linear Method, Errors and Statistics

Stability constants are not directly measurable but must be calculated from an observed response function of a fixed, but experimentally adjustable, variable. Since the response data are subject to random error and indeed may be subject to systematic errors if we have not controlled the experiment well, the stability constants will be calculated with limited precision. However, it is important to estimate the precision of any calculated constants, as it will indicate the reliability of the value obtained and in turn the efficiency of the experiment. In addition we need to have a mathematical model for describing the data.

2.2.1.1 Model Building

Experiments attempts to find some functional form for the way quantities in nature are related. We try to build up a mathematical model which may be an assumed one, in which case we need to measure of how good the model is in describing our data, or it may be derived from first principles and then tested experimentally. The model could be an approximated one, which initially may be acceptable and then refined or modified in the further experimental observations. The typical experiment consists of fixing one group of known values variables called independent variables and then making observations of another dependent variables. In stability constant work, the independent variables might be temperature, ionic strength, or the concentration of one or more components and dependent variables might be e.m.f. or pH or absorbance of the solution. We then calculate or estimate the parameters of interest from the assumed function by relating the dependent to the independent variables.

The parameters for our model are calculated by fitting them to the experimental data. This may be done either graphically or by a mathematical procedure, such as least-squares. The latter calculates the values of the parameters

which sum of the squares of the residuals is defined as the difference between the observed and calculated data points at each fixed minimum value of the independent variable. In addition the method of least-squares allows us to obtain the estimated errors of the interested parameters and to estimate the 'goodness of fit' of the assumed model, that is, it allows us to test alternative hypotheses.

2.2.1.2 Random Errors

Random or observational errors are assumed to follow a Gaussian or normal distribution , expressed mathematically as

$$f(r_x) = \frac{1}{\sqrt{2}\sigma_x} e^{-r_x^2 / 2\sigma_x^2} \qquad (2.13)$$

where r_x is the residual of x or observed value - true value ,σ_x^2 is the variance of x and σ_x is the standard deviation.

The probability of observing the i th residual, P_i in the region r_{xi} to $r_{xi} + dr_{xi}$ is:

$$dP_i = \frac{1}{\sqrt{2}\sigma_x} e^{-r_{xi}^2 / 2\sigma_x^2} dr_{xi} \qquad (2.14)$$

Now the probability for a given set of n observations, where P is the product of the probabilities of i th measurements is

$$dP = \prod_{i=1}^{i=n} dP_i = \prod_{i=1}^{i=n} \left(\frac{d\, r_{xi}}{\sqrt{2}\, \sigma_x} \right) e^{-\left(1/2\sigma_x^2\right)\sum r_{xi}^2} \qquad (2.15)$$

Based on the statistical principle of maximum likelihood this probability becomes a maximum when the sum of the squares residuals is a minimum.

$$\sum_{i=1}^{n} r_{xi}^2 = \text{minimum} \qquad (2.16)$$

Hence the origin of the term 'least squares ' is apparent.

The discussion so far has assumed that the measurements of x have all come from the same population distribution, that is, the variance of the residuals are equal. If this is not so, equation (2.14) should be rewritten as :

$$d P_i = \frac{1}{\sqrt{2}\sigma_{xi}} e^{-r_{xi}^2 / 2\sigma_{xi}^2} dr \qquad (2.17)$$

and the equation (2.58) becomes

$$dP = \prod_{i=1}^{i=n} dp_i = \prod_{i=1}^{i=n} \left(\frac{d r_{x_i}}{\sqrt{2}\ \sigma_{xi}} \right) e^{-\frac{1}{2}\sum\left(r_{xi}^2 / \sigma_{xi}^2\right)} \qquad (2.18)$$

and the least-squares principle gives:

$$\sum_{i=1}^{i=n} \left(\frac{r_{xi}^2}{\sigma_{xi}^2} \right) = \text{minimum} \qquad (2.19)$$

A quantity inversely proportional to the variance is termed the weight of an observation. Hence:

$$w_{xi} = \frac{\sigma_0^2}{\sigma_{xi}^2} \qquad (2.20)$$

where σ_0^2 is known as the variance of an observation of unit weight. In practice σ_0^2 will often have the value of unity. The quantity now to be minimized is the sum of the weighted squares of the residuals.

$$\sum_{i=1}^{i=n} w_{xi} r_{xi}^2 = \text{minimum} \qquad (2.21)$$

In practice we cannot know the true value of x , but the principle of least-squares attempts to adjust the estimate of x according to equation (2.21). Generally

the experimental data are function of the parameter x so that r_{xi} in equation (2.21) is defined as:

$$r_{xi} = \left[f(x_i) - f(\bar{x}) \right] \tag{2.22}$$

and $\bar{x}$ is the least-squares estimator of the true value of the parameter.

2.2.1.3 Systematic Errors

Systematic errors are caused by the limitations of the apparatus, or experimentalist, and introduce bias into the data resulting in inaccurate parameters. Thus it is possible to obtain high precision with poor accuracy, as indicated diagrammatically in Figure 2.1.

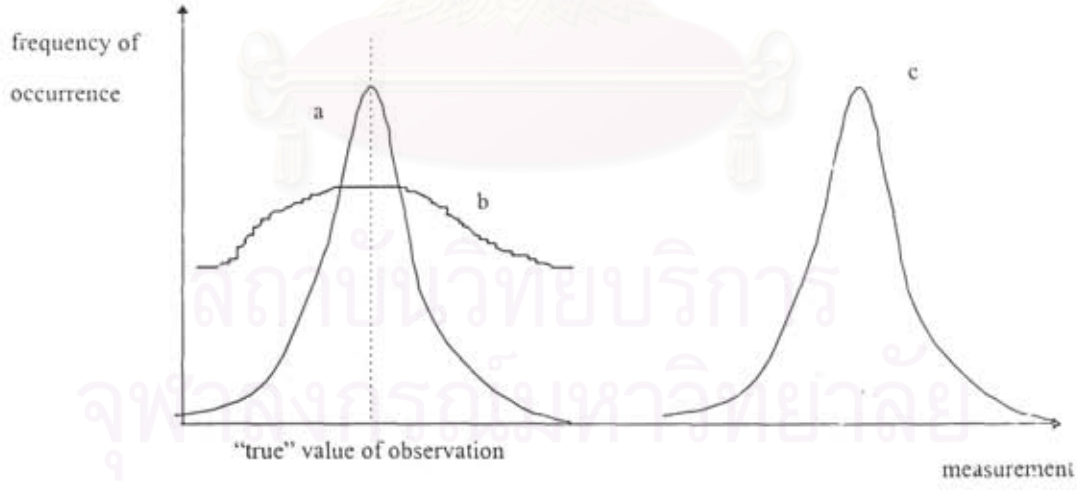


Figure 2.1 Diagrammatic representation types of experimental error : (a) high precision, high accuracy ; (b) low precision, high accuracy (due to large random errors); (c) high precision, poor accuracy (due to systematic errors).

2.2.2 Non-Linear Parameter Estimation

2.2.2.1 Least -squares-extension case

To extend least-squares theory to the non-linear case, that is the situation where the dependent variables are non-linear functions of the independent variables, we take equation and express the dependent variables (observables) as a function of the unknowns by a Taylor series expansion. Thus if the initial estimates of the parameter values are $\left(x_1^0\ x_2^0\ \ldots x_m^0\right)$ then the observables are expressed about this point in parameter space by:

$$o_i = f_i\left(x_1^o \ldots x_m^0\right) + \left(\frac{\partial f_i}{\partial x_i}\right)_0 \left(x_1 - x_1^o\right) + \ldots + \left(\frac{\partial f_i}{\partial x_m}\right)_0 \left(x_m - x_m^o\right) \qquad (2\ 23)$$

that is

$$o_i = f_i\left(x_1^o \ldots x_m^0\right) + \sum_{j=1}^{j=m}\left(\frac{\partial f_i}{\partial x_j}\right)_0 \Delta x_j \qquad (2.24)$$

where terms higher than first order have been neglected. Therefore the change in the observables Δo_i on making the corrections Δx_j are given by

$$\Delta o_i = o_i - f_i\left(x_1^o \ldots x_m^0\right) = \sum_{j=1}^{j=m}\left(\frac{\partial f_i}{\partial x_j}\right)_0 \Delta x_j \qquad (2.25)$$

2.2.2.2 Hypothesis testing

Another quantity which has been used in non-linear estimation situations is the Halmilton *R*-factor. In this procedure the *R*-factor defined by :

$$R = \left[\frac{\sum_{i=1}^{i=n} w_i \left(o_i^{calc} - o_i^{obs} \right)^2}{\sum_{i=1}^{i=n} w_i \left(o_i^{obs} \right)^2} \right]^{\frac{1}{2}} \qquad (2.26)$$

is compared with R_{lim} calculated from :

$$R_{lim} = \left[\frac{\sum_{i=1}^{i=n} w_i \; e_i^2}{\sum_{i=1}^{i=n} w_i \left(o_i^{obs} \right)^2} \right]^{\frac{1}{2}} \qquad (2.27)$$

where e_i is the residual in the i th equation calculated from the estimated errors in all the experimental quantities using error propagation rules, o_i^{calc} and o_i^{obs} are the calculated and the observed values of the response variable respectively, w_i are the appropriate weighting factors. A satisfactory fit is assumed if $R < R_{lim}$.

2.3 Calculation of Equilibrium Constants

The acidity and basicity constants were calculated by fitting the pH data to the SUPERQUAD program [13] which has been widely used to calculate the equilibrium constants of many ligands in solution. The formation constants are determined by minimization of an error-square sum based on measure electrode potentials. The SUPERQUAD program also permits refinement of any reactant concentration or standard electrode potential. The refinement is incorporated into new procedure which can be used for model selection. The assumptions for computation of formation constants by SUPERQUAD could be described as follows.

Assumptions : There are number of assumptions underlying the whole treatment, and each needs to be considered explicitly.

1. For each chemical species $A_aB_b...$ in the soiution equilibria, there is a chemical constant, the formation constant, which is expressed as a concentration quotient in equation (2.28).

$$\beta_{ab....} = \frac{[A_aB_b...]}{[A]^a[B]^b...} \qquad (2.28)$$

A, B... are the reactants (SUPERQUAD allows up to four of them) and [A], [B] are the concentrations of free reactant; electrical charges may be attached to any species, but they are omitted for sake of simplicity in this discussion. Since the thermodynamic definition of a formation constant is as an activity quotient, it is to be assumed that the quotient of the activity coefficients is constant, an assumption usually justified by performing the experiments with a medium of high ionic strength .

2. Each electrode present exhibits a pseudo-Nernstian behavior, equation (2.29), where [A] is the concentration of the electro-active ion,

$$E = E^\circ + S_L \log [A] \qquad (2.29)$$

E is the measured potential, and E° is the standard electrode potential. The ideal value of the slope S_L is of course RT/nF, but we assume only that it is a constant for a given electrode. The value of E° and S_L are usually obtained in a separate calibration experiment. Further there is a modified Nernst equation.

$$E = E^\circ + S_L \log [H^+] + r [H^+] + s [H^+]^{-1} \qquad (2.30)$$

This equation was first suggested as means of taking into account junction potentials in strongly acidic and strongly basic condition.

3. Systematic errors must be minimized by careful experimental work. Sources of systematic error include electrode calibration, sample weightings and dilutions, standardization of reagents (use of carbonate-free alkali in particular), temperature variation and water quality. The last-named factor is more significant today than it was in the past, as water may be contaminated by titrable species which

can pass through distillation columns by surface action. All statistical tests are based on the assumption that systematic errors are absent from the data.

