K_{eq} AND ΔH FOR THE ESTERIFICATION OF GLYCINE IN ALCOHOL-WATER SYSTEMS

by

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TABLE OF CONTENTS

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LIST OF FIGURES

INTRODUCTION

THE DETERMINATION OF Keq VALUES FOR THE ESTERIFICATION OF GLYCINE WITH ETHANOL IN THREE DIFFERENT ETHANOL-WATER SOLVENT SYSTEMS

A protein is a macromolecule made up of amino acid residues linked together by peptide bonds. How the peptide bond was formed and thus how a protein made was not known in the early days of protein research. At that time it was thought that there might be only one or a few unspecific enzymes for amino acid activation for protein formation. A similarity was noted between the initial step in amino acid activation and acetate activation by DeMoss, et al. (1). The reaction used to show this was

$$\text{Acetate} + \text{AP}\sim\text{PP}_i + \text{CoASH} \rightleftharpoons \text{Acetyl}\sim\text{SCoA} + \text{PP}_i + \text{AMP} .$$

From the study of this reaction, it was concluded that the activation and transfer of an amino acid in an animal system was the function of one enzyme. It was also discovered that there was a set of ca 20 amino acids required for incorporation into a protein. In 1957, Hoagland, Zamecnik, and Stephenson demonstrated that the activated amino acid could be transferred to soluble RNA (now called transfer RNA) and joined to it in a relatively unstable linkage (2). Zachau, et al. then confirmed that an amino acid was esterified to the 2' or 3' position of a ribose moiety in RNA and it was concluded that amino acids were transferred from an initial activation product to the terminal adenosine moiety in sRNA (tRNA) (3). From these findings it was shown that amino acid-activating enzymes bring together only one particular amino acid with its specific tRNA molecule.

These amino acid-activating enzymes are really amino acyl RNA synthetases. These enzymes, of which each is specific for only one amino acid, take an amino acid and cause it to bind to its acceptor RNA (tRNA) through an ester link to the 2' or 3' OH group of the 3-terminal nucleotidyl ribose moiety. The general reactions that are catalyzed by these synthetases are

$$AMP\text{-}PP_i + RCHNH_3^+COO^- + Enzyme \overset{Mg^{2+}}{\rightleftharpoons} Enz\text{-}AMP\text{-}COCHNH_3^+R + PP_i$$

$$Enz\text{-}AMP\text{-}COCHNH_3^+R + RNA\text{-}OH \rightleftharpoons RNA\text{-}OCOCHNH_3^+R + AMP + Enz$$

OVERALL:

$$AMP\text{-}PP_i + RCHNH_3^+COOH + RNA\text{-}OH \overset{Mg^{2+}}{\rightleftharpoons} RNA\text{-}OCOCHNH_3^+R + AMP + PP_i \quad (4).$$

To understand the thermodynamics involved in the above reaction, the equilibrium constants (Keq's) were determined for reactions involving various amino acids (5). The Keq's of interest in this thesis research, however, are those of simpler amino acid esters since very little is known about the thermodynamics of reactions involving this type of compound. Some Keq's found include the Keq for the esterification of N-acetyl-L-tryptophan methyl ester by α chymotrypsin, Keq = 12.2 (6); the esterification of N-acetyl-L-tryptophan ethyl ester, Keq = 32.6 (6); and the Jencks, et al. value of 2.3 for the Keq of the esterification of glycine in water at 39°C. (7). These Keq's for the esterification of amino acids are of interest because the esterification of an amino acid is one of the steps that occurs in the synthesis of a protein. Amino acid esters are "high energy compounds", a high energy compound being one that when hydrolyzed exhibits a large negative $\Delta G^{\circ\prime}$. For the hydrolytic reaction "energy rich" means that some 7000 to 14,000 cal/mole are liberated when the reactions occur at some pH, usually 7 (7). This helps to explain the ready reversibility of amino acid activation by ATP to the amino acyl-RNA ester.

The Keq value of 2.3 for the esterification of glycine found by Jencks, et al. does not agree with the value of 0.82 previously obtained in this laboratory (8). This possibly could be due to the difference in temperature and/or solvent used for experimentation. Jencks, et al. used only a dilute aqueous solvent in their system for measuring the Keq value for glycine esterification. In the work done previously in this laboratory, an equimolar ethanol-water solvent system was used. The discrepency could also be due to the methods used for assay of the reactants and/or products. Jencks, et al. used the Hestrin alkaline hydroxylamine method and assayed only for the amount of ester formed (7,9). The method used in this laboratory involved the micro-Kjeldahl method and the analysis for both the free glycine and ester. A third possibility for a disagreement of the Keq values could be

due to the use of concentrations or mole quantities rather than activities of the different species present.

The purpose of the work described in this thesis is to determine the equilibrium constants associated with the esterification of glycine and the hydrolysis of glycine ethyl ester in a dilute aqueous or 0:1 molar ratio ethanol-water solvent system, a 0.5:1 molar ratio ethanol-water solvent system, and an equimolar or 1:1 molar ratio ethanol-water solvent system. (NOTE: In this thesis, the 0:1 molar ratio ethanol-water solvent system will be referred to as the 0:1 or dilute aqueous solvent system interchangeably. For the 1:1 molar ratio ethanol-water solvent system, the two designations, 1:1 and equimolar ethanol-water solvent system, will be used interchangeably.) This work was done to determine if solvents are important to a reaction. It is known that water, for instance, is an important solvent for many compounds. The solvation energies involved are likely to be contributing to the reactivity of various species in a solution to a much greater extent than is now thought. One example of how important this phenomenon is can be observed through the exergonic characteristics of the aminolysis of the amino acid esters of tRNA that are formed during the biosynthesis of a protein. This can be explained in terms of the stronger solvation of the products, i.e., the amino acid and tRNA compared to the reactants, water and amino acid-tRNA (10). Ths purpose of this thesis is to describe more fully through thermodynamic quantities the basic reaction occurring in the various alcohol-water solvent systems.

$$\text{Glycine} + \text{Alcohol} \overset{\text{HCl}}{\rightleftharpoons} \text{Glycine Ethyl Ester} + \text{Water}$$

From this work a comparison will be made between the thermodynamic quantities involved in the reaction in a mixed solvent system and those involved in a dilute aqueous solvent system.

EXPERIMENTAL APPROACH

PART I

As was previously mentioned, there are two experimental parts to the problem addressed in this thesis. The first has to do with determining the Keq's for the esterification of glycine and hydrolysis of glycine ethyl ester reactions. These Keq's are determined for these reactions in a dilute aqueous solvent system, a 0.5:1 ethanol-water solvent system, and an equimolar ethanol-water solvent system. The second part of the experimental work pertains to finding the enthalpy change associated with the esterification of glycine.

The general procedure used for finding the Keq's for glycine esterification and the hydrolysis of glycine ethyl ester, was to begin by placing glycine ethyl ester hydrochloride, water, ethanol and HCl or glycine, water, ethanol and HCl into a stoppered volumetric flask, shaking the flask at 20°C until equilibrium was reached and then to sample the mixture. The Keq of the reaction was determined using radioactive labels and a liquid scintillation counter.

This method varies in some ways from the one used by Jencks, et al. (7). They studied the same reaction but only in a dilute aqueous solvent system whereas the reaction was studied in this thesis in not only a dilute aqueous solvent system but also in a 0.5:1 and 1:1 molar ratio ethanol-water solvent system. The method of assay used by Jencks, et al. was the Hestrin alkaline hydroxylamine method (9). Their work was done at 39°C.

The above method of Jencks, et al. and the method reported in this thesis were used to find a Keq value for the esterification of glycine. In order to understand what the value of a Keq means, one needs to know how that Keq is defined. There are many conventions used for defining Keq. Because of this the value given for Keq must be accompanied by an explanation of how it was calculated. Many times, Keq values are defined on the basis of the concentrations of products and reactants that occur in solution instead of the activities. The convention used for defining the value of Keq in this thesis will be:

$$\text{Keq} = \frac{(\text{total moles all species of glycine ethyl ester})(\text{moles water})}{(\text{total moles all species of glycine})(\text{moles ethanol})} \quad .$$

In this convention the Keq value is defined in terms of the moles of all reactants and products including water. This convention was also used by Jencks, et al. In all systems, no correction was made for the different species present for both glycine and ester since in all cases the glycine and ester existed as cations almost exclusively.

The materials used in this research consisted of aminoacetic acid (glycine) from Mallinckrodt Chemical Works, St. Louis, Mo., glycine ethyl ester hydrochloride Lot # 24C-2930 from Sigma Chemical Co., St. Louis, Mo., (1-^{14}C) glycine, batch # 1175-977 and glycine 1-^{14}C ethyl ester hydrochloride, batch # 471-279 both from Research Products International Corp. (made by the Commissariat A L'Energie Atomique), and Trizma Base, Lot # 78C-5000 from Sigma Chemical Co., St. Louis, Mo. All other materials used were of reagent grade.

The purity of the 1-^{14}C glycine and 1-^{14}C glycine ethyl ester hydrochloride was checked using thin layer chromatography (Figure 1). It was noticed that the ester spot (spot 2) had a higher Rf value than the ester spot from the equilibrium reaction mixtures containing an excess of HCl (Figure 2). Using thin layer chromatography, it was thought that the commercial 1-^{14}C ester having been lyophilized during preparation had probably lost HCl and thus became less polar than the ester in the equilibrium mixtures (Figure 3, spots 2 and 3). A similar phenomenon was noticed by McBride-Warren and Mueller where it was found that the pH of a reconstituted acidic solution rises after lyophilization (11).

The experimental procedure consisted of the following steps. First, ca 1,2 and 3N HCl solutions were made and standarized by potentiometric titration using a Radiometer/Copenhagen pH Meter 26 and Tris as the standard base. The solutions for the equilibrium experiments were then set up by first weighing on a Mettler balance a 100 ml volumetric flask which would ultimately contain the reaction of interest. Once this was done, each reactant was added separately to the flask. Between each addition of reactant, the flask and its contents were weighed. In this way, not only the solid glycine or ester hydrochloride were added by weight but also the liquid reactants, the alcohol, water, and the standardized HCl. This way, too, instead of knowing that ca 30 ml of reactant had been added, for example, the weight was taken and thus the moles of each reactant and its mole fraction could be calculated. This made calculation of the Keq simpler. The reactions were set up so that both the esterification and hydrolysis of glycine and the ester, respectively, could be studied and thus a Keq determined for each system.