4. The independent variable is not subject to error. Errors in the dependent variable are assumed to have a normal distribution. If these assumptions are true, use of the principle of least squares will yield a maximum likelihood result, and computed residuals should not show systematic trends.

5. There exits a model of the equilibrium system, which adequately accounts for the experimental observations. The model is specified by a set of coefficients a, b , ..., one for each species formed. All least-squares refinements are performed in terms of an assumed model. Examination of a sequence of models should yield a best model which is not significantly different from the true model. Choice of the best model is known as species selection.

2.4 Inert Background Electrolyte

To study acid-base characteristics of ligand and their complexation properties toward metal, ionic strength is controlled by inert background electrolyte present at a concentration far in excess that of the reacting ionic species under investigation. Inert background electrolyte is sometime called inert background solution or supporting electrolyte which is defined as electrolyte which does not react with any of reacting species such as metal ion, ligand or metal-ligand species in the equilibrium being studied. The main function of the inert background electrolyte is to keep the overall ionic strength and activity coefficient constant. Properties of the chosen inert background electrolyte must meet the following requirements

1. a strong and non reacting (inert) electrolyte,
2. no part of electrolyte involved in equilibrium under investigation,
3. its cation must not associate with the ligand and with the complex species,
4. its anion must not associate with the central metal ion and with the complex species,

5. redox reaction must not occur between the constituents of the inert electrolyte and the central ion or ligand,
6. its solubility has to be large enough,
7. its contribution to the measured physical or chemical property must be negligible.

Inert background electrolytes that are commonly used in aqueous solvent are sodium salts such as the perchlorate or nitrate e.g. sodium perchlorate ($NaClO_4$), sodium nitrate ($NaNO_3$), perchlorate is usually more suitable than any other ions. Sodium chloride (NaCl) has been used as an inert background electrolyte, but its use is less common than that of perchlorate or nitrate because chloride ions often form complexes with metal ions under study. Potassium salts such as potassium nitrate (KNO_3) and potassium chloride (KCl) have also been used occasionally, but potassium perchlorate ($KClO_4$) is unsuitable due to its low solubility in water.

CHAPTER III

EXPERIMENTAL

3.1 Chemicals and Equipment

3.1.1 Chemicals

■ Potassium nitrate	Analar grade, Fluka, Switzerland
■ Sodium hydroxide 1.0 M	Volumetric grade, Merck, Germany
■ Hydrochloric acid 1.0 M	Volumetric grade, Merck, Germany
■ N-acetylaspartic acid	Analar grade, Sigma , U.S.A.
■ Aspartic acid	Analar grade, Sigma , U.S.A.
■ Aspartylaspartic acid	Analar grade, Sigma , U.S.A.
■ Asp-Asp-Asp	Analar grade, Sigma , U.S.A.
■ Asp-Asp-Asp-Asp	Analar grade, Sigma , U.S.A.
■ Asp-Asp-Asp-Asp-Asp	Analar grade, Sigma , U.S.A.
■ Asp-Asp-Asp-Asp-Asp-Asp	Analar grade, Sigma , U.S.A.
■ Potassium hydrogenphthalate	Analar grade, Carlo Erba, Italy
■ Argon gas	Ultra high purity grade, TGI, Thailand

3.1.2 Equipment

- Automatic titrator, Mettler, Model DL 25, Switzerland
- Thermostat bath, Model DT-2, Denmark
- Combined pH electrode, Mettler, Model DG 111-SC, Switzerland
- Personal Computer, 486/DX2, RAM 16 MB

3.2 Preparation of solution

Inert background electrolyte, used in the research, was 0.1 M KNO_3 which obtained by dissolution of dried KNO_3, AR grade from FLUKA in double distillation water. Stock solutions of the ligands of N-acetyl aspartic acid, aspartic acid and aspartylaspartic acid used in the titrations were 0.01 M in 0.1 M KNO_3. For the titrations of the Asp-Asp-Asp, Asp-Asp-Asp-Asp, Asp-Asp-Asp-Asp-Asp and Asp-Asp-Asp-Asp-Asp-Asp, their solid powder were directly added into the titration beaker which contained 10 cm^3 of 0.1 M KNO_3. The solutions of 0.05 M NaOH and 0.05 M HCl in 0.1 M KNO_3, used to adjust pH of the working solution, were prepared by adding a weighed quantity of dried KNO_3 in the dilution process of standard solution of 1 M HCl. The solutions of 0.05 M NaOH and 0.05 M HCl in 0.1 M KNO_3 were diluted from standard solution of 1.0 M NaOH and 1.0 M HCl respectively and standardized by the standard solution of 0.05 M KHP.

3.3 The Calibration of Electrode

An automatic titrator, Mettler DL25 including combined pH electrode of Mettler DG113-SC was used in the titration. The pH electrode was calibrated by standard pH buffers of pH 4.00 and 7.00 at 25 ± 0.1 °C. Accuracy of the pH measurement was indicated by the Nernstian slope value of exceeding 99% based on the isopotential point of pH 7.00 = 0.0 mV; the Nernstian slope is a ratio of the pH and potential in unit of millivolt. The calibration of pH electrode and all potentiometric titrations were carried out at 25 ± 0.1 °C.

3.4 Potentiometric Titration

Titration of the N-acetyl aspartic acid, aspartic acid and aspartylaspartic acid were carried out by adding 1 to 3 cm^3 of 0.01 M of the ligand in 0.1 M KNO_3 into the titrating beaker containing 10 cm^3 of 0.1 M KNO_3. For the titration of the Asp-Asp-Asp, Asp-Asp-Asp-Asp, Asp-Asp-Asp-Asp-Asp and Asp-Asp-Asp-Asp-Asp-Asp, their solid powder were added directly into the titration beaker which contained 10 cm^3 of 0.1 M KNO_3. The 0.05 M NaOH in 0.1 M KNO_3 was used as the titrant and 0.05 M HCl in 0.1 M KNO_3 was used to adjust pH of the working solution.

The titrations were performed under ultrapure argon gas, saturated by 0.1 M potassium nitrate vapour, through the titration beaker. The titration beaker was kept constantly at 25 °C with deviation of ± 0.1 °C by the external circulation control of thermostat bath. Each titration, at least 50 titrating data were recorded and at least 3 titrations were performed for each ligand .

3.5 Experimental Data

The titrating data for determination of acidity and basicity constants of aspartic acid compounds were evaluated by the computer refinement program. The calculations were performed on the microcomputer PC 486/DX4. The titrating data obtained from the measurements were used in the evaluation and the optimization process by the SUPERQUAD program (18). The range of titrating data for the titration of the N-acetyl aspartic acid, aspartic acid, aspartylaspartic acid, Asp-Asp-Asp, Asp-Asp-Asp-Asp, Asp-Asp-Asp-Asp-Asp and Asp-Asp-Asp-Asp-Asp-Asp are shown in Table 3.1, 3.2, 3.3, 3.4, 3.5 and 3.6 respectively.

Table 3.1 Titration data range of N-acetyl aspartic acid in 0.1 M KNO_3 at 25 °C.

Titration	Initial Concentration (mM)		pH range	Data point
	Ligand	Proton		
1	2.27	-	2.95 - 11.09	51
2	2.08	4..13	2.44 - 10.27	50
3	1.92	7.62	2.15 - 8.51	50

Table 3.2 Titration data range of aspartic acid in 0.1 M KNO_3 at 25 °C.

Titration	Initial Concentration (mM)		pH range	Data point
	Ligand	Proton		
1	1.16	-	3.51- 11.24	50
2	2.22	-	3.37 - 10.91	50
3	2.15	2.12	2.78 - 10.43	50

Table 3.3 Titration data range of Asp-Asp in 0.1 M KNO_3 at 25 °C.

Titration	Initial Concentration (mM)		pH range	Data point
	Ligand	Proton		
1	1.82	4.50	2.82 - 10.72	59
2	1.67	-	3.49 - 11.34	72
3	1.61	1.98	2.89 - 11.30	66
4	2.01	-	3.47 - 10.88	63

Table 3.4 Titration data range of Asp-Asp-Asp in 0.1 M KNO_3 at 25 °C.

Titration	Initial Concentration (mM)		pH range	Data point
	Ligand	Proton		
1	0.854	-	3.31 - 11.53	66
2	0.854	-	3.38 - 11.72	75
3	0.854	8.17	2.17 - 11.93	66

Table 3.5 Titration data range of Asp-Asp-Asp-Asp in 0.1 M KNO_3 at 25 °C.

Titration	Initial Concentration (mM)		pH range	Data point
	Ligand	Proton		
1	0.806	-	3.32 - 11.49	72
2	0.806	-	3.19 - 11.83	72
3	0.652	7.69	2.14 - 11.48	68
4	0.671	8.30	2.13 - 11.35	65

Table 3.6 Titration data range of Asp-Asp-Asp-Asp-Asp in 0.1 M KNO_3 at 25 °C.

Titration	Initial Concentration (mM)		pH range	Data point
	Ligand	Proton		
1	0.805	-	3.23 - 11.48	76
2	0.691	8.49	2.12 - 11.01	71
3	0.691	8.49	2.16 - 8.22	52

Table 3.7 Titration data range of Asp-Asp-Asp-Asp-Asp-Asp in 0.1 M KNO_3 at 25 °C.

Titration	Initial Concentration (mM)		pH range	Data point
	Ligand	Proton		
1	0.575	8.334	2.11 - 11.33	76
2	0.575	4.167	2.42 - 10.96	76
3	0.575	8.334	2.13 - 11.16	100

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Equilibrium Constant of N-Acetylaspartic Acid

The chemical equilibria of the N-acetylaspartic acid (symbolized as LH_2) in aqueous solution (0.1 M KNO_3) are written as following equations

$$K_1 : LH_2 \rightleftharpoons LH^- + H^+$$

$$K_2 : LH^- \rightleftharpoons L^{2-} + H^+$$

K_1 and K_2 are acidity constants and their logarithm values are shown in Table 4.1. The titration curves of the N-acetylaspartic acid in 0.1 M KNO_3 are shown in Figure 4.1. The log K_1 and log K_2 of N-acetylaspartic acid are acidity constants that correspond to the terminal acid proton and the side acid proton respectively. The acidity constants of the N-acetylaspartic acid and the sites of their corresponding protons are shown in Figure 4.2.