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Figure 1. An autoradiogram showing the purity of the 1-^{14}C glycine, spot 1 - left, and 1-^{14}C glycine ethyl ester hydrochloride, spot 2 - right. The thin layer plate the autoradiogram was made from was spread with microcrystalline cellulose (Avicel) and the solvent used was butanol, acetic acid, and water, 60:15:25 v/v/v.

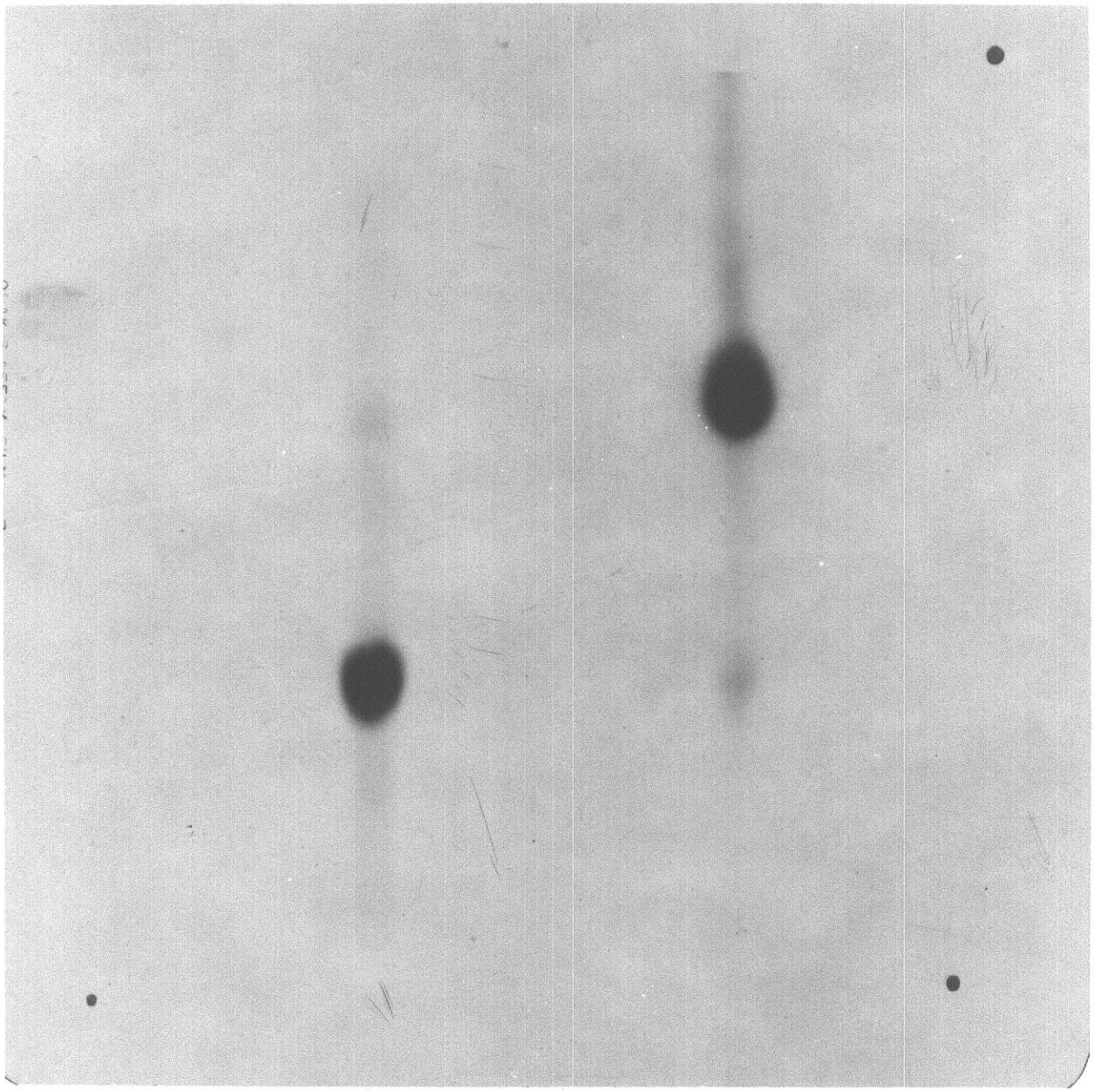

Figure 2. An autoradiogram showing a typical result for a pair of reactions at equilibrium (bottom spots = glycine, top spots = ester). The thin layer plate the autoradiogram was made from was spread with microcrystalline cellulose (Avicel) and the solvent used was butanol, acetic acid and water, 60:15:25 v/v/v.

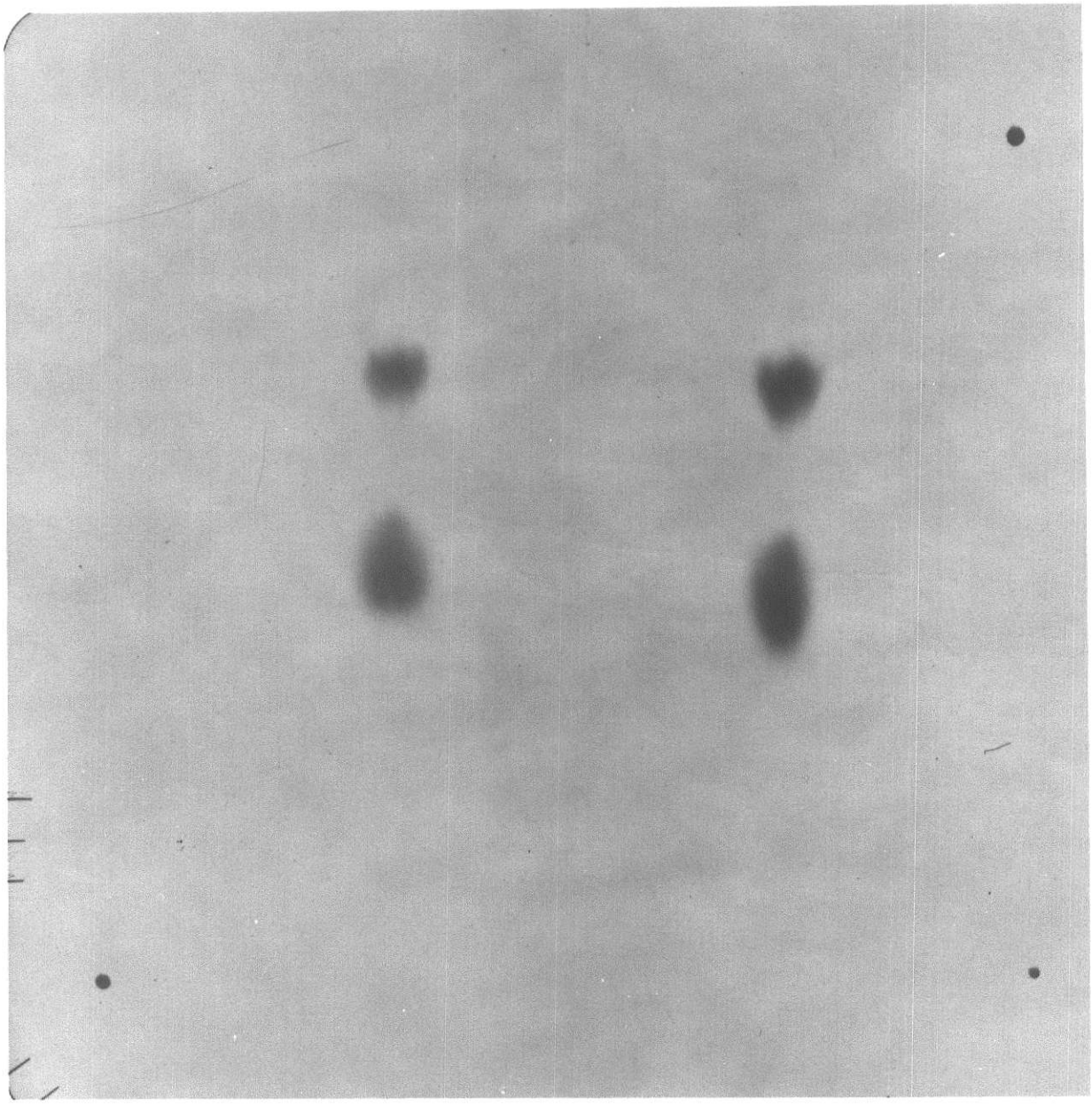

Figure 3. An autoradiogram showing the effect of lyophilization of ester on the mobility of the ester (L→R spot 1 = 1-^{14}C glycine, spot 2 = 1-^{14}C glycine ethyl ester hydrochloride, spot 3 = typical reaction mixture at equilibrium, spot 4 = 1-^{14}C glycine ethyl ester hydrochloride + water, spot 5 = 1-^{14}C glycine ethyl ester hydrochloride + 1N HCl) The thin layer plate the autoradiogram was made from was spread with microcrystalline cellulose (Avicel) and the solvent used was butanol, acetic acid and water, 60:15:25 v/v/v. This figure should be viewed with the page number in the lower right-hand corner of the page.

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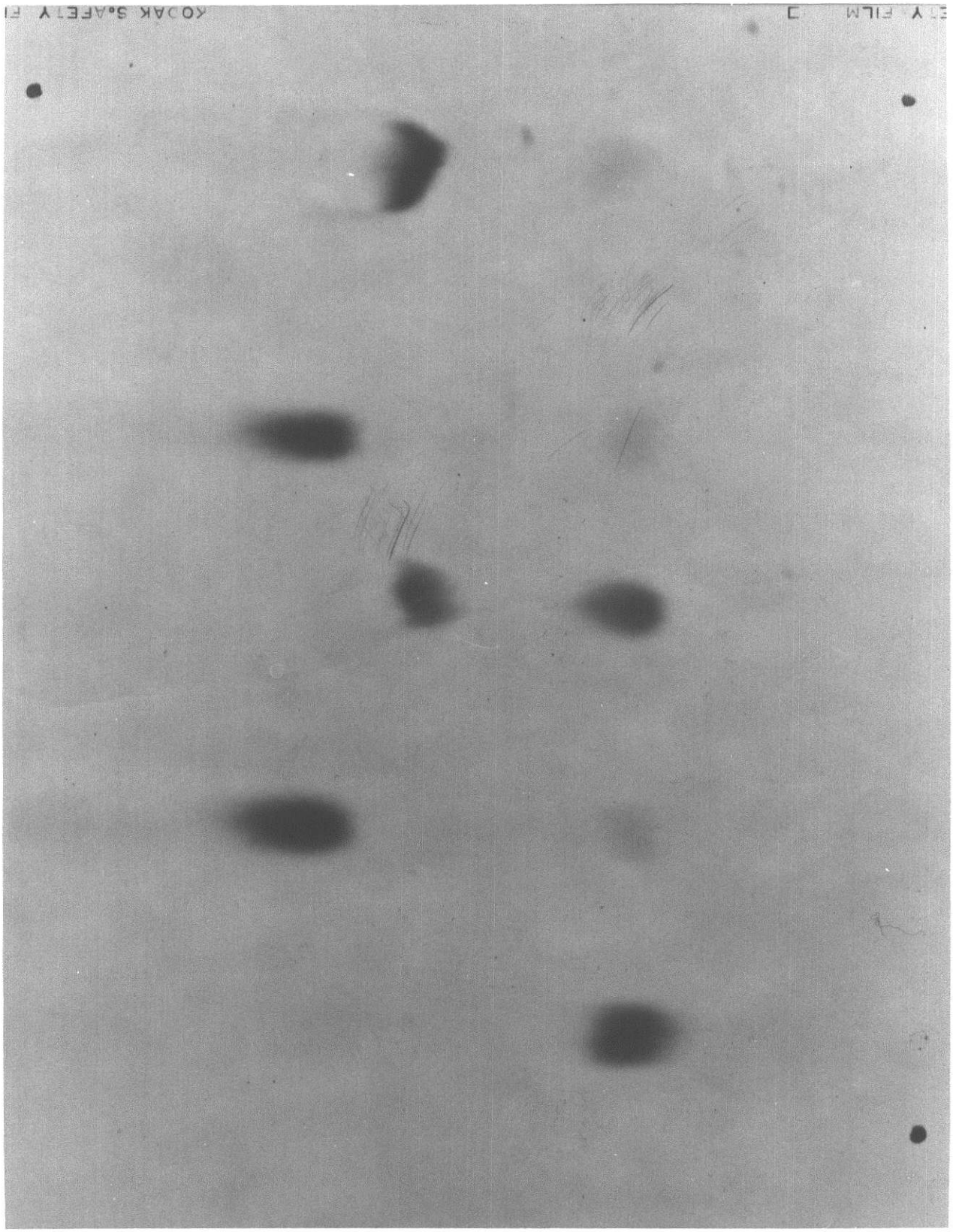

By taking samples of each of the systems, it could be determined when each system had reached equilibrium.

There were actually three different systems of esterification and hydrolysis reactions. One system of six reactions consisted of those reactions in a solution of distilled water with a small amount of alcohol, ca 0:1 molar ratio alcohol-water mixture. Another system of six reactions was set up in a 0.5:1 molar ratio alcohol-water mixture and the third system in a solvent system where the molar ratio of alcohol-water was ca 1:1. The six reactions in each system were set up so the mole fractions of glycine, ester and HCl remained the same. Only the alcohol and water mole fractions changed. The reactions in each system of six reactions were paired. One reaction in each pair began with glycine as a reactant whereas the other began with ester. Each pair of reactions differed from each of the other two pairs in the system of six reactions in the concentration of HCl used. One pair was made with ca 1N HCl, another with ca 2N HCl and the third with ca 3N HCl. The densities of all reaction mixtures were measured.

As mentioned above, work previously done in this laboratory used the micro-Kjeldahl method to analyze for product whereas in this thesis work, radioactivity was used as the method of assay. Samples of the reaction mixtures in the 100 ml volumetric flasks were taken and placed into 3 ml reaction vessels Supelco, Inc.). Into each weighed vessel, ca 1 ml of reaction mixture was placed and weighed. Then each vessel was spiked with enough ^{14}C glycine or ^{14}C glycine ethyl ester hydrochloride by use of an Eppendorf micropipet to give ca 1.0×10^6 CPM. The amount of ^{14}C glycine or ^{14}C ester added changed the molar concentration of the glycine or ester not more than 0.1%. These reaction vessels and the 100 ml volumetric flasks containing the bulk of the nonradioactive reaction mixtures were placed in an Eberbach water-bath shaker at shaker setting 2½ at 20°C. They shook continuously until reaching equilibrium. The set of reactions containing ca 3N HCl as catalyst reached equilibrium in ca 70 days. The set with ca 2N HCl as catalyst reached equilibrium in ca 90 days and the set made with ca 1N HCl as catalyst came to equilibrium in ca 180 days.

After equilibrium had been reached, a 10 µl sample was removed from each reaction vessel of the set and spotted on a 0.75 mm thick microcrystalline cellulose (Avicel) thin layer plate. Two spots were put on each plate. The two spots were the pair of esterification-hydrolysis reactions which were in the same solvent and had the same concentration of HCl for catalyst. Once these spots had dried, the plate was placed in a developing tank. The solvent

used was butanol, acetic acid and water, 60:15:25 v/v/v. After the solvent had risen close to one inch of the top of the plate, the plate was removed from the solvent and allowed to dry overnight. This was to insure that all solvent was removed from the plate prior to the making of the autoradiogram.

The next step was to make an autoradiogram of the plate. This was done by placing a piece of Kodak X-ray film on top of the microcrystalline layer on the plate, and then putting another glass plate on top. The plate and film were then wrapped in aluminum foil and taped with blue Time Tape. This sandwich was then placed in a light-tight folder consisting of black cardboard lined with aluminum foil. This allowed the film to be taken out of the darkroom without danger of exposure to light. The autoradiogram was allowed to develop for ca 24 hours. The film was then taken off the plate, in the darkroom, and developed in Kodak D-11 developer. Each developed film exhibited four spots (Figure 2). Two spots were from one reaction in the pair of reactions and the other two were from the other reaction in the pair. The top spot was from the glycine ethyl ester and the lower spot from the glycine in the reaction mixture. The identity of the spots was found by chromatographing nonradioactive glycine and ester under identical conditions but using ninhydrin rather than autoradiography to locate each of these solutes. NOTE: Ninhydrin was not used with the radioactive components since the ^{14}C would have been lost as radioactive formaldehyde--a gas at room temperature.

Once the position of the spots had been determined with the autoradiogram, they could be isolated from the thin layer plate. This was done by placing the plate on top of the film and shining light through both of them using a light box. The spots were scored around on the plate using a plastic template. Each spot was removed from the thin layer plate using a Kensington scintered glass sample collection tube. One end of the tube was fitted into a rubber hose that was attached to an aspirator. The other end was used to suck up the spot on the plate. Once all the spot was removed from the plate, the aspirator was turned off and the tube pulled from the hose, the contents of the tube were shaken into a glass scintillation vial. To the vial was added 5 ml of Research Products International Corp. 3a70B scintillation fluid. The vial was then counted in a Beckman LS-200B scintillation counter. The instrument settings were: Gain = 2.5, Window = ^{14}C, Low Sample Reject (CPM) = off, Preset Error = 0.2%, Background = 10.0 CPM. All samples were counted for one minute.

The numbers from the counting were obtained for each spot on each of the plates. In order to determine how accurately these spots could be isolated, an experiment was done where 1 to 10 µl of ^{14}C ester or glycine was spotted on a thin layer plate and isolated. From the results, it was determined that the mean percent recovery of applied radioactivity was ca 93% over the range of ca 10,000 to 100,000 CPM and that the amount of radioactivity isolated in each spot increased in a linear fashion from 1 to 10 µl. The ratio of CPM glycine ethyl ester to CPM glycine was obtained for each reaction in a set of six reactions. From this ratio, the Keq for the pair of reactions could be determined. This calculation was done with two computer programs (Appendix). The first program was used to calculate the number of moles and mole fractions of glycine, glycine ethyl ester hydrochloride, HCl, water and ethanol for each system. The second computer program used the ratio of glycine ethyl ester to glycine from each pair of reactions to calculate the Keq associated with them. The data used to calculate the Keq values can be found in the Appendix. Here is an example of how a ratio and a Keq value are calculated.

REACTANT	CPM	CPM ESTER/GLYCINE
Glycine Ethyl Ester HCl	23674.4	
		0.9596
Glycine	24670.4	

Initial mmoles of:

Glycine = 3.9856

Glycine Ethyl Ester = 0.0000

Water = 514.3660

Ethanol = 500.2300

CALCULATION OF:

1) Final mmoles of Glycine:

if Glycine + Ester = 3.9856 (initial mmoles of Glycine)

if Ester/Glycine = 0.9596

then Glycine = 2.0339 mmoles

and Ester 1.9517 mmoles

2) Final mmoles of Water:

Final mmoles of Water = Initial mmoles of Water + Final mmoles of Ester

516.3177 = 514.3660 + 1.9517

3) Final mmoles of Ethanol:

Final mmoles of Ethanol = Initial mmoles Ethanol - Final mmoles of Ester

498.2783 = 500.2300 - 1.9517

To Calculate the observed Keq of esterification:

$$\text{Observed Keq of Esterification} = \frac{(\text{mmoles Water})(\text{mmoles Glycine Ethyl Ester})}{(\text{mmoles Glycine})(\text{mmoles Ethanol})}$$

$$= \frac{(516.3177)(1.9517)}{(2.0339)(498.2783)}$$

$$= 0.9943$$

RESULTS

PART I

Once all the systems had come to equilibrium, six observed Keq values were obtained for each of the three different solvent systems using the methods described in the Experimental Approach section of this thesis. These values were plotted versus mole fraction HCl (Figures 4,5,6). The effect of the different concentrations of HCl can be seen on the plots. The points furthest from the ordinate are from the reactions in which the added ca 3N HCl was the catalyst. The middle points are from the reactions where ca 2N HCl was used as the catalyst and the points nearest the ordinate are from the reactions where ca 1N HCl was the catalyst. The method of least squares was used to calculate the best line for each solvent system and to determine the corresponding Keq at zero mole fraction of HCl. The extrapolated values of Keq that were obtained were: for the dilute aqueous solvent system, of ethanol-water, Keq = 4.6 ± 0.5; for the 0.5:1 molar ratio of ethanol-water, Keq = 1.0 ± 0.07; and for the equimolar ethanol-water solvent system, Keq = 0.8 ± 0.09. The standard deviation shown for these observed Keq values are the standard deviations of the intercepts of the least squares lines (12). The ΔG° values calculated for these systems were found to be: for the dilute aqueous solvent system, $\Delta G^{\circ} = -0.9$ kcal/mole; for the 0.5:1 molar ratio ethanol-water solvent system, $\Delta G^{\circ} = 0.0$ kcal/mole; and for the equimolar ethanol-water solvent system, $\Delta G^{\circ} = +0.1$ kcal/mole (Appendix).