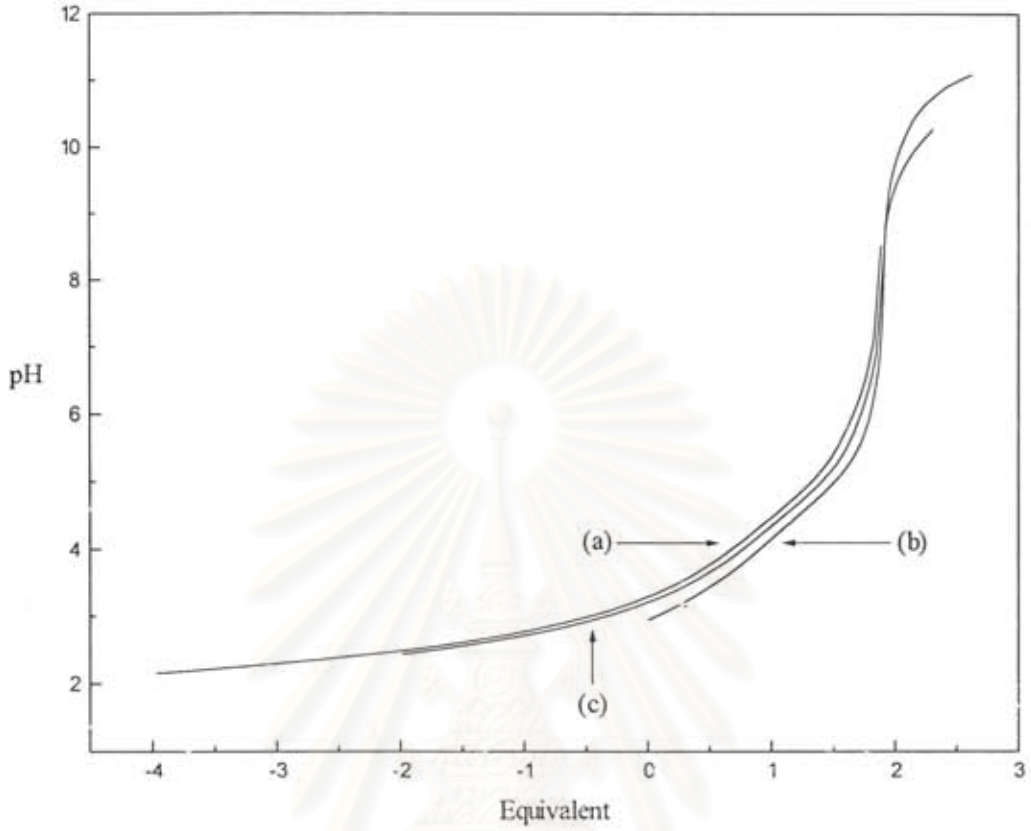


Figure 4.1 Potentiometric titration curves of N-acetyl aspartic acid in 0.1 M KNO_3 at 25 °C, based on the initial concentration ratio of the ligand to proton of (a) 1.92 mM : 7.62 mM (b) 2.08 mM : 4.13 mM (c) 2.70 mM : 0 mM ; equivalent is defined as the ratio of $(n_{OH^-} - n_{acid})$ to n_{ligand}.

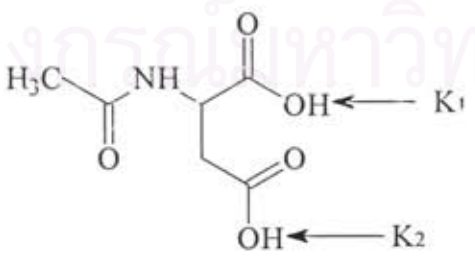


Figure 4.2 Acidity of constants of N-acetylaspartic acid and the sites of their corresponding protons.

4.2 Equilibrium Constant of Aspartic Acid

The chemical equilibria of the aspartic acid (symbolized as LH_2) in aqueous solution (0.1 M KNO_3) are written as following equations

$$K_1 : LH_3^+ \rightleftharpoons LH_2 + H^+$$

$$K_2 : LH_2 \rightleftharpoons LH^- + H^+$$

$$K_b : L^{2-} + H^+ \rightleftharpoons LH^-$$

K_1 and K_2 are acidity constants and K_b is basicity constant. Their logarithm values are shown in Table 4.1. The titration curves of the aspartic acid in 0.1 M KNO_3 are shown in Figure 4.3. K_1 and K_2 of aspartic acid are acidity constants that correspond to the terminal acid proton and the side acid proton respectively. The acidity and basicity constants of the aspartic acid and the sites of their corresponding protons are shown in Figure 4.4.

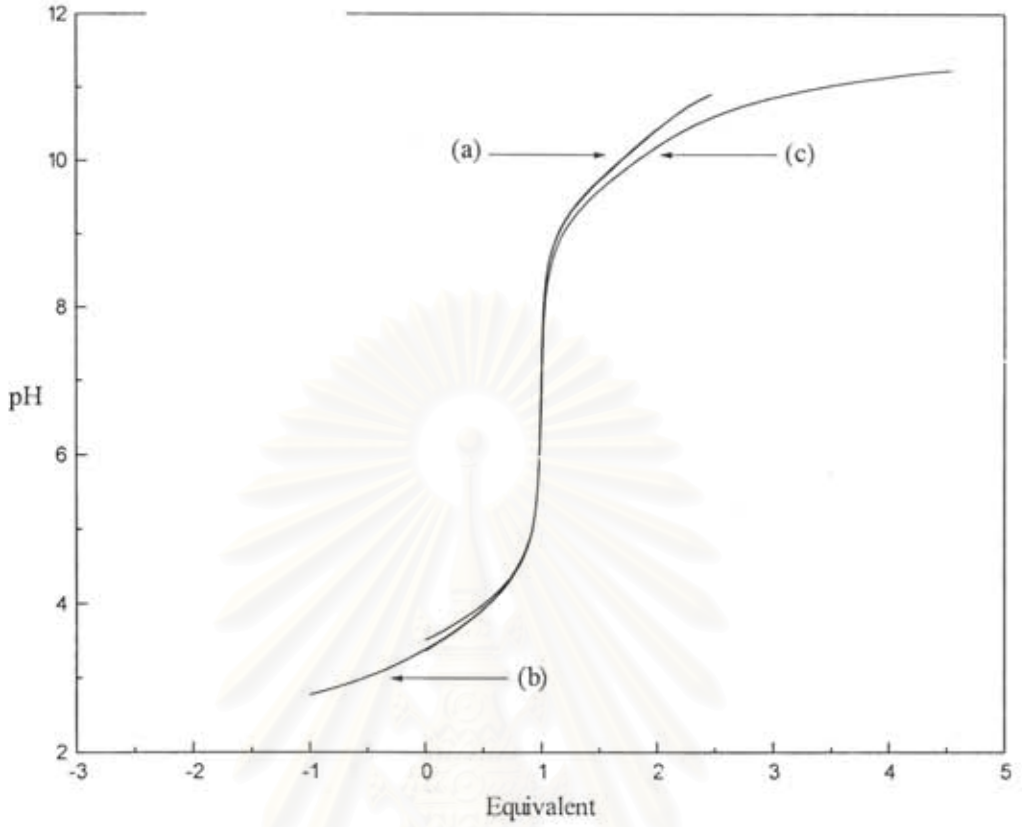


Figure 4.3 Potentiometric titration curves of aspartic acid in 0.1 M KNO_3 at 25 °C, based on the initial concentration ratio of the ligand to proton of (a) 1.16 mM : 0 mM (b) 2.22 mM : 0 mM (c) 2.15 mM : 1.12 mM; equivalent is defined as the ratio of $(n_{OH^-} - n_{acid})$ to n_{ligand}.

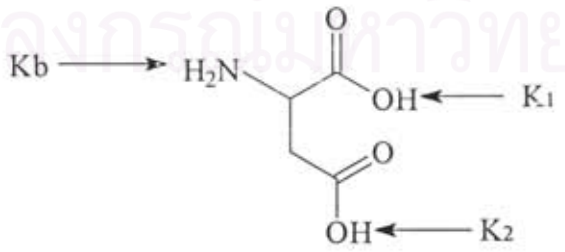


Figure 4.4 Acidity and basicity constants of aspartic acid and the sites of their corresponding protons.

4.3 Equilibrium Constant of Aspartylaspartic Acid

The chemical equilibria of the aspartic acid (symbolized as LH_3) in aqueous solution (0.1 M KNO_3) are written as following equations

$$K_1 : LH_4^+ \rightleftharpoons LH_3 + H^+$$

$$K_2 : LH_3 \rightleftharpoons LH_2^- + H^+$$

$$K_3 : LH_2^- \rightleftharpoons LH^{2-} + H^+$$

$$K_b : L^{3-} + H^+ \rightleftharpoons LH^{2-}$$

K_1, K_2 and K_3 are acidity constants and K_b is basicity constant. Their logarithm values are shown in Table 4.1. The titration curves of the aspartic acid in 0.1 M KNO_3 are shown in Figure 4.5. K_1, K_2 and K_3 of aspartylaspartic acid are acidity constants that correspond to the terminal acid proton and the side acid protons respectively. The acidity and basicity constants of the aspartic acid and the sites of their corresponding protons are shown in Figure 4.6.

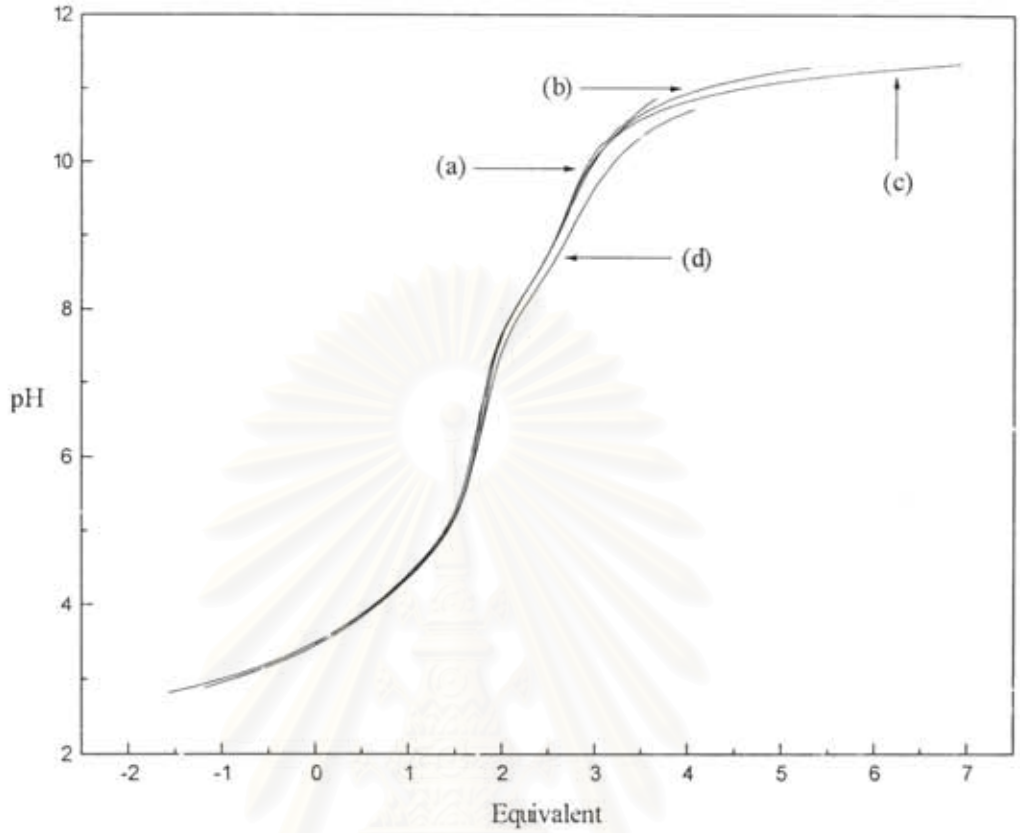


Figure 4.5 Potentiometric titration curves of aspartylaspartic acid in 0.1 M KNO_3 at 25 °C, based on the initial concentration ratio of the ligand to proton of (a) 2.01 mM : 0 mM (b) 1.61 mM : 1.98 mM (c) 1.67 mM : 0 mM and (d) 1.82 mM : 4.50 mM; equivalent is defined as the ratio of $(n_{OH^-} - n_{acid})$ to n_{ligand}.

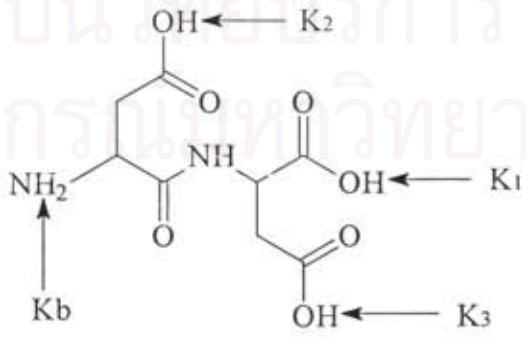


Figure 4.6 Acidity and basicity constants of aspartylaspartic acid and the sites of their corresponding protons.