Figure 4. Graph of observed Keq vs mole fraction HCl for the 0:1 molar ratio ethanol-water solvent system

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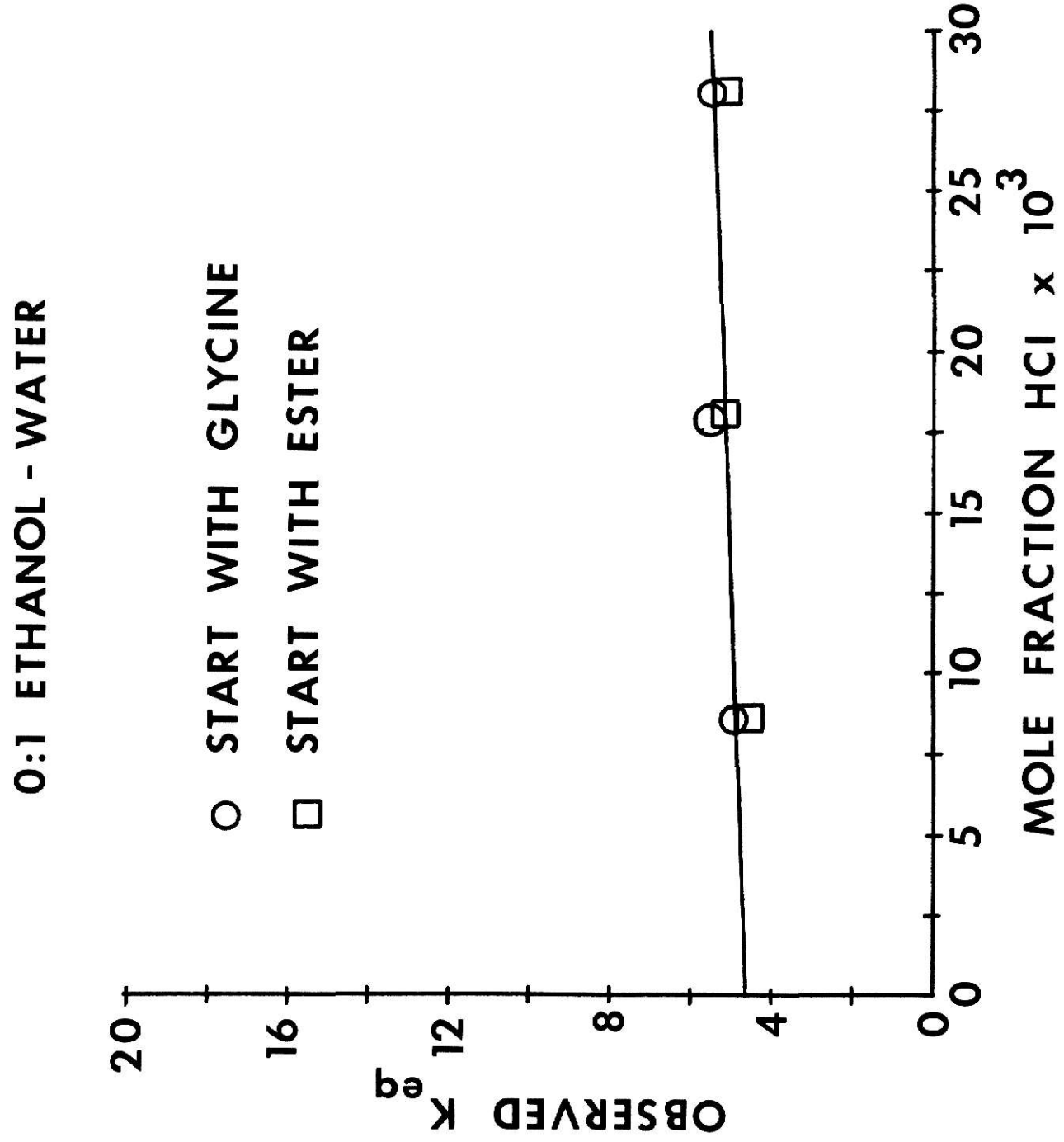

0:1 ETHANOL - WATER
○ START WITH GLYCINE
□ START WITH ESTER
OBSERVED K_{eq}
20
16
12
8
4
0
0
5
10
15
20
25
30
MOLE FRACTION HCl x 10^3

Figure 5. Graph of observed Keq vs mole fraction HCl for the 0.5:1 molar ratio ethanol-water solvent system

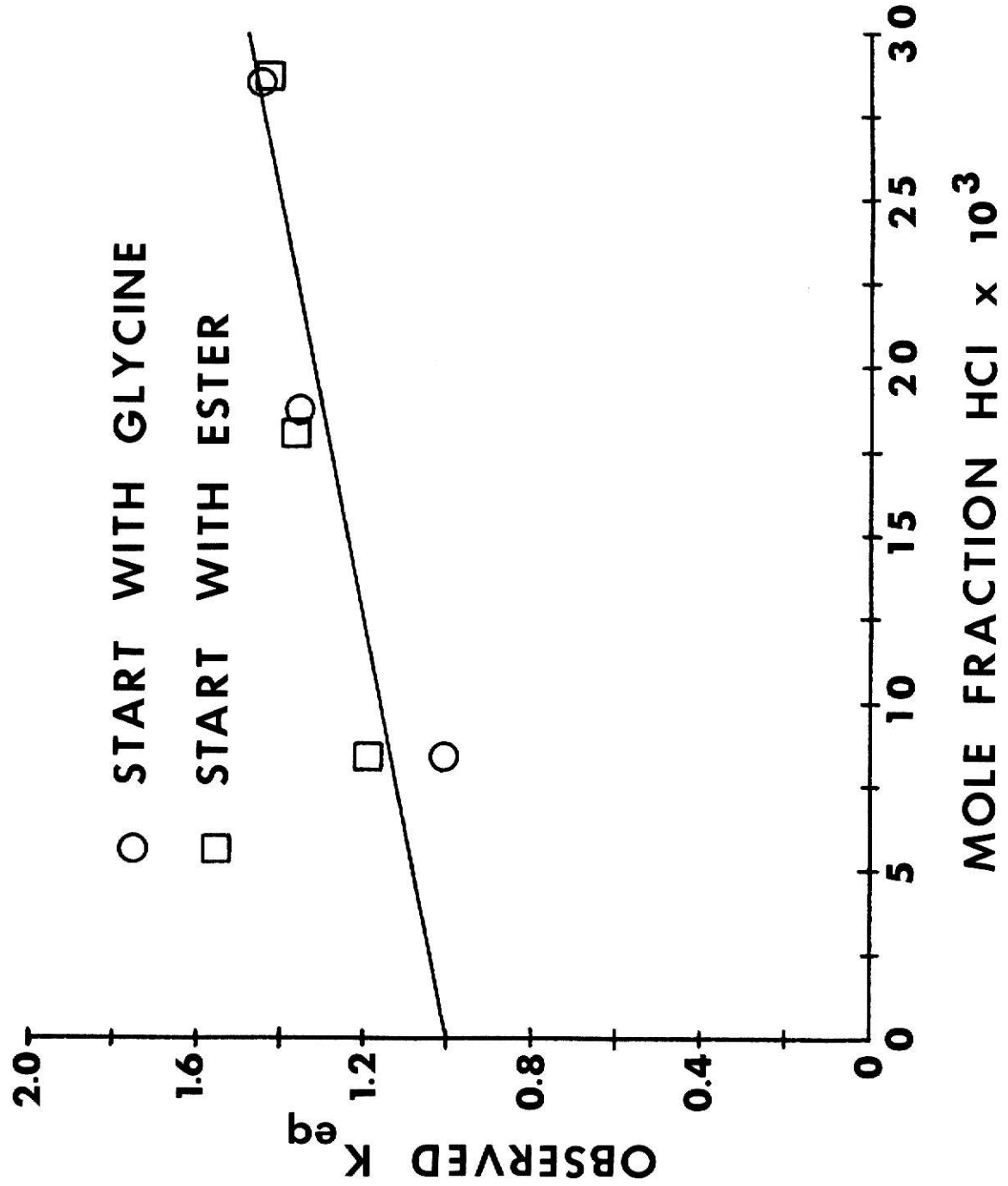
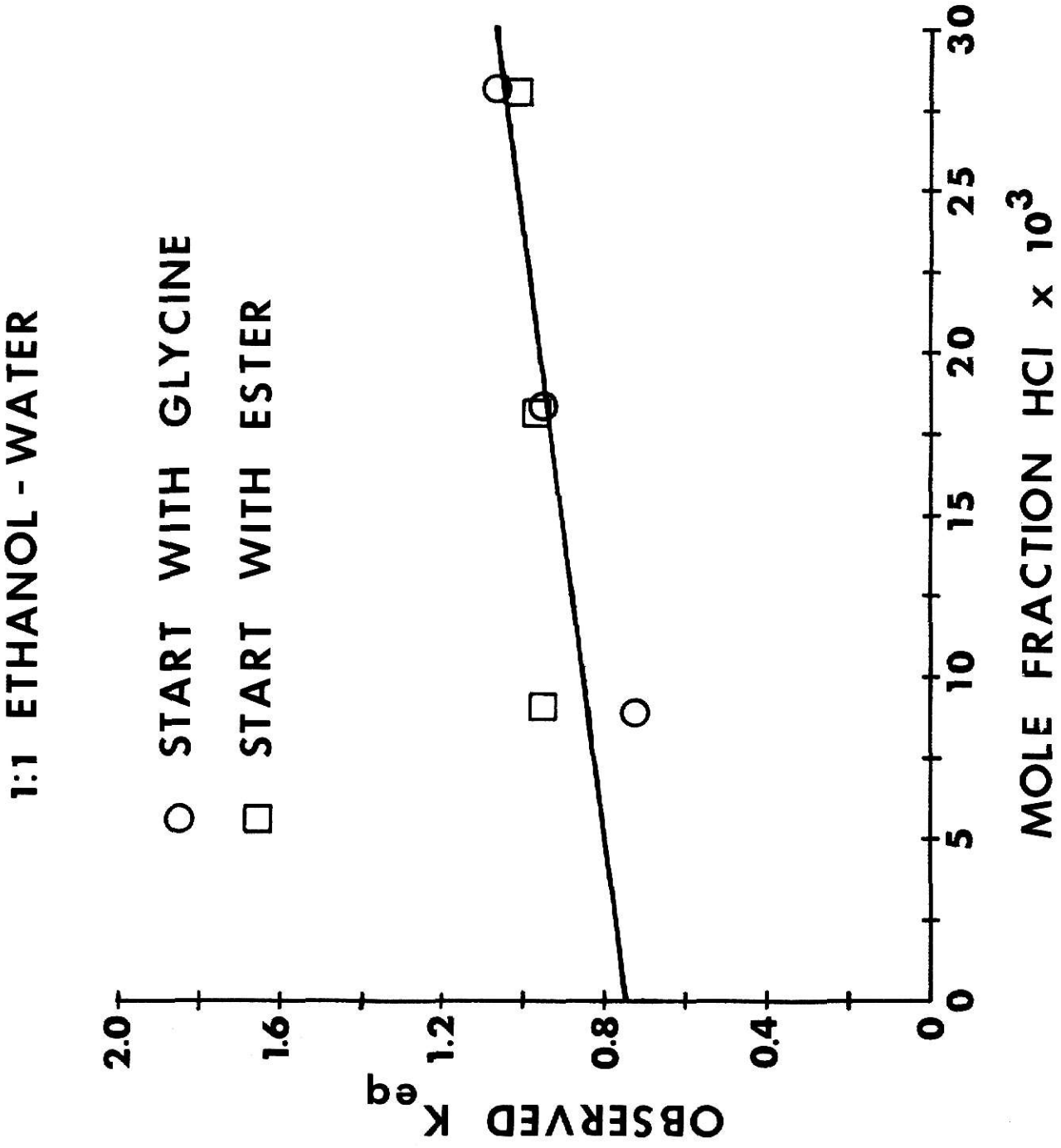

0.5:1 ETHANOL - WATER
○ START WITH GLYCINE
□ START WITH ESTER
OBSERVED K_{eq}
2.0
1.6
1.2
0.8
0.4
0
0
5
10
15
20
25
30
MOLE FRACTION HCl x 10^3

Figure 6. Graph of observed Keq vs mole fraction HCl for the 1:1 molar ratio ethanol-water solvent system


1:1 ETHANOL - WATER
○ START WITH GLYCINE
□ START WITH ESTER
OBSERVED K_{eq}
2.0
1.6
1.2
0.8
0.4
0
0
5
10
15
20
25
30
MOLE FRACTION HCl x 10^3

INTRODUCTION

THE DETERMINATION OF ΔH FOR THE ESTERIFICATION OF GLYCINE WITH ETHANOL

In Part I of this thesis, the Keq's for the esterification of glycine were found in three solvent systems. From them the corresponding ΔG° values can be calculated. The calculation of ΔG° for each of the three systems of interest is only one parameter that is needed for describing this esterification reaction thermodynamically. The corresponding changes in enthalpy and entropy are quantities that also need to be determined if the thermodynamic description of this reaction is to be complete. Therefore, if the ΔH can be experimentally determined, using the equation: $\Delta G = \Delta H - T\Delta S$, ΔS may be calculated and thus a thermodynamic description of this esterification reaction for glycine ethyl ester may be completed. In this thesis work, only the ΔH for the system containing 1:1 molar ratio ethanol-water solvent was determined experimentally. The ΔH for the dilute aqueous solvent system can be obtained from the literature and a comparison made between the thermodynamic quantities obtained for the esterification of glycine in both these solvent systems.

EXPERIMENTAL APPROACH

PART II

The chemicals used for this research were the same as those in Part I of this thesis work except that no radioactivity was used. Sodium hydroxide was also used in this part of the research work and was purchased from Mallinckrodt, Inc., St. Louis, Mo. The measurement of the ΔH value was done using a heat burst batch calorimeter built by G. L. Dohm and R. K. Burkhard. The glass reaction cells were from Beckman Instrument Company, the power supply used was a Keithley 150B Microvolt Ammeter and Power Supply, and the chart recorder was a Leeds and Northrup Company Speedomax G.

In order to find the ΔH for the esterification of glycine in a 1:1 molar ratio ethanol-water solvent system

$$NH_3^+CH_2COOH + CH_3CH_2OH \longrightarrow NH_3^+CH_2COOCH_2CH_3 + H_2O$$

the ΔH values for four component reactions had to be found and their ΔH values added together to give the overall ΔH value for the glycine esterification reaction. All reactions were done in the 1:1 molar ratio ethanol-water solvent system. The four reactions were:

1) $NH_3^+CH_2COOCH_2CH_3 + 2\ OH^- \longrightarrow NH_2CH_2COO^- + CH_3CH_2OH + H_2O$

2) $NH_2CH_2COO^- + H_2O \longrightarrow NH_3^+CH_2COO^- + OH^-$

3) $NH_3^+CH_2COO^- + H^+ \longrightarrow NH_3^+CH_2COOH$

4) $H_2O \longrightarrow H^+ + OH^-$

These four reactions give the overall hydrolysis reaction of glycine ethyl ester. To get the ΔH value for the esterification reaction of glycine, the sign on the ΔH value determined from the four reactions above must be changed.

To find the ΔH's associated with each of the four reactions listed above, each reaction had to be set up in the microcalorimeter so that a spontaneous reaction would take place upon mixing. With this instrument, the heat given off by mixing the two reactants could be measured. The

experimental result of one of these reaction runs in the microcalorimeter can be seen in Figure 7. The area under this curve was measured, and by knowing this, the calibration constant for the microcalorimeter, and the number of moles of the limited reactant in the reaction mixture, the ΔH could be computed as cal/mole.

Experimentally, the determination of a value of ΔH for one of the component reactions was done in the following way. As an example reaction, glycine and sodium hydroxide will be used. To begin, the 1:1 molar ratio ethanol-water solvent was mixed by weight. Then, enough glycine was added to this solvent to make a 0.02M glycine solution in 1:1 molar ratio ethanol-water solvent. The 0.1N NaOH solution was made by adding the appropriate amount of NaOH to the 1:1 molar ratio ethanol-water solvent.

The reaction was set up in the microcalorimeter in the following manner. A cell was loaded by microburet on one side with 5 ml 0.02M glycine. The other side of the cell was loaded with 5 ml 0.1N NaOH. The second cell had its compartments loaded with 5 ml 0.02M glycine and 5 ml 1:1 molar ratio ethanol-water solvent. This cell was used to subtract the heat produced by the dilution of the ester with the ethanol-water solvent in the glycine-NaOH reaction. An analogous experiment was done with NaOH and 1:1 molar ratio ethanol-water to determine the heat of dilution of the NaOH so that it could also be subtracted from the reaction of interest.

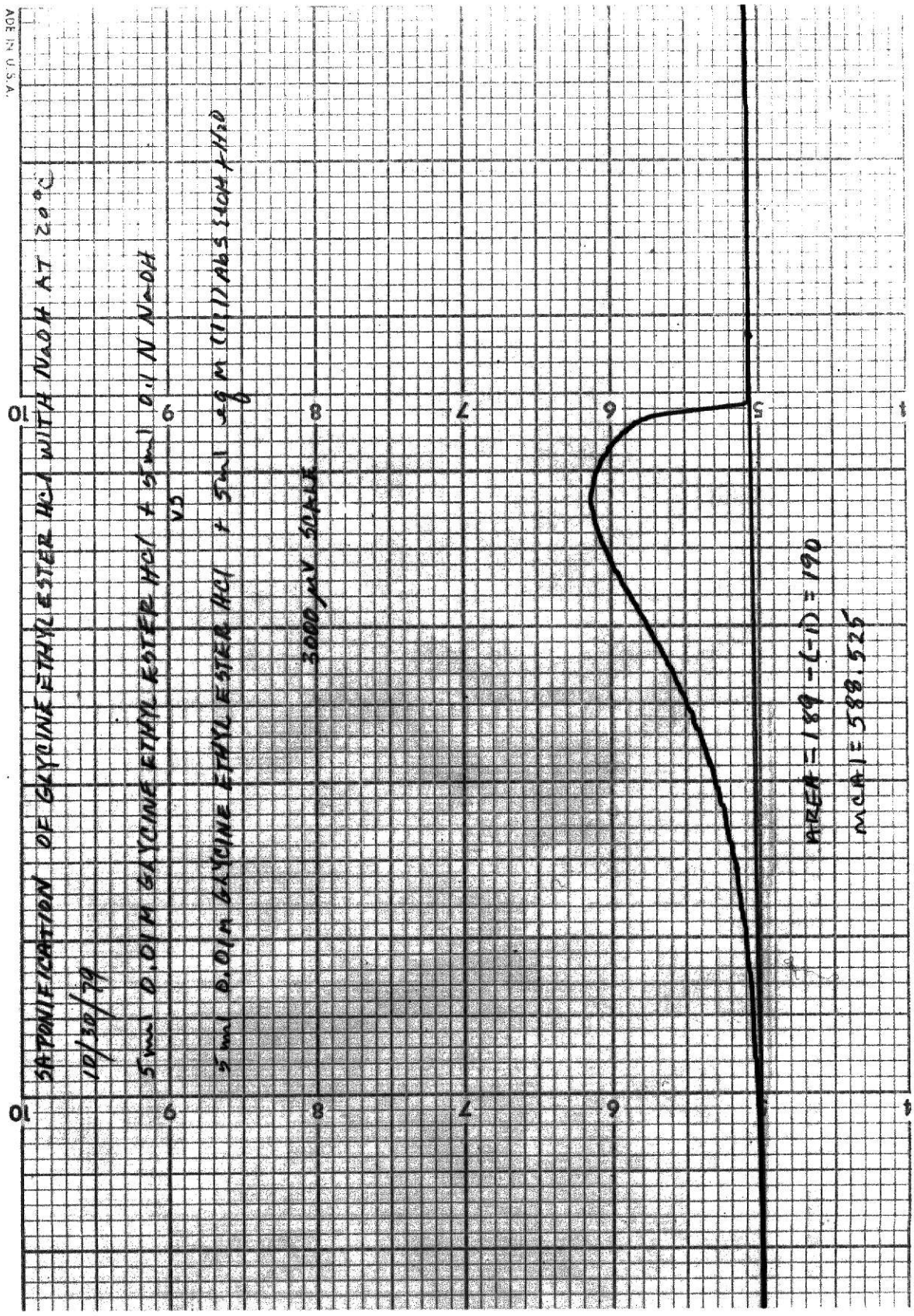
From the area under the cooling curve obtained for the reaction of 0.02M glycine and 0.1N NaOH, a ΔH was calculated for this reaction. An example of how this was done can be seen in the following set of calculations.