Table 4.1 Logarithm of acidity and basicity constants of N-acetylaspartic acid, aspartic acid and aspartylaspartic acid in 0.1 M KNO_3 at 25 °C.

Amino acid	log K_b	log K_1	log K_2	log K_3
N-acetylaspartic acid	-	-3.41± 0.09	-5.13 ± 0.04	-
aspartic acid	9.80 ± 0.007	-2.38 ± 0.02	-3.80 ± 0.01	-
aspartylaspartic acid	8.79 ± 0.05	-3.23 ± 0.20	-3.47 ± 0.17	-5.38± 0.11

Species distribution curves of N-acetylaspartic acid in 0.1 M KNO_3 at 25 °C as shown in Figure 4.7 indicates that over 20 % of species LH^- exists within pH range of 3.0 to 6.0. The di-deprotonated species of N-acetylaspartic acid, L^{2-} can be found at pH above 4.0. Above pH 6.0, species LH_2, can not survive in the solution.

The corresponding acid protons of the acidity constants for aspartylaspartic acid, derived from known systems of N-acetylaspartic acid and aspartic acid, were identified by following reasons :

1. K_1 corresponds to dissociation of the strongest acid proton at the right position of the aspartylaspartic acid's structure (see Figure 4.6).
2. K_2 corresponds to dissociation of the secondly strong acid proton at the left position of its structure (see Figure 4.6).
3. K_3 corresponds to dissociation of the weakest acid proton at the bottom right position of its structure (see Figure 4.6).

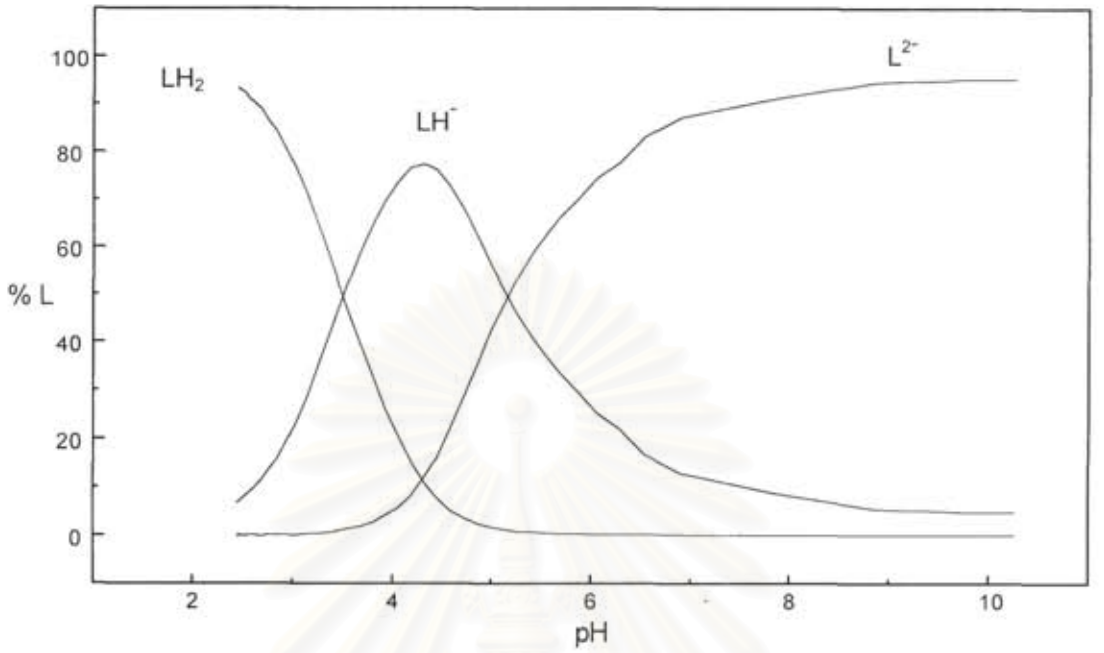


Figure 4.7 Species distribution curves of N-acetylaspartic acid in 0.1 M KNO_3 at 25 °C, with initial concentration of 2.08 x 10^{-3} M.

It has been known that the terminal amino-nitrogen of aspartic acid and aspartylaspartic acid can be easily protonated in aqueous solution to form zwitter ion. Basicity constants of aspartic acid and aspartylaspartic acid, expressed as log K_b are 9.80 and 8.79 respectively. The acidity and basicity constants of aspartic acid and aspartylaspartic acid at the corresponding acid protons are labeled as shown in Figure 4.4 and 4.6 respectively.

Species distribution of aspartic acid in 0.1 M KNO_3 at 25 °C, plotted according to the observed data obtained from the titrations and the evaluated equilibrium constants is shown in Figure 4.8. Species domination of aspartic acid depends on the pH of the solution in which the species LH_3^+ exists in the solution at the pH below 4.0 and LH_2 presents at the pH below 6.0.

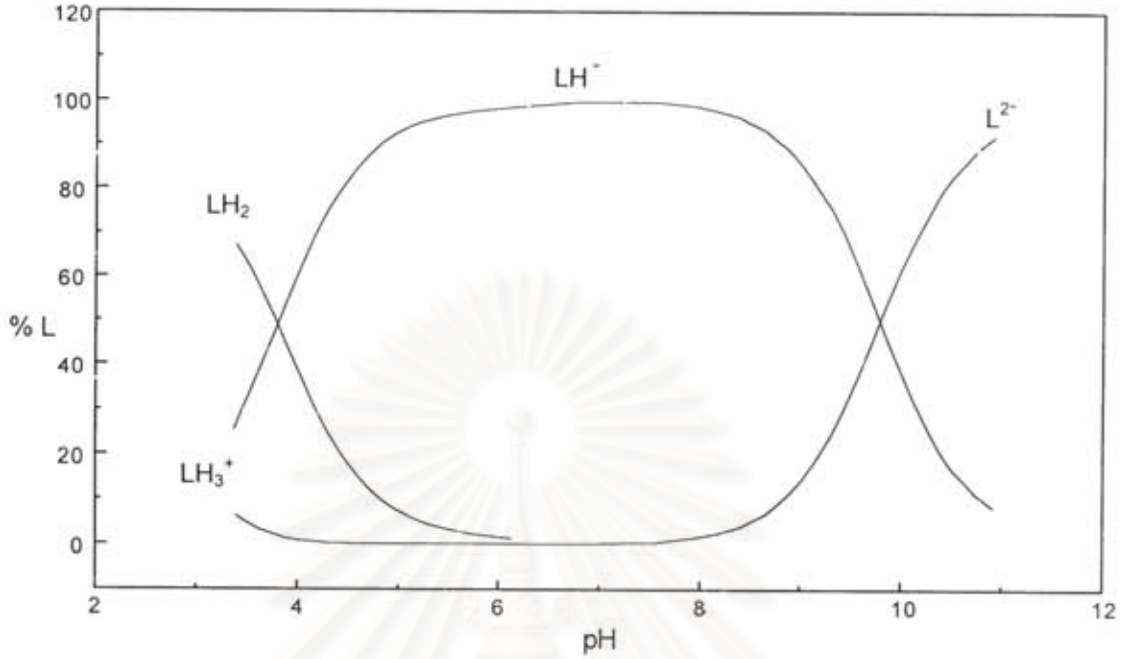


Figure 4.8 Species distribution curves of aspartic acid in 0.1 M KNO_3 at 25 °C, with initial concentration of 2.22×10^{-3} M.

The LH^- is a dominant species spreading in the wide pH range of 3.5 to 10.5. Over 80 % of LH^- exist within the pH range of 4.5 to 7.0. The complete deprotonated species, L^{2-}, can survive only in the solution of the pH above 8.0. The acidity and basicity constants of aspartylaspatic acid which correspond to the carboxylic acid protons and terminal amino-nitrogen, respectively, are shown in the Figure 4.6.

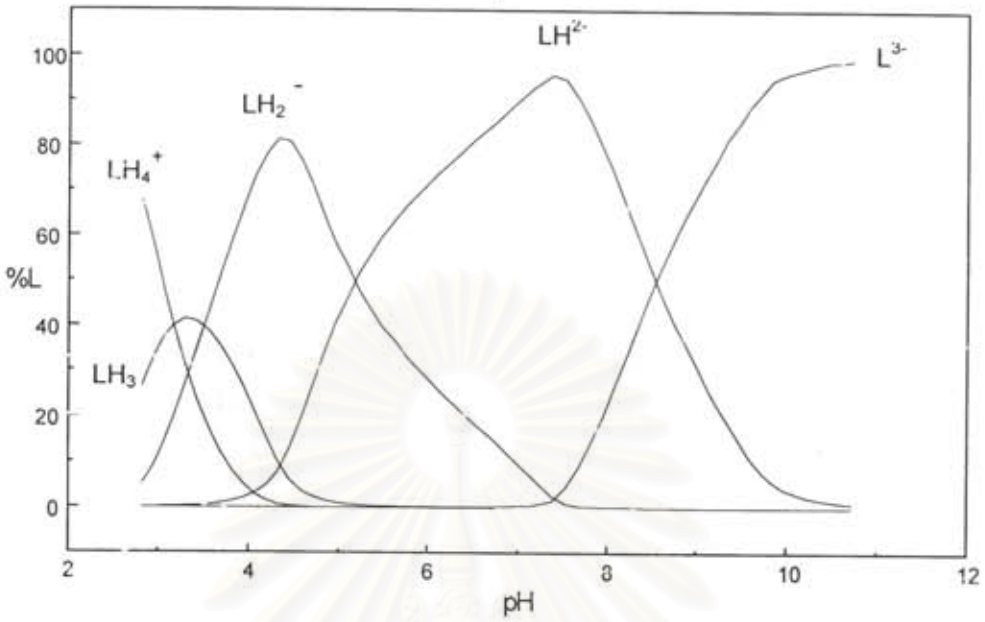


Figure 4.9 Species distribution curves of aspartylaspartic acid in 0.1 M KNO_3 at 25 °C, with initial concentration of 8.10×10^{-4} M.

Species distribution of aspartylaspartic acid (LH_3) in 0.1 M KNO_3 at 25°C, shown in Figure 4.9 composes of the species LH_4^+, LH_3, LH_2^-, LH^{2-} and L^{3-}. At the high acidic solution, pH below 3.0, LH_4^+ is a dominant species. The maximum population of LH_3 located at pH ~ 3.25 is about 40 % . The maximum population of LH_2^- and LH^{2-} located at pH 4.4 and 7.5, respectively, are over 80 %. The LH^{2-} is a species that exists within the widest pH range; over 50 % exist within the pH range of 5.5 to 8.5. At pH above 11, L^{3-} can survive only in the solution.

4.4 Equilibrium Constant of Asp-Asp-Asp

The chemical equilibria of the asp-asp-asp (symbolized as LH_4) in aqueous solution (0.1 M KNO_3) are written as following equations

$$K_1 : LH_5^+ \rightleftharpoons LH_4 + H^+$$
$$K_2 : LH_4 \rightleftharpoons LH_3^- + H^+$$
$$K_3 : LH_3^- \rightleftharpoons LH_2^{2-} + H^+$$
$$K_4 : LH_2^{2-} \rightleftharpoons LH^{3-} + H^+$$
$$K_b : L^{4-} + H^+ \rightleftharpoons LH^{3-}$$

K_1, K_2, K_3 and K_4 are acidity constants and K_b is basicity constant. Their logarithm values are shown in Table 4.2. The titration curves of the asp-asp-asp in 0.1 M KNO_3 are shown in Figure 4.10. Acidity and basicity constants of asp-asp-asp and the proposed sites of their corresponding protons are shown in Figure 4.11.

Table 4.2 Logarithm of acidity and basicity constants of asp-asp-asp in 0.1 M KNO_3 at 25 °C.

Equilibrium constant	Log K
K_1	-3.0 ± 0.2
K_2	-3.37 ± 0.11
K_3	-3.86 ± 0.08
K_4	-5.09 ± 0.05
K_b	8.34 ± 0.02

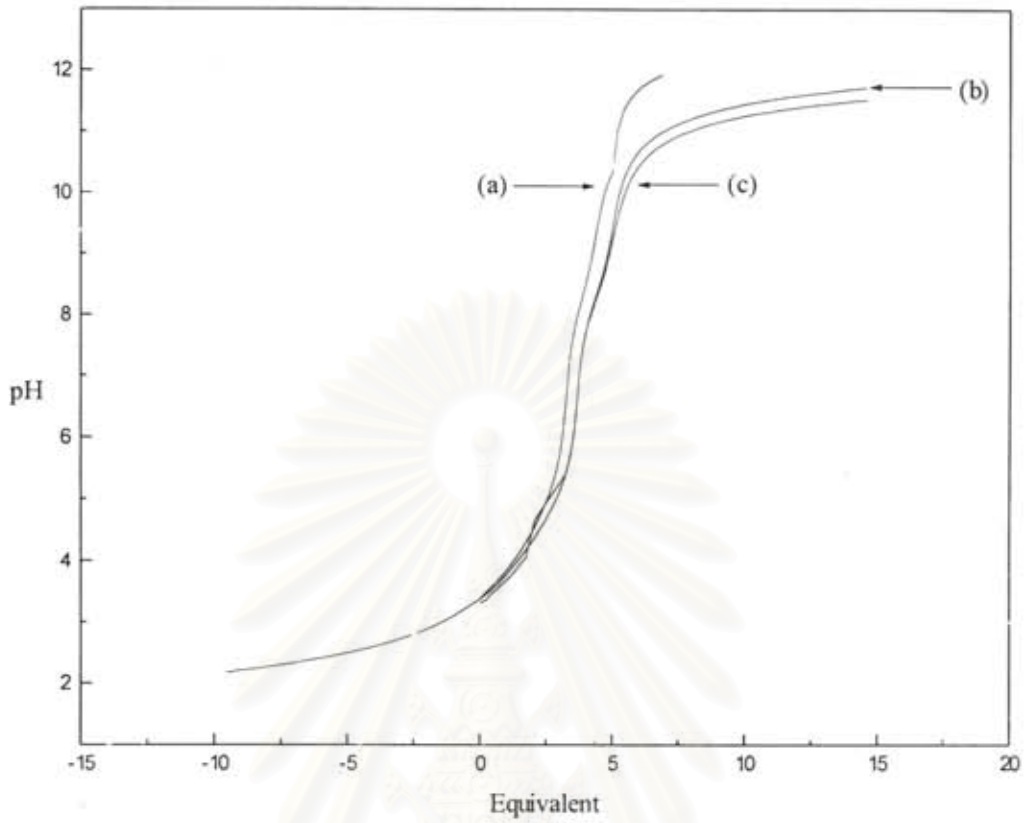


Figure 4.10 Potentiometric titration curves of asp-asp-asp in 0.1 M KNO_3 at 25 °C, based on the initial concentration ratio of the ligand to proton of (a) 0.85 mM : 8.17 mM (b) 0.85 mM : 0 mM (c) 0.85 mM : 0 mM ; equivalent is defined as the ratio of $(n_{OH^-} - n_{acid})$ to n_{ligand}.

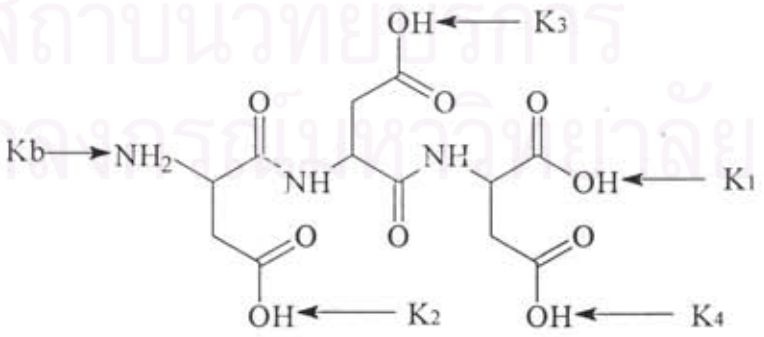


Figure 4.11 Acidity and basicity constants of asp-asp-asp and the proposed sites of their corresponding protons.

The proposed sites of acid protons corresponding to the acidity constants of asp-asp-asp as shown in Figure 4.11 were derived from the acidity constants of aspartylaspartic acid as following principals :

1. K_1 corresponds to dissociation of the strongest acid proton at the right position of the asp-asp-asp's structure (see Figure 4.11).
2. K_2 corresponds to dissociation of the secondly strong acid proton at the first position from the left of its structure (see Figure 4.11).
3. K_3 corresponds to dissociation of the thirdly strong acid proton at the second position from the left of its structure (see Figure 4.11).
4. K_4 corresponds to dissociation of the weakest acid proton at the bottom right position of its structure (see Figure 4.11).

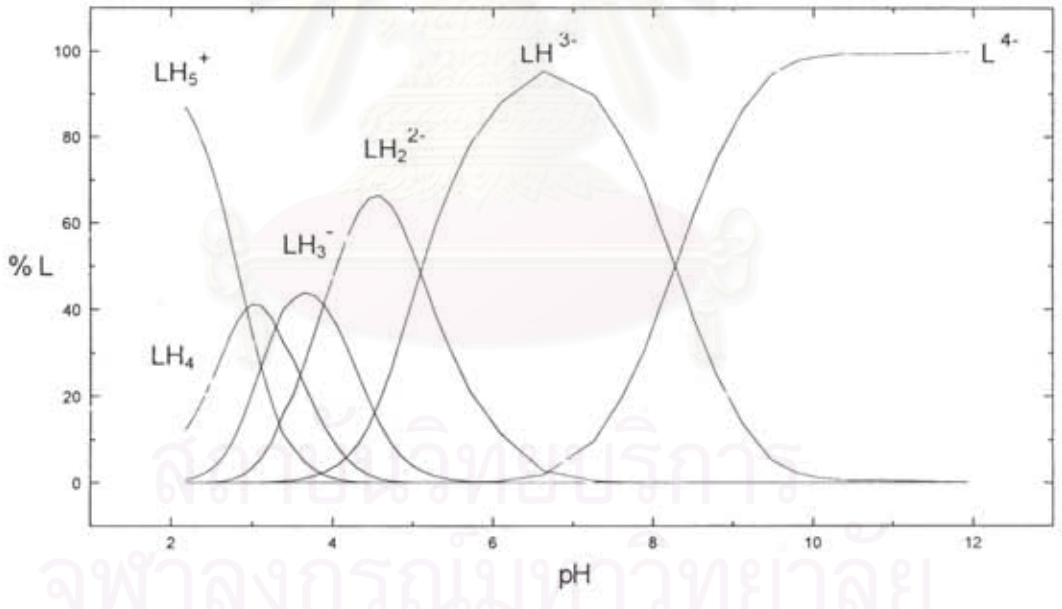


Figure 4.12 Species distribution curves of asp-asp-asp in 0.1 M KNO_3 at 25 °C, with initial concentration of 8.54×10^{-4} M.

Species distribution of asp-asp-asp (LH_4) in 0.1 M KNO_3 at 25°C, Figure 4.12 shows that species LH_4, LH_3^-, LH_2^{2-} exist within the pH range of 2 - 4.5, 2.2 - 5.2 and 2.8 - 7.2, respectively. The maximum population of LH_4, LH_3^-, LH_2^{2-} located at 3.0, 3.5 and 4.5 are about 40 %, 45 % and 65 %, respectively. The over 30 % of LH_5^+ species presents at pH below 3. The neutral solution (pH = 7), the LH^{3-} species is predominant and exists within the widest pH range (pH 4 to 10). At pH above 10.5, only L^{4-} exists in the solution.

4.5 Equilibrium Constant of Asp-Asp-Asp-Asp

The chemical equilibria of the asp-asp-asp-asp (symbolized as LH_5) in aqueous solution (0.1 M KNO_3) are written as following equations

$$K_1 : LH_6^+ \rightleftharpoons LH_5 + H^+$$
$$K_2 : LH_5 \rightleftharpoons LH_4^- + H^+$$
$$K_3 : LH_4^- \rightleftharpoons LH_3^{2-} + H^+$$
$$K_4 : LH_3^{2-} \rightleftharpoons LH_2^{3-} + H^+$$
$$K_5 : LH_2^{3-} \rightleftharpoons LH^{4-} + H^+$$
$$K_b : L^{5-} + H^+ \rightleftharpoons LH^{4-}$$

K_1, K_2, K_3, K_4 and K_5 are acidity constants and K_b is basicity constant. Their logarithm values are shown in Table 4.3. The titration curves of the asp-asp-asp-asp in 0.1 M KNO_3 are shown in Figure 4.13. The asp-asp-asp-asp's acid protons of their corresponding acidity constants derived by the same principle applying on the asp-asp-asp were proposed as shown in Figure 4.14.

Table 4.3 Logarithm of acidity and basicity constants of asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C.

Equilibrium constant	Log K
K_1	-3.0 ± 0.2
K_2	-3.12 ± 0.16
K_3	-3.89 ± 0.11
K_4	-4.42 ± 0.08
K_5	-5.37 ± 0.05
K_b	8.50 ± 0.02

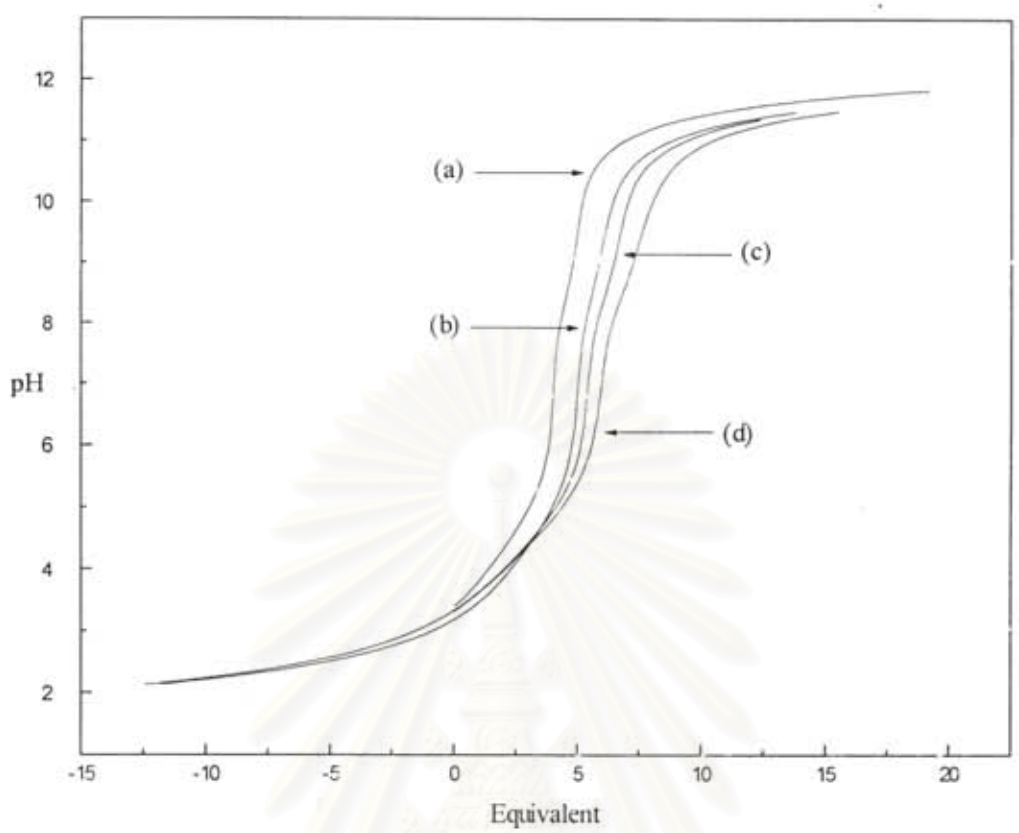


Figure 4.13 Potentiometric titration curves of asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C, based on the initial concentration ratio of the ligand to proton of (a) 0.806 mM : 0 mM (b) 0.806 mM : 0 mM (c) 0.652 mM : 7.69 mM and (d) 0.671 mM : 8.30 mM ; equivalent is defined as the ratio of $(n_{OH^-} - n_{acid})$ to n_{ligand}.