Area Under Curve Using a Keuffel and Esser Planimeter: 186 units
Voltage Setting on the Power Supply: 1000 μV
Instrument Calibration Constant: 0.0010325
Heat Evolved in mcal = (Area of Peak)(Microvolt Setting)(Calibration Constant)
= (186)(1000)(0.0010325)
= 192 mcal

ΔH CALCULATION:

Glycine Used: 1.0190×10^{-1} mmoles
ΔH = (mcal)/(mmoles glycine)
= $(192)/(1.0190 \times 10^{-1})$
= 1.88×10^{3} cal/mole

Figure 7. A typical cooling curve obtained from a calorimetry run.


SAPONIFICATION OF GLYCINE ETHYL ESTER HCl WITH NaOH AT 20°C
10/30/79
5 ml 0.01M GLYCINE ETHYL ESTER HCl + 2 ml 0.1 N NaOH
VS
2 ml 0.01M GLYCINE ETHYL ESTER HCl + 5 ml 0.1 N NaOH
3000 mV SCALE
AREA = 189 - (-1) = 190
mCM = 588.525
MADE IN U.S.A.

By repeating the above experiment three times, the mean and standard deviation for the three runs were determined. By subtracting the ΔH associated with the heat of dilution of NaOH, the ΔH value for the reaction of glycine and NaOH could be determined.

The ΔH values for the other reactions mentioned above were found in the same way. One thing must be pointed out, however. All solutions were prepared for running in the calorimeter in the same way as in the example above except for reaction #4, the Heat of Neutralization of HCl with NaOH. For this reaction, since the calorimeter is sensitive to dissolved carbon dioxide, the water used in making the reactant solutions was boiled for 30 minutes and cooled under a stream of nitrogen. The 1:1 molar ratio ethanol-water solution was made from the boiled water and NaCl was added to it. The addition of NaCl eliminated the error incurred by the glass binding chloride ions (13). Therefore, the ca 0.01N HCl solution and the 0.02N NaOH solution were made with 0.0002M NaCl in 1:1 molar ratio ethanol-water solvent.

After all corrections were made for heats of dilution, the ca 0.01N HCl solution standardized with Tris, and the ΔH's for the four component reactions calculated, an overall ΔH was found for the esterification of glycine giving ΔH the opposite sign than was computed (since the ΔH of esterification was desired). ΔH for the esterification of glycine was then found. A ΔS value for the reaction in 1:1 molar ratio ethanol-water was then calculated from this value (Appendix). This was done using the equation $\Delta G = \Delta H - T\Delta S$.

RESULTS

PART II

The ΔH value obtained from the component reactions for the esterification of glycine in an equimolar ethanol-water solvent system was ΔH of esterification = $+1.7 \pm 0.65$ kcal/mole or $+7.2 \pm 2.5$ kJ/mole. The calculated ΔS value was found to be +5.4 cal/mole-^{o}K or $+2.3 \times 10^{1}$ J/mole-^{o}K.

DISCUSSION

In Part I of this thesis, Keq values for the esterification of glycine by ethanol in three different solvent systems were determined. They were: for the 0:1 molar ratio ethanol-water solvent system, Keq = 4.6 $\pm$ 0.5; for the 0.5:1 molar ratio ethanol-water solvent system, Keq = 1.0 $\pm$ 0.07; and for the 1:1 molar ratio ethanol-water solvent system, Keq = 0.8 $\pm$ 0.09. These Keq's suggest that the relative amount of ester being formed becomes larger as the amount of water is increased in the solvent system. However, the Keq for a reaction is a constant that, in theory, for ideal systems, does not change even if the reaction undergoes a change in solvent system. The Keq's reported above are based on mole quantities, not activities. The discrepency observed between them is probably due to their individual differences from ideality. Further experimentation is needed to determine the exact cause of these differences.

Jencks, et al. also calculated a value for the Keq for this esterification reaction of glycine in a dilute aqueous system. Their Keq value (observed) was 2.3 (7). The discrepency that is seen between the value in this thesis of Keq = 4.6 and the Jencks, et al. observed value, Keq = 2.3, can be explained as due to a number of reasons.

First, experimental error may have been significant enough to have caused such a large discrepency. In the Hestrin method of assay (9) used by Jencks, et al., only the ester was assayed for. Since the amount of ester present in the reaction mixture was very much less than the amount of glycine, a large amount of error may have resulted. In the ^{14}C method of assay used in this thesis, the amount of error incurred in the Keq and ΔG^{o} results was thought to be much less. This was because even though the ester may have been in much smaller amounts than the glycine in some reaction mixtures, both ester and glycine were assayed and the ratio of the CPM results for both of these was always used in calculations rather than the absolute amount of only one component as was done by Jencks, et al.

Another reason for the discrepency between the apparent Keq values could be the way the values were calculated. Mathematically, Jencks, et al. used concentrations to calculate the Keq; here moles of reactant and product were used. Although there is really no difference between these methods of calculation themselves, neither calculation gives a Keq based on activities. It

may be that the difference between these two values arises from this simplification.

A final reason for the differences between the apparent Keq values found in this thesis work and by Jencks, et al., could be due to the slightly different conditions used for the reaction. Jencks, et al. did their studies at 39°C whereas in this thesis, the reaction temperature was 20°C.

From the apparent Keq values found here and by Jencks, et al., ΔG° values may be calculated. As was stated previously, the ΔG° value for the 0:1 molar ratio ethanol-water solvent system is -8.9×10^{-1} kcal/mole; for the 0.5:1 molar ratio ethanol-water solvent system, $\Delta G^{\circ} = 0.0$ kcal/mole; and for the 1:1 molar ratio ethanol-water solvent system, $\Delta G^{\circ} = +1.3 \times 10^{-1}$ kcal/mole. The $\Delta G^{\circ\prime}$ of hydrolysis value calculated for the dilute aqueous system was -7.1 kcal/mole (Appendix). The $\Delta G^{\circ\prime}$ esterification value calculated using the Jencks, et al. Keq value of 2.3 is -516 cal/mole and the $\Delta G^{\circ\prime}$ of hydrolysis calculated by Jencks, et al. was -8400 cal/mole (7). From these ΔG° and $\Delta G^{\circ\prime}$ of hydrolysis results from this thesis work and from Jencks, et al., it would appear that the dilute aqueous solvent system describes the most favorable situation for glycine esterification. The 0.5:1 molar ratio ethanol-water solvent system seems to indicate a situation where the amount of ester produced is equal to the amount of glycine present. And, the 1:1 molar ratio ethanol-water solvent system, not having a favorable ΔG° value, does not appear to depict a situation that is favorable like that described by the dilute aqueous system. However, if the ratios of ester to glycine are looked at more closely, the most ester is produced in the 1:1 molar ratio ethanol-water solvent system (Appendix). In fact, the dilute aqueous solvent system has the least amount of ester formed of all the systems even though its ΔG° is the most favorable. Perhaps the differences between the Keq-ΔG° and CPM ratio results can be explained by the fact that the Keq and ΔG° value calculations were based on mole quantities and not activities and that the CPM ratios reflect the Law of Mass Action. Since chemically it also makes more sense that the esterification reaction would go more favorably in a more nonpolar solution it would also appear that the 1:1 molar ratio ethanol-water solvent system favors esterification more than the dilute aqueous solvent system.

If the CPM ratio results of this thesis research and thus the Law of Mass Action can be used to infer something about an in vivo system, they

might be used to describe the solvent conditions needed to make not only esterification of the amino acid to its specific tRNA molecule more favorable but also the whole process of protein synthesis more favorable. These results may be indicating that to make these processes more favorable in a cell, the esterification and/or protein synthesis may be occurring in a pocket or area of the cell that is isolated from the main cell solvent, water.

In Part II of this thesis, using microcalorimetry the ΔH was found for the esterification of glycine in an equimolar ethanol-water solvent system. The value obtained was $+1.7 \pm 0.65$ kcal/mole. A ΔH was also calculated for this esterification reaction in a dilute aqueous solvent system. The value obtained was $H = +1.1 \pm 0.43$ kcal/mole. One could also use the van't Hoff equation to determine the ΔH for this esterification reaction. If this is done for the dilute aqueous solvent system with the Jencks, et al. observed Keq value at 39°C and the observed Keq value obtained in this thesis at 20°C for this solvent system, the ΔH for the esterification of glycine is -6.6 kcal/mole. This value is much different from the ΔH of esterification found in this thesis. This shows that the better of the two ways to get ΔH values for any particular system is to determine them calorimetrically. It also shows that the difference between these two Keq's does not appear to be due to temperature differences but rather to other factors such as methodology.

As was mentioned earlier, in order to get the ΔH value for the esterification reaction in the 1:1 molar ratio ethanol-water solvent system, the ΔH's of the component reactions must first be obtained and the sign changed to reflect the enthalpy change for the reaction as written. The reactions and their ΔH's are as follows.

	ΔH cal/mole in 1:1 EtOH-H_2O
1) $^{+}NH_3CH_2COOCH_2CH_3 + 2\ OH^- \longrightarrow$	
$NH_2CH_2COO^- + CH_3CH_2OH + H_2O$	$-1.1 \times 10^4 \pm 6.0 \times 10^2$
2) $NH_2CH_2COO^- + H_2O \longrightarrow$	
$^{+}NH_3CH_2COO^- + OH^-$	$+1.1 \times 10^3 \pm 1.7 \times 10^2$

	ΔH cal/mole in 1:1 EtOH-H_2O
3) $^{+}NH_3CH_2COO^- + H^+ \longrightarrow {}^{+}NH_3CH_2COOH$	$-8.4 \times 10^2 \pm 1.0 \times 10^2$
4) $H_2O \longrightarrow H^+ + OH^-$	$+9.7 \times 10^3 \pm 1.3 \times 10^2$

NET:

$$^{+}NH_3CH_2COOCH_2CH_3 + H_2O \longrightarrow {}^{+}NH_3CH_2COOH + CH_3CH_2OH \qquad \Delta H = -1.7 \times 10^3 \pm 6.5 \times 10^2$$

Therefore, the ΔH for the hydrolysis of glycine ethyl ester in a 1:1 molar ratio ethanol-water solvent system is -1.7 ± 0.65 kcal/mole or -7.2 ± 2.5 kJ/mole.