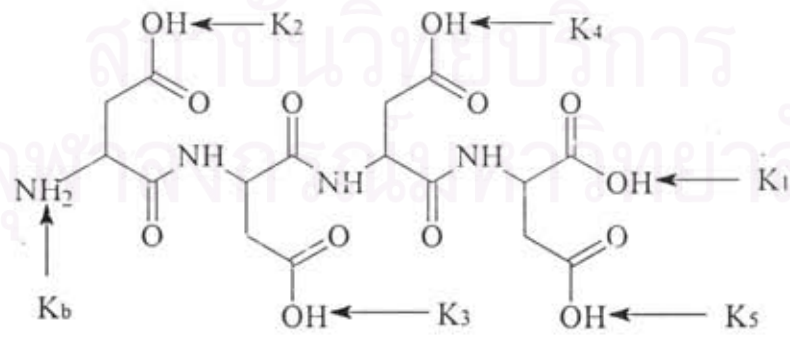


Figure 4.14 Acidity and basicity constants of asp-asp-asp-asp and the proposed sites of their corresponding protons.

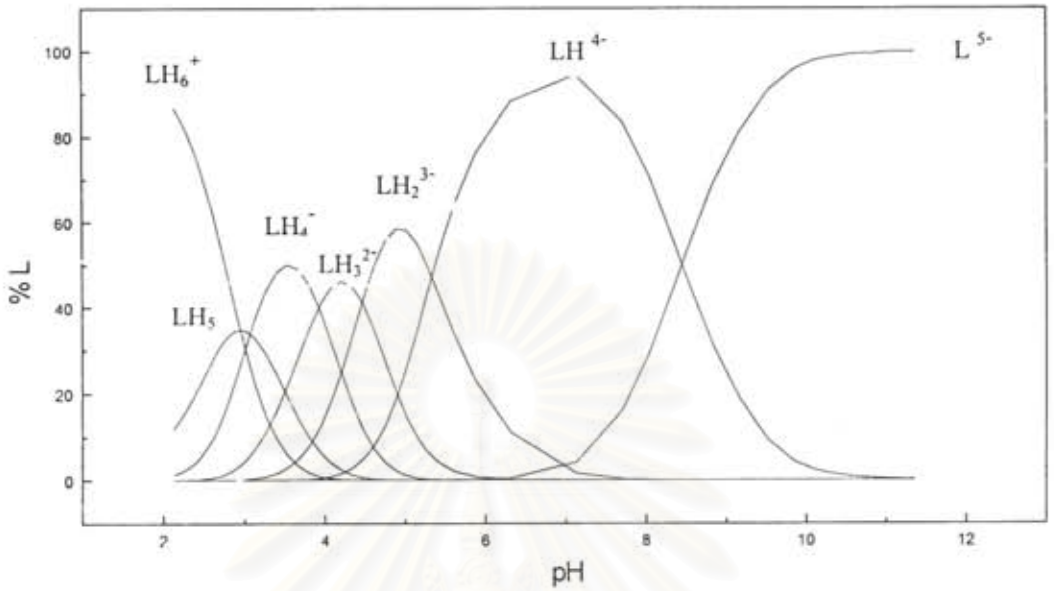


Figure 4.15 Species distribution curves of asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C, with initial concentration of 6.52×10^{-4} M.

Species distribution of asp-asp-asp-asp in 0.1 M KNO_3 at 25°C, Figure 4.15 shows that species of LH_5, LH_4^-, LH_3^{2-} and LH_2^{3-} present within the pH range of 2 - 4.5, 2.2 - 5.1, 2.5 - 5.6 and 3 - 7, respectively. The maximum population of LH_5, LH_4^-, LH_3^{2-} and LH_2^{3-} located at 2.9, 3.4, 4.2 and 4.8 are about 35 %, 50 %, 45 % and 58 %, respectively. The over 30 % of LH_6^+ species presents at pH below 3. The neutral solution (pH = 7), the LH^{4-} species is predominant and exists within the widest pH range (pH 4 to 10). At pH above 10.5, only L^{5-} exists in the solution.

4.6 Equilibrium Constant of Asp-Asp-Asp-Asp-Asp

The chemical equilibria of the asp-asp-asp-asp-asp (symbolized as LH_6) in aqueous solution (0.1 M KNO_3) are written as following equations

K_1 : $LH_7^{+} \rightleftharpoons LH_6 + H^{+}$

K_2 : $LH_6 \rightleftharpoons LH_5^{-} + H^{+}$

K_3 : $LH_5^{-} \rightleftharpoons LH_4^{2-} + H^{+}$

K_4 : $LH_4^{2-} \rightleftharpoons LH_3^{3-} + H^{+}$

K_5 : $LH_3^{3-} \rightleftharpoons LH_2^{4-} + H^{+}$

K_6 : $LH_2^{4-} \rightleftharpoons LH^{5-} + H^{+}$

K_b : $L^{6-} + H^{+} \rightleftharpoons LH^{5-}$

K_1, K_2, K_3, K_4, K_5 and K_6 are acidity constants and K_b is basicity constant. Their logarithm values are shown in Table 4.4. The titration curves of the asp-asp-asp-asp in 0.1 M KNO_3 are shown in Figure 4.16. The asp-asp-asp-asp-asp's acid protons of their corresponding acidity constants derived by the same priciple applying on the asp-asp-asp were proposed as shown in Figure 4.17.

Table 4.4 Logarithm of acidity and basicity constants of asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C.

Equilibrium constant	Log K
K_1	-3.0 ± 0.2
K_2	-3.0 ± 0.2
K_3	-3.54 ± 0.29
K_4	-4.66 ± 0.20
K_5	-4.68 ± 0.10
K_5	-6.06 ± 0.15
K_b	8.56 ± 0.06

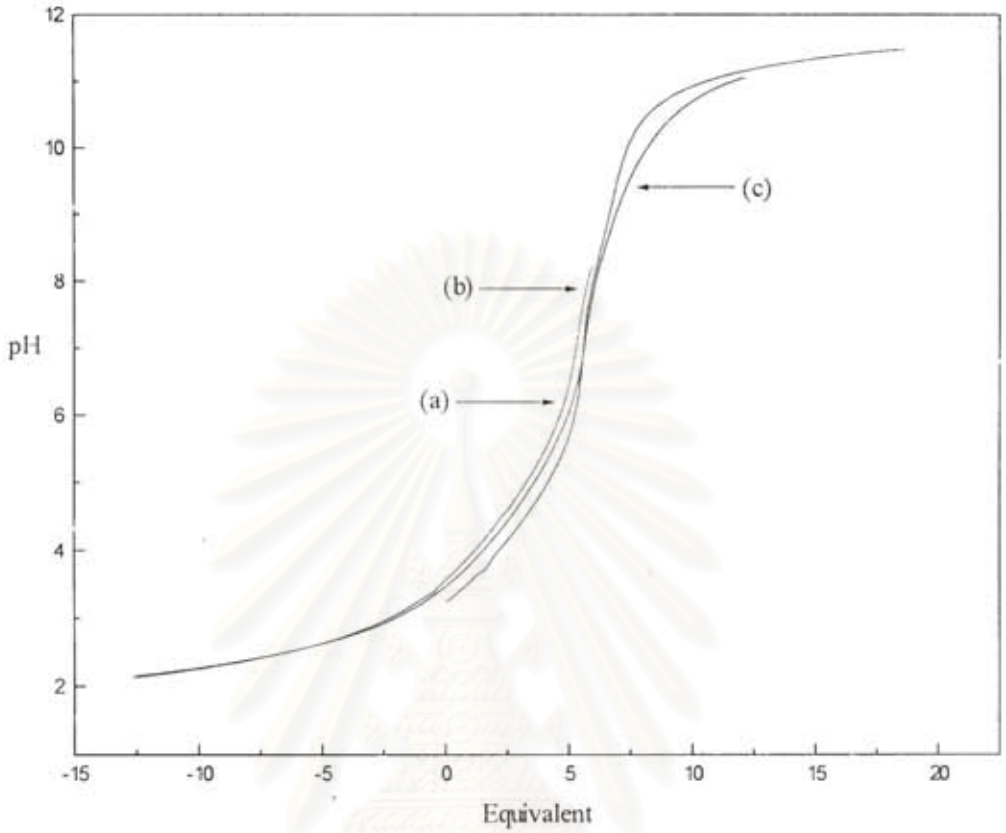


Figure 4.16 Potentiometric titration curves of asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C, based on the initial concentration ratio of the ligand to proton of (a) 0.805 mM : 8.49 mM (b) 0.805 mM : 8.49 mM and (c) 0.805 mM : 0 mM ; equivalent is defined as the ratio of $(n_{OH^-} - n_{acid})$ to n_{ligand}.

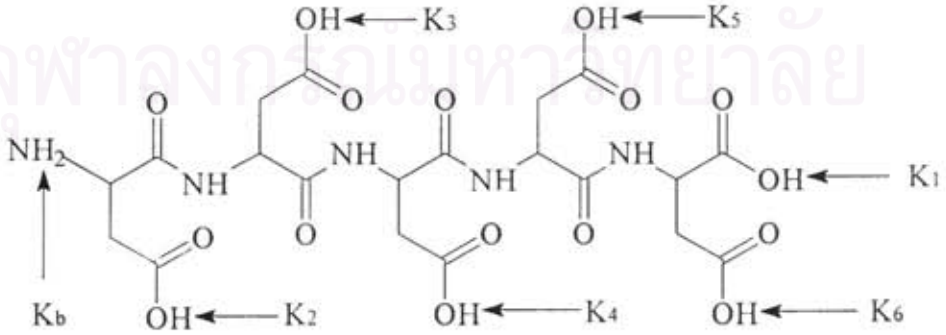


Figure 4.17 Acidity and basicity constants of asp-asp-asp-asp-asp and the proposed sites of their corresponding protons.