In order to find the ΔH for the esterification reaction of glycine in a dilute aqueous solvent system, the same basic procedure can be used. However, reactions 2 and 3 used in the overall ester hydrolysis reaction in this dilute aqueous system were not the same as reactions 2 and 3 used for this same reaction in the equimolar solvent system. This is because while reactions analogous to reactions 2 and 3 for the dilute aqueous system could have been studied in the calorimeter one might encounter problems regarding stoichiometry. For example, to determine the enthalpy change for the protonation of the amino group of glycine one should make sure this group is completely protonated but without concomitant protonation of the carboxyl group. This can be avoided by performing the reverse of reaction 2 for the alcohol-water system. The sign of the ΔH determined from these experimental runs was then changed to reflect the enthalpy change for the reaction as written. The reactions used to find the ΔH of esterification in the dilute aqueous solvent system and their corresponding ΔH values are as follows.

	ΔH cal/mole in 0:1 EtOH-H_2O
1) $^{+}NH_3CH_2COOCH_2CH_3 + 2\ OH^- \longrightarrow NH_2CH_2COO^- + CH_3CH_2OH + H_2O$	$-1.7 \times 10^4 \pm 4.3 \times 10^2$

	ΔH cal/mole in 0:1 EtOH-H_2O	
2) $NH_2CH_2COO^- + H^+ \longrightarrow$ $^+NH_3CH_2COO^-$	$-1.1 \times 10^4 \pm 0.05$	(14)
3) $^+NH_3CH_2COO^- + H^+ \longrightarrow$ $^+NH_3CH_2COOH$	$-9.8 \times 10^2 \pm 0.05$	(15)
4) $2\ H_2O \longrightarrow 2\ H^+ + 2\ OH^-$	$+1.4 \times 10^4 \pm 0.04$	(16)

NET:

$$^+NH_3CH_2COOCH_2CH_3 + H_2O \longrightarrow {}^+NH_3CH_2COOH + CH_3CH_2OH \qquad \Delta H = -1.1 \times 10^3 \pm 4.3 \times 10^2$$

Therefore, the H for the hydrolysis of the ester in a dilute aqueous system is $\Delta H = -1.1 \pm 0.4$ kcal/mole or $-4.8 \pm$ kJ/mole. The ΔH's for reactions 2, 3 and 4 used in determining the ΔH of esterification in the dilute aqueous system were obtained from the literature as noted. The ΔH for reaction 1 was found using the microcalorimeter. Reaction 4 was also done calorimetrically in this thesis research although the reference value is shown above. The value obtained in this research, $+1.4 \times 10^4 \pm 4.3 \times 10^2$ cal/mole, agreed with the literature value. This suggested that the low ΔH value found for the ionization of water in the equimolar ethanol-water solvent system, -9.7 ± 0.13 kcal/mole, was not due to experimental error.

As can be seen, the ΔH of esterification found for the equimolar ethanol-water solvent system is virtually the same as the dilute aqueous system's ΔH of esterification. What this indicates is that in order for glycine to be esterified in either the 0:1 or the 1:1 molar ratio ethanol-water solvent system, heat must be added to the system. Actually, since these ΔH of esterification values are small, it means that the enthalpy change really contributes little to the favorability of the reaction under these different solvent conditions. By examining the ΔG° values and the ΔS values a better idea of which solvent condition is more favorable for this glycine esterification reaction should be able to be obtained.

The calculated ΔG° of esterification for the 0:1 molar ratio ethanol-water solvent system was -8.9×10^{-1} kcal/mole; for the 0.5:1 molar ratio ethanol-water solvent system, $\Delta G^{\circ} = 0.0$ kcal/mole; and for the 1:1 molar ratio ethanol-water solvent system, $\Delta G^{\circ} = +1.3 \times 10^{-1}$ kcal/mole. From these it appears that the ΔG° of esterification for the dilute aqueous solvent system is the more favorable. However, since the ΔG° of esterification calculations were based on the Keq values calculated from mole quantities and not activities, the differences in the ΔG° of esterification values could be misleading. Because calculation of the corresponding entropy changes entails using these ΔG° values, the results from such calculations could also be misleading. However, if one uses these free energy changes and the corresponding enthalpy changes the entropy changes for the esterification of glycine in two of these systems would be +7.0 cal/mole-$^{\circ}$K and +5.4 cal/mole-$^{\circ}$K for the 0:1 and 1:1 molar ratio ethanol-water solvent systems, respectively. It should be emphasized, however, that these values as well as the free energy changes are not based on activities and could be misleading. As can be seen, since the ΔS values are basically the same magnitude and calculated from the ΔG° and ΔH values found previously, no certain final conclusion can be reached as to which of the solvent systems provides the more favorable conditions for the glycine esterification reaction.

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APPENDIX

I. Computer program for computing the number of moles and mole fractions of glycine, glycine ethyl ester, HCl, ethanol, and water using PL/C

```
MOLES:  PROCEDURE OPTIONS(MAIN);
        DECLARE  GLYORE FLOAT DEC, /*G Glycine or Ester Weighed*/
                 MGLYORE FLOAT DEC, /*Mw Glycine or Ester*/
                 GHCL FLOAT DEC, /*G HCl Weighed*/
                 NHCL FLOAT DEC, /*Normality of HCl*/
                 PHCL FLOAT DEC, /*Density of HCl*/
                 MHCL FLOAT DEC, /*Mw HCl*/
                 GHCL1 FLOAT DEC, /*G HCl in HCl Solution*/
                 GETOH FLOAT DEC, /*G EtOH*/
                 GH2O FLOAT DEC, /*G H2O Weighed*/
                 GH2O1 FLOAT DEC, /*G H2O in HCl Solution*/
                 MH2O FLOAT DEC, /*Mw H2O*/
                 COUNT FIXED DEC, /*No. of Reactions*/
                 PROD FLOAT DEC, /*mmoles Glycine or Ester*/
                 PROD1 FLOAT DEC, /*mmoles HCl*/
                 PROD2 FLOAT DEC, /*mmoles Ethanol*/
                 PROD3 FLOAT DEC, /*mmoles H2O in HCl Solution + Additional
                                   H2O*/
                 SUM FLOAT DEC, /*Total Moles in Reaction*/
                 SUM1 FLOAT DEC, /*1/Sum*/
                 MMOLE FLOAT DEC, /*Mole Fraction Glycine or Ester*/
                 MMOLE1 FLOAT DEC, /*Mole Fraction HCl*/
                 MMOLE2 FLOAT DEC, /*Mole Fraction Ethanol*/
                 MMOLE3 FLOAT DEC, /*Mole Fraction H2O*/
                 TWT FLOAT DEC, /*Total Weight of Reaction*/
                 SYSTEM FIXED DEC,
                 RXN# FIXED DEC;
        PROD=0;
        PROD1=0;
        PROD2=0;
        PROD3=0;
        SUM=0;
        SUM1=0;
        MMOLE=0;
        MMOLE1=0;
        MMOLE2=0;
        MMOLE3=0;
        TWT=0;
        GET LIST (COUNT);
        PROD_LOOP:  DO WHILE (COUNT > 0);
/*COMPUTE THE MOLES GLYCINE OR ESTER*/
        GET LIST (GLYORE, MGLYORE);
        PROD=GLYORE*(MGLYORE**-1)*1E+3;
/*COMPUTE THE MOLES HCL*/
        GET LIST (GHCL, NHCL, PHCL, MHCL);
        GHCL1=NHCL*1E-3*MHCL*GHCL*(PHCL**-1);
        PROD1=GHCL1*(MHCL**-1)*1E+3;
/*COMPUTE THE MOLES ETOH*/
        GET LIST (GETOH, METOH);
```

```
        PROD2=GETOH*(METOH**-1)*1E+3;
/*COMPUTE THE MOLES H2O*/
        GET LIST (GH2O, MH2O);
        GH2O=GHCL - GHCL1;
        PROD3=(GH2O1*(MH2O**-1))+(GH2O*(MH2O**-1)))*1E+3;
/*CALCULATION OF TOTAL MMOLES IN THE RXN*/
        SUM=SUM + PROD + PROD1 + PROD2 + PROD3;
        SUM1=SUM**-1 + SUM1;
/*CALCULATION OF MOLE FRACTION OF GLYCINE OR ESTER*/
        MMOLE=SUM1*PROD;
/*CALCULATION OF MOLE FRACTION OF HCL*/
        MMOLE1=SUM1*PROD1;
/*CALCULATION OF MOLE FRACTION OF ETOH*/
        MMOLE2=SUM1*PROD2;
/*CALCULATION OF MOLE FRACTION OF H2O*/
        MMOLE3=SUM1*PROD3;
/*CALCULATION OF TOTAL WEIGHT OF THE RXN*/
        TWT=GLYORE + GHCL1 + GETOH + (GH2O1 + GH2O);
        GET LIST (SYSTEM, RXN#);
        PUT SKIP(2) LIST ('SYSTEM', 'REACTION #', 'G GLYCINE/ESTER');
        PUT LIST ('MMOLES GLYCINE/ESTER', 'MOLE FR. GLYCINE/ESTER');
        PUT SKIP LIST (SYSTEM, RXN#, GLYORE);
        PUT LIST (PROD, MMOLE);
        PUT SKIP LIST ('SYSTEM', 'REACTION #', 'G HCL');
        PUT LIST ('MMOLES HCL', 'MOLE FR. HCL');
        PUT SKIP LIST (SYSTEM, RXN#, GHCL);
        PUT LIST (PROD1, MMOLE1);
        PUT SKIP LIST ('SYSTEM', 'REACTION #', 'G ETOH');
        PUT LIST ('MMOLES ETOH', 'MOLE FR. ETOH');
        PUT SKIP LIST (SYSTEM, RXN#, GETOH);
        PUT LIST (PROD2, MMOLE2);
        PUT SKIP LIST ('SYSTEM', 'REACTION #', 'G H2O');
        PUT LIST ('MMOLES H2O', 'MOLE FR. H2O');
        PUT SKIP LIST (SYSTEM, RXN#, GH2O);
        PUT LIST PROD3, MMOLE3);
        PUT SKIP LIST ('SYSTEM', 'REACTION #', 'TOTAL MOLES IN RXN');
        PUT LIST ('TOTAL WEIGHT OF RXN');
        PUT SKIP LIST (SYSTEM, RXN#, SUM);
        PUT LIST (TWT);
        PROD=0;
        PROD1=0;
        PROD2=0;
        PROD3=0;
        SUM=0;
        SUM1=0;
        MMOLE=0;
        MMOLE1=0;
        MMOLE2=0;
        MMOLE3=0;
        TWT=0;
        COUNT=COUNT-1;
        END PROD_LOOP;
END MOLES;
```

II. Computer program for computing the Keq's of the 0:1, 0.5:1 and 1:1 molar ratio ethanol-water solvent systems using PL/C

```
KEQ:  PROCEDURE OPTIONS(MAIN);
      DECLARE (COUNT FIXED DEC,
               NUMBER FIXED DEC,
               CPMGLY FLOAT DEC, /*CPM of Glycine*/
               CPMEST FLOAT DEC, /*CPM of Ester*/
               RATIO FLOAT DEC, /*Ratio of CPMEST to CPMGLY*/
               GLYCINE FLOAT DEC, /*Initial mmoles Glycine*/
               ESTER FLOAT DEC, /*Initial mmoles Glycine Ester*/
               H2O FLOAT DEC, /*Initial mmoles H2O*/
               ETOH FLOAT DEC, /*Initial mmoles Ethanol*/
               GLYF FLOAT DEC, /*Equilibrium mmoles Glycine*/
               ESTERF FLOAT DEC, /*Equilibrium mmoles Ester*/
               H2OF FLOAT DEC, /*Equilibrium mmoles H2O*/
               ETOHF FLOAT DEC, /*Equilibrium mmoles Ethanol*/
               KEQ1 FLOAT DEC, /*Equilibrium Constant*/
/*COMPUTE THE RATIO OF GLYCINE*/
      GET LIST (COUNT);
      EQUIL_LOOP:  DO WHILE (COUNT>0);
/*COMPUTE THE GLYCINE/ESTER CPM RATIO*/
      GET LIST (CPMEST, CPMGLY);
      RATIO = (CPMEST)*(CPMGLY**-1);
      GET LIST (GLYCINE, H2O, ETOH, ESTER, NUMBER);
      IF NUMBER = 1
         THEN DO; GLYF=(GLYCINE)*((RATIO+1**-1);
                  ESTERF=GLYCINE-GLYF;
                  H2O=H2O + ESTERF;
                  ETOHF=ETOH - ESTERF; END;
         ELSE DO; GLYF=(ESTER)*((RATIO + 1)**-1);
                  ESTERF=ESTER - GLYF;
                  H2OF=H2O - GLYF;
                  ETOHF=ETOH + GLYF; END;
      KEQ1=(H2OF)*(ESTERF)*(((GLYF)*(ETOHF))**-1);
      PUT SKIP(2) LIST ('EQ GLY', 'EQ ESTER', 'EQ H2O', 'EQ ETOH', 'KEQ');
      PUT SKIP LIST (GLYF, ESTERF, H2OF, ETOHF, KEQ1);
      COUNT= COUNT - 1;
      END EQUIL_LOOP;
END KEQ;
```

III. Results From the Standardization of HCl in Water

ca 1N HCl: 0.9393N

ca 2N HCl: 1.9699N

ca 3N HCl: 2.9827N

ca 0.01N HCl: 0.00934N

IV. Results From the Standardization of HCl in Ethanol-Water (1:1)

ca 0.01N HCl: 0.00922N

V. Data for the Calculation of Keq

A. For the 0:1 Molar Ratio Ethanol-Water Solvent System

Wt. Glycine (g)	Wt. Ester (g)	N HCl
0.6243	0.0	0.9393
0.0	1.1496	0.9393
0.6233	0.0	1.9699
0.0	1.1851	1.9699
0.6383	0.0	2.9827
0.0	1.1863	2.9827

Wt. HCl (g)	Wt. H_2O (g)	Wt. EtOH (g)	Reaction Density (g/ml)
19.9419	19.0331	0.5750	1.0069
19.7965	19.0045	0.0	1.0137
20.2504	19.0812	0.6053	1.0136
20.3530	18.9470	0.0	1.0211
21.6917	18.8402	0.5228	1.0270
21.6307	18.8704	0.0	1.0306

B. For the 0.5:1 Molar Ratio Ethanol-Water Solvent System

Wt. Glycine (g)	Wt. Ester (g)	N HCl
0.3575	0.0	0.9393
0.0	0.7601	0.9393
0.3686	0.0	1.9699
0.0	0.6821	1.9699
0.3690	0.0	2.9827
0.0	0.6829	2.9827

Wt. HCl (g)	Wt. H_2O (g)	Wt. EtOH (g)	Reaction Density (g/ml)
11.2230	3.8245	18.9190	0.9032
11.4793	3.8456	18.8465	0.9062
12.3159	3.4395	18.2855	0.9136
11.4385	3.7110	17.9815	0.9129
12.6930	3.5148	18.3287	0.9178
12.7835	3.5010	18.2974	0.9209

C. For the 1:1 Molar Ratio Ethanol-Water Solvent System

Wt. Glycine (g)	Wt. Ester (g)	N HCl
0.3066	0.0	0.9393
0.0	0.5593	0.9393
0.2996	0.0	1.9699
0.0	0.5582	1.9699
0.2992	0.0	2.9827
0.0	0.5720	2.9827

Wt. HCl (g)	Wt. H_2O (g)	Wt. EtOH (g)	Reaction Density (g/ml)
10.0079	0.0	22.4341	0.8708
10.1144	0.0	22.3207	0.8749
9.9905	0.0	22.9355	0.8698
10.0264	0.0	23.6371	0.8717
10.3436	0.0	23.0499	0.8784
10.4792	0.0	23.5668	0.8814

VI. Calculation of the ΔG° Values

A. For the 0:1 Molar Ratio Ethanol-Water Solvent System

$$\Delta G^{\circ} = -RT\ln Keq$$

$$= -(1.987 \text{ cal/mole-deg})(293^{\circ}K)\ln(4.6)$$

$$= -8.9 \times 10^{-1} \text{ kcal/mole}$$

B. For the 0.5:1 Molar Ratio Ethanol-Water Solvent System

$$\Delta G^{\circ} = -RT\ln Keq$$

$$= -(1.987 \text{ cal/mole-deg})(293^{\circ}K)\ln(1.0)$$

$$= 0.0 \text{ kcal/mole}$$

C. For the 1:1 Molar Ratio Ethanol-Water Solvent System

$$\Delta G^{\circ} = -RT\ln Keq$$

$$= -(1.987 \text{ cal/mole-deg})(293^{\circ}K)\ln(0.8)$$

$$= +1.3 \times 10^{-1} \text{ kcal/mole}$$

VII. Calculation of the ΔS for the 1:1 Molar Ratio Ethanol-Water Solvent System

$$\Delta G = \Delta H - T\Delta S$$

$$+130 \text{ cal/mole} = +1725 \text{ cal/mole} - (293\ ^{\circ}K)(\Delta S)$$

$$\Delta S = +5.44 \text{ cal/mole-}^{\circ}K$$

VIII. Calculation of $\Delta G^{\circ\prime}$ for the 0:1 Molar Ratio Ethanol-Water Solvent System (pH = 7, Water Assumed to have Unit Activity)

$$Keq = \frac{(Glycine)(EtOH)}{(H_2O)(Glycine\ Ethyl\ Ester)}$$

$$(Ka)(Keq) = \frac{(Glycine^{-})(EtOH)}{(H_2O)(Ester)} \qquad (17)$$

$$(H_2O)(Ka)(Keq) = \frac{(Glycine^{-})(EtOH)}{(Ester)}$$

$$= Keq'$$

$$(H_2O) = 1$$

$$Ka = 0.0043 \text{ calculated from } pKa = \frac{A'}{T} - C' + D'T \qquad (18)$$

$$A' = 1300.53$$

$$C' = 5.5277$$

$$D' = 0.0118$$

$$T = 293\ ^{\circ}K$$

$$Keq' = 1.1581 \times 10^{4}$$

$$\Delta G^{\circ\prime} = -RT\ln Keq$$

$$= -(1.987 \text{ cal/mole-deg})(293^{\circ}K)\ln (1.978 \times 10^{5})$$

$$= -7099 \text{ cal/mole or ca } -7.1 \text{ kcal/mole}$$

IX. Calculation of the CPM Ratio of Ester to Glycine

A. For the 0:1 Molar Ratio Ethanol-Water Solvent System

CPM Ester	CPM Glycine	Ratio
5877.0	205850.0	0.0285
4980.0	236790.0	0.0174
6145.0	181795.5	0.0338
5626.0	277762.7	0.0203
5045.0	173201.7	0.0291
5415.0	274117.2	0.0198

B. For the 0.5:1 Molar Ratio Ethanol-Water Solvent System

CPM Ester	CPM Glycine	Ratio
19627.2	37612.2	0.5218
35021.0	59166.4	0.5919
25569.0	39825.4	0.6420
37323.8	55358.1	0.6742
27675.7	40322.2	0.6864
39678.3	57282.8	0.6927

C. For the 1:1 Molar Ratio Ethanol-Water Solvent System

CPM Ester	CPM Glycine	Ratio
24397.1	37519.6	0.6502
41702.5	48666.1	0.8569
25647.8	28067.2	0.9138
47539.0	49176.0	0.9667
27521.0	26799.8	1.0269
45312.9	45295.6	1.0004

Keq AND ΔH FOR THE ESTERIFICATION OF GLYCINE IN ALCOHOL-WATER SYSTEMS

by

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B. S., Pennsylvania State University, 1978

AN ABSTRACT OF A MASTER'S THESIS

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The esterification of glycine with ethanol was studied at 20 ^{o}C in 0:1, 0.5:1, and 1:1 molar ratio ethanol-water solvents to determine the Keq of the esterification reaction. In the 0:1 molar ratio ethanol-water solvent system, Keq = 4.6; in the 0.5:1 molar ratio ethanol-water solvent system, Keq =1.0; and in the 1:1 molar ratio ethanol-water solvent system, Keq = 0.8. From these Keq values, ΔG^{o} values were calculated. The enthalpy change was calorimetrically found for the reaction in the 1:1 molar ratio ethanol-water solvent. It was $\Delta H = +1.7 \pm 0.65$ kcal/mole or $+7.2 \pm 2.5$ kJ/mole.