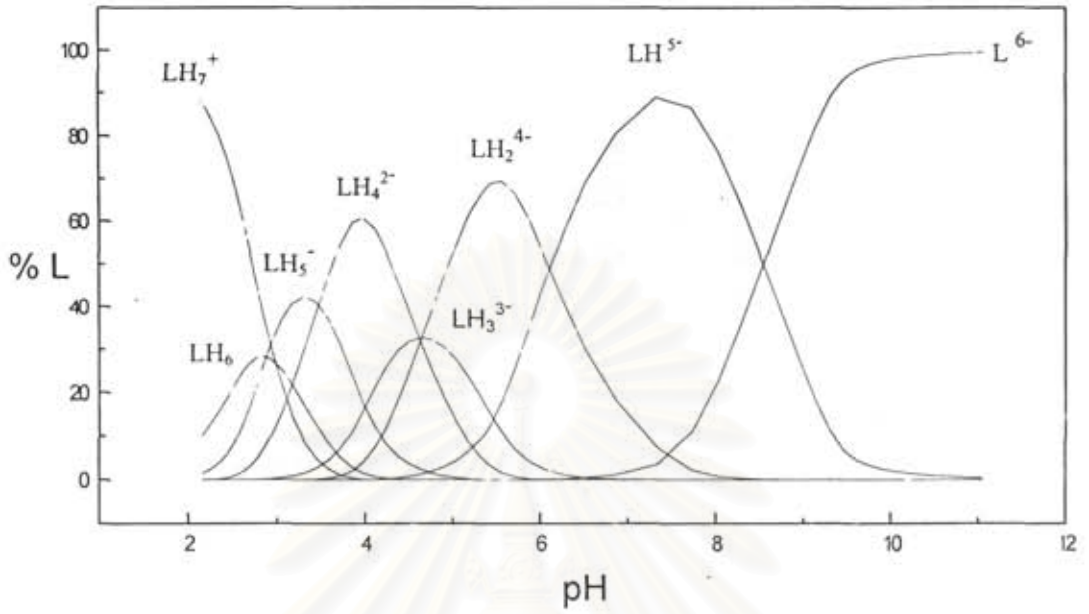


Figure 4.18 Species distribution curves of asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C, with initial concentration of 6.91×10^{-4} M.

Species distribution of asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25°C, Figure 4.18 shows that species of LH_6, LH_5^-, LH_4^{2-} and LH_3^{3-} present within the pH range of 2 - 4.1, 2.0 - 5.0, 2.5 - 5.6 and 3.0 - 6.4, respectively. The maximum population of LH_6, LH_5^-, LH_4^{2-} and LH_3^{3-} located at 2.8, 3.3, 4.0 and 4.6 are about 30 %, 42 %, 61 % and 32 %, respectively. The over 80 % of LH_7^+ species presents at pH below 2. The LH_2^{4-} is dominant species that exists within the pH range of 3.5 to 8.1 and presents over 70 % at pH = 5.5. The LH^{5-} species presents and within the pH range of pH 4.5 to 10.5 and its maximum population is located at 6.4. At pH above 6.7, only L^{6-} exists in the solution.

4.7 Equilibrium Constant of Asp-Asp-Asp-Asp-Asp-Asp

The chemical equilibria of the asp-asp-asp-asp-asp-asp (symbolized as LH_7) in aqueous solution (0.1 M KNO_3) are written as following equations

K_1 : $LH_8^+ \rightleftharpoons LH_7 + H^+$

K_2 : $LH_7 \rightleftharpoons LH_6^- + H^+$

K_3 : $LH_6^- \rightleftharpoons LH_5^{2-} + H^+$

K_4 : $LH_5^{2-} \rightleftharpoons LH_4^{3-} + H^+$

K_5 : $LH_4^{3-} \rightleftharpoons LH_3^{4-} + H^+$

K_6 : $LH_3^{4-} \rightleftharpoons LH_2^{5-} + H^+$

K_7 : $LH_2^{5-} \rightleftharpoons LH^{6-} + H^+$

K_b : $L^{7-} + H^+ \rightleftharpoons LH^{6-}$

K_1, K_2, K_3, K_4, K_5, K_6 and K_7 are acidity constants and K_b is basicity constant. Their logarithm values are shown in Table 4.5. The titration curves of the asp-asp-asp-asp-asp-asp in 0.1 M KNO_3 are shown in Figure 4.19. The asp-asp-asp-asp-asp-asp's acid protons of their corresponding acidity constants derived by the same principle applying on the asp-asp-asp were proposed as shown in Figure 4.20.

Table 4.5 Logarithm of acidity and basicity constants of asp-asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C.

Equilibrium constant	Log K
K_1	-3.0 ± 0.2
K_2	-3.0 ± 0.2
K_3	-3.0 ± 0.2
K_4	-3.0 ± 0.2
K_5	-3.87 ± 0.24
K_6	-4.52 ± 0.24
K_7	-5.98 ± 0.14
K_b	8.99 ± 0.06

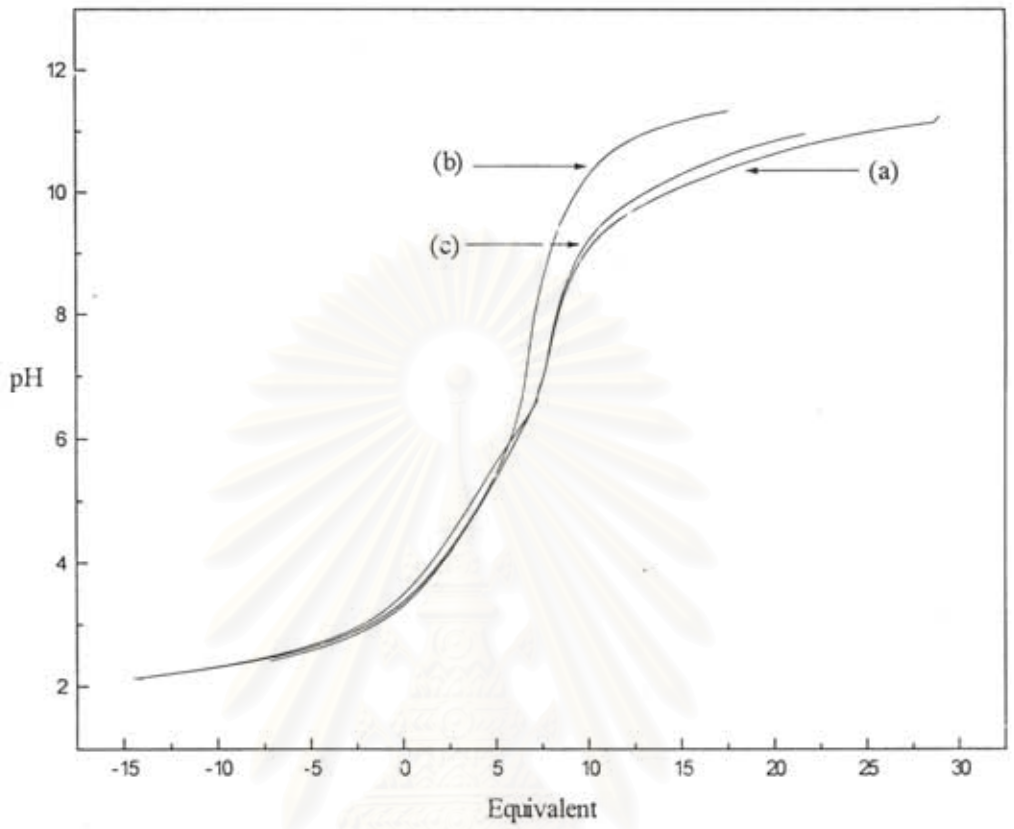


Figure 4.19 Potentiometric titration curves of asp-asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C, based on the initial concentration ratio of the ligand to proton of (a) 0.575 mM : 8.334 mM (b) 0.575 mM : 4..167 mM and (c) 0.575 mM : 8.334 mM ; equivalent is defined as the ratio of $(n_{OH^-} - n_{acid})$ to n_{ligand}.

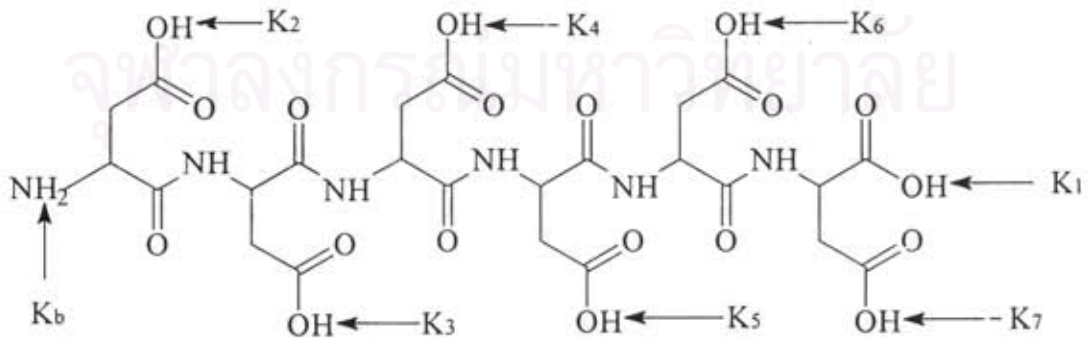


Figure 4.20 Acidity and basicity constants of asp-asp-asp-asp-asp-asp and the proposed sites of their corresponding protons.

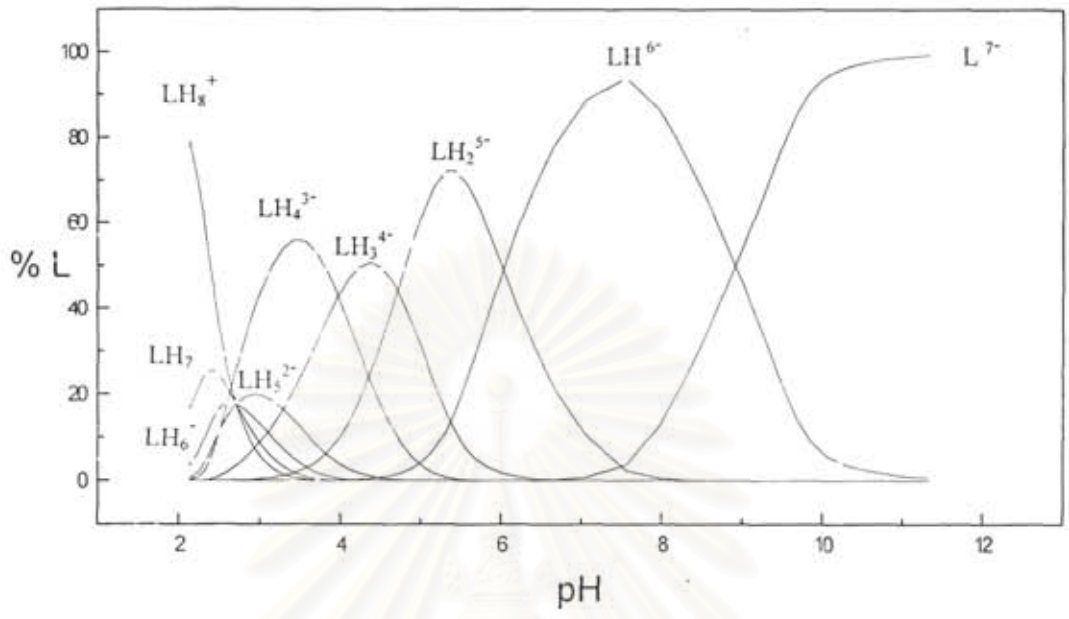


Figure 4.21 Species distribution curves of asp-asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25 °C, with initial concentration of 5.75×10^{-4} M.

Species distribution of asp-asp-asp-asp-asp-asp in 0.1 M KNO_3 at 25°C, Figure 4.21 shows that species of LH_7, LH_6^- and LH_5^{2-} present within the narrow pH range of 2 to 4. The species of LH_4^{3-}, LH_3^{4-} and LH_2^{5-} distribute within the pH range of 2.0 - 5.5, 2.5 - 6.5 and 3.5 - 8.0, respectively. The maximum peaks of species LH_4^{3-}, LH_3^{4-} and LH_2^{5-} located at 3.5, 4.5 and 5.5 are about 55 %, 53 % and 73 %, respectively. The LH_8^+ species presents in the solution at pH below 3.4. The LH^{6-} is dominant species that exists within the pH range of 4.5 to 11.0 and presents over 90 % at pH = 7.5. At pH above 7.0, only L^{7-} exists in the solution. The relation between the log K_b of the compounds of aspartic acid and the number of their aspartic unit(s) is plotted in Figure 4.22. The log K_b of asp-asp-asp (n =3) is the smallest magnitude. Basicity constants, expressed as log K of aspartic acid (asp) and polyaspartic acids $(asp)_n$, when n=2 to 6, are 9.80 and 8.79, 8.34, 8.50, 8.56 and 8.99 respectively. The number of acidity constant of polyaspartic acids $(asp)_n$ can be calculated by n+1 that is equivalent to the number of carboxylic acid in the molecule. The pI (defined as $-\log[H^+]$ at the isoelectric point) of aspartic acid, aspartylaspartic acid , asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp

in aqueous solution of 0.1 M potassium nitrate at 25 °C, estimated from the involving species of corresponding curves of species distribution, are tabulated in table 4.6 and plot of the n (number of aspartic unit) against the pI is shown in Figure 4.23.

According to the species distribution curves of all aspartic acid compounds. Most of their protonated species present in the acidic range (at pH below 7). The aspartic acid compounds of the high number of aspartic unit, at least three protonated species of which acid protons are simultaneously dissociated in the high acidic solution. As the possibility of complexation of many amino acids (12-16) depending on their binding atoms, charges, molecular structure and also number of protonated species, the high number of aspartic unit is, therefore, expected to form complexes with various cations. The aspartic acid compounds of high aspartic unit which have a high possibility of complexation are also expected to form the high stable complexes and cation selectivity. Therefore, the complexation study of aspartic acid compounds is a very interesting research for determination of their stability constants and their selectivity for various cations. One of the most important application for cation selectivity of aspartic acid compounds is the seperation of toxic cations for environmental improvement.

Basicity of the polyaspartic acids depends on the number of aspartic unit as the function of log K_b = f(n), indicated by the basicity curve as shown in Figure 4.22. Figure 4.22 shows that the basicity of terminal nitrogen of asp-asp-asp is the weakest one.

The pI of the methanolic solution of aspartic acid compounds in 0.1 M potassium nitrate at 25 °C can be calculated from the fitted equation as written below.

$$pI = 3.75 - 0.225\,n \qquad (4.1)$$

The equation (4.1) represents the pI of the polyaspartic acid, $(asp)_n$ where $1< n <7$. Deviation between the calculated pI according to the equation (4.1) and the observed values is approximately $\pm$ 0.08.

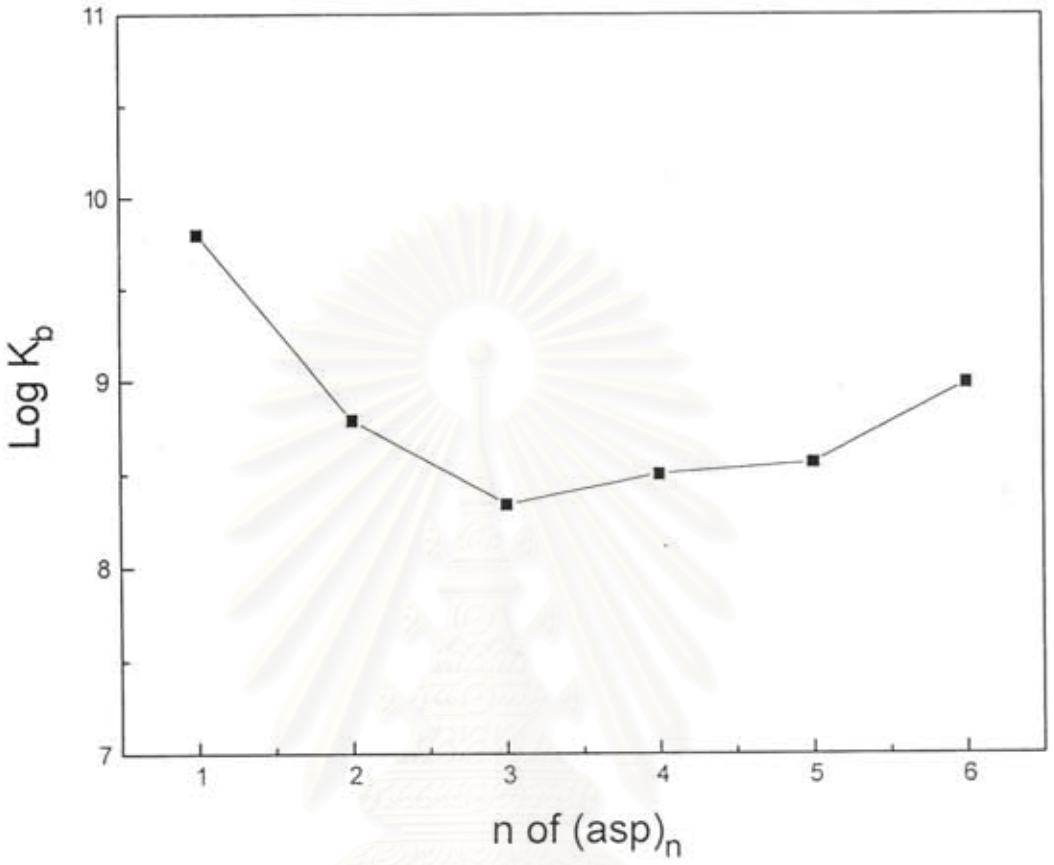


Figure 4.22 Plot of log K_b of the compounds of aspartic acid against the number of aspartic unit, $(asp)_n$.

We can conclude that the aspartic unit's number of polyaspartic acid affects the number of existing species depending on the pH of its solution and the basicity of its terminal nitrogen.

Table 4.6 The pI of the compounds of aspartic acids existing in 0.1 M KNO_3 at 25 °C.

compounds	n of $(asp)_n$	pI
asp	1	3.1
asp-asp	2	3.3
asp-asp-asp	3	3.1
asp-asp-asp-asp	4	2.8
asp-asp-asp-asp-asp	5	2.7
asp-asp-asp-asp-asp-asp	6	2.4

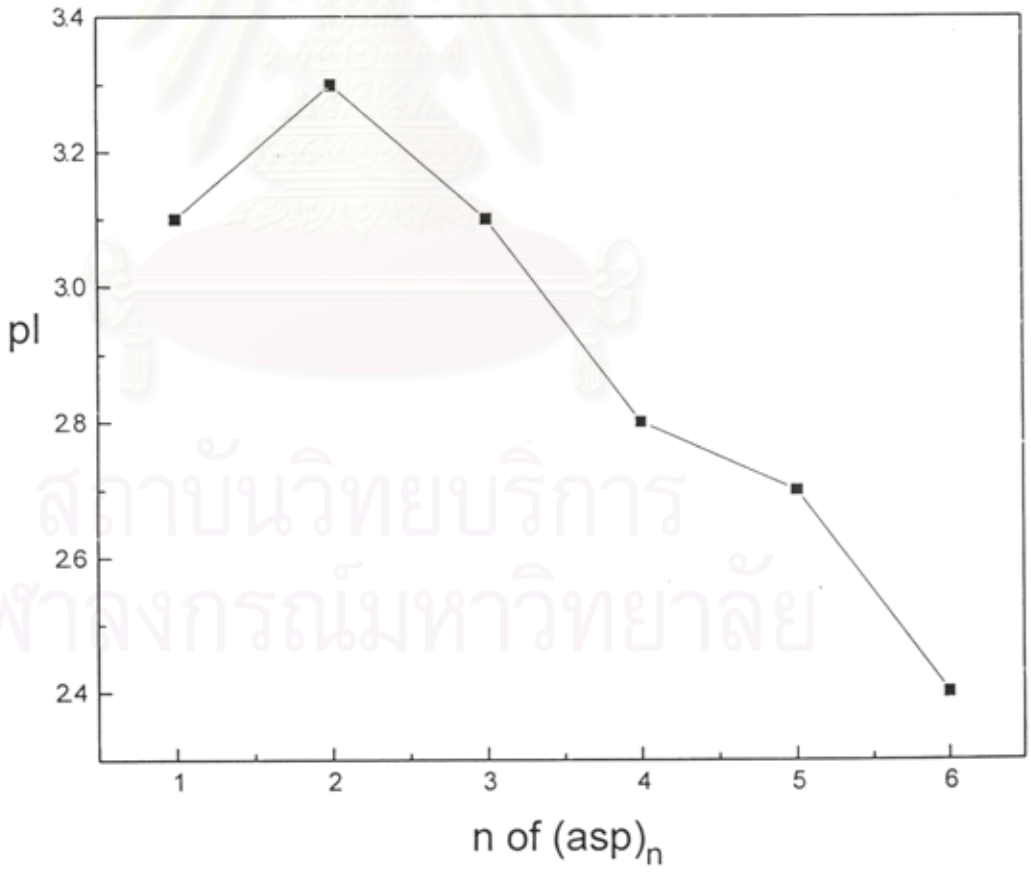


Figure 4.23 Plot of pI against the number of aspartic unit, $(asp)_n$.

CHAPTER V

CONCLUSION

Acidity and basicity constants of N-acetylaspartic acid (NA) , aspartic acid (A1), aspartylaspartic acid (A2), asp-asp-asp (A3), asp-asp-asp-asp (A4), asp-asp-asp-asp-asp (A5) and asp-asp-asp-asp-asp-asp (A6) in 0.1 M potassium nitrate at 25 °C, expressed as log K are tabulated in table below.

Amino Acid	log K_b	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6	log K_7
NA	-	-3.41	-5.13	-	-	-	-	-
A1	9.80	-2.38	-3.80	-	-	-	-	-
A2	8.79	-3.23	-3.47	-5.38	-	-	-	-
A3	8.34	-3.0	-3.37	-3.86	-5.09	-	-	-
A4	8.50	-3.0	-3.12	-3.89	-4.42	-5.37	-	-
A5	8.56	-3.0	-3.0	-3.54	-4.66	-4.68	-6.06	-
A6	8.99	-3.0	-3.0	-3.0	-3.0	-3.87	-4.52	-5.98

The number of acidity constant of polyaspartic acids $(asp)_n$ can be calculated by n+1 which is equivalent to the number of carboxylic acid in molecule of aspartic acids. The pI of aspartic acid, aspartylaspartic acid , asp-asp-asp, asp-asp-asp-asp, asp-asp-asp-asp-asp and asp-asp-asp-asp-asp-asp in 0.1 M potassium nitrate at 25 °C are 3.1, 3.3, 3.1, 2.8, 2.7 and 2.4 respectively.

We can conclude that the number of the existing species of aspartic acid compounds is n+3 and the corresponding species is (are) $LH_{(n+3-i)}^{(i-2)-}$, where i = 1, 2, to (n+3). Existing order of dominant species of $(asp)_n$ as increasing of pH is $LH_{(n+2)}^{-}$, $LH_{(n+1)}$, LH_n^{-}, ... $L^{(n+1)-}$.

Suggestion For the Future Work

In order to confirm the proposed sites of corresponding protons for each polyaspartic acid, electron densities of neighboring atoms of their acidic protons should be computed by quantum chemical calculations. The structures of those compounds of aspartic acids should be also optimized in order to obtain the most stable form of each compound. Protonation energies for their acid protons of the compounds of aspartic acids can be evaluated by quantum chemical method.

Thermodynamic terms (ΔH, ΔG and ΔS) corresponding to the protonation of these compounds of aspartic acids should be determined by Differential Scanning Calorimeter and be evaluated also by quantum chemical calculations. Comparison of the results between two methods will lead to obtain the important information to understand and reveal many properties of the compounds of aspartic acids.

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