THE ADSORPTION OF AMINO ACIDS, PEPTIDES, AND RELATED SUBSTANCES ON SILICA:

A STUDY IN ADSORPTION AT INORGANIC SURFACES.

By

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A Thesis submitted to the University of Glasgow in fulfilment of requirements for the Ph.D. degree in Science.

MAY, 1951.

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C O N T E N T S.

A C K N O W L E D G E M E N T S.

The author wishes to record his thanks to Professors P. D. Ritchie and W. M. Cumming, for providing the necessary laboratory facilities, and for their continued interest and advice during this investigation.

Thanks are also due to Professor G. Hibberd and Dr. M. Gordon, for stimulating discussions during the course of this work, and to the late Dr. J. R. Campbell who supervised its early stages.

This investigation was part of a pneumoconiosis research programme financed by the National Coal Board (Scottish Division).

The author is also indebted to Dr. C. E. Dent (London Univ. College Hospital) who kindly supplied a small sample of gamma-aminobutyric acid, and to Mr. A. Clunie and staff (Tech. Chem. Dept. (Workshops)), and to Mr. Barbour (Glass-blower), for their help in preparing various pieces of apparatus.

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INTRODUCTION.

Section 1.

Theories on the nature of Silicosis.

Silicosis, one of the major industrial hazards of the present day, has been extensively studied, and at various times theories on the nature and cause of the disease have been advanced. At present, the explanation of the pathogenic action of silica, which is most in favour, is known as the 'solubility' theory.(1) Although the 'solubility' of a given sample of silica *in vitro* is correlated closely with its surface area, its correlation with its pathogenicity, although close, is not perfect, as has been shown by King.(1) To offset certain anomalies, King comments: "either the noxious dust releases something which is harmful to the living cell or it has special surface properties which cause abnormal reactions to take place in certain cell constituents which become adsorbed on its faces."

Considering such surface reactions King debates the possibility of a transformation of the 'lesser proteins' into reticulin or collagen at a particle surface, but considers this unlikely since there is involved an alteration of the physical properties of a protein, its molecular size, and also its amino acid content.

Heffernan (2) has strongly advocated such surface activity on the part of aerosols harmful to tissues, as an explanation of the pathogenic nature of siliceous dusts. He reviews the evidence of X-ray diffraction and other methods for the existence of free unsatisfied valency forces at the surface of freshly cloven particles, which can

take up any reactive material which is available. However, it was necessary for Heffernan to postulate, in support of the theory, that only freshly fractured dust was active; but in view of other experimental evidence,[3, 4] this assumption would appear to be unjustifiable since it is most probable that the fresh surfaces reach equilibrium with the surrounding atmosphere in a matter of seconds or minutes, and even if they reach the alveoli in an active condition, their activity would presumably be destroyed as soon as they are wetted by the alveolar fluids.

The inhibition of the biological activity of silica by coating its surface with Aluminium [5] has been explained by Heffernan [6] and others on the 'freshly fractured' surface theory. However, although this phenomenon suggests that the activity of a silica particle is concentrated at its surface, it is not essential that these surfaces should have been freshly formed. When a silica dust, whether 'freshly-fractured' or 'old', comes in contact with aqueous solutions, the silica particles acquire a negative charge, and consequently surface reactions, such as adsorption, may still occur on an 'old' dust.

This present thesis is an endeavour to examine in detail a specific surface reaction, namely, the adsorption of amino acids and related substances on a silica dust, and to determine the fundamental forces involved. The individual amino acids are of a less complex nature than when combined in proteins and consequently in this thesis fundamental conclusions concerning this surface reaction can be derived from the experimental data. In conclusion, it is significant that the biological activity of silica has been shown to be inhibited by the adsorption of

of aluminium or iron oxide, and in view of the present limited knowledge of the possible adsorption reactions on the surface of the silica it is not inconceivable that the aluminium or iron oxide may be preferentially adsorbed on the silica and thus inhibit the adsorption of other cell constituents.

Section 2.

Theories on the adsorption of amino acids and proteins.

In 1916, Adams [7] suggested that in solution an amino acid exists in the form which is now known as the zwitterion (or dipolar ion) and in 1923 Bjerrum [8] formulated this idea more completely. From that time, further arguments for the existence of zwitterions have been advanced and among them may be mentioned heats of neutralisation of amino acids and proteins [9], studies of Raman and infra-red spectra of the amino acids in solution [10], and their high dielectric constants [10]. The nature and behaviour of amino acids, peptides, and proteins in aqueous solution has been discussed in detail by Cohn and Edsall [10].

In aqueous suspension, quartz and silica particles have been found to be negatively charged [11]. Proteins have been widely studied on account of the changes in their physico-chemical properties on adsorption at solid-liquid interfaces. Freundlich and Abramson [12] have studied the change in zeta-potential of egg-albumin adsorbed on quartz and many other workers [13, 14, 15, 16] have studied the mobility of proteins by techniques involving their adsorption on fine quartz particles. Lindau and Rhodius [17] investigating the adsorption of egg-albumin and gelatin on powdered quartz, found that multimolecular layers of egg-albumin were adsorbed. They also found that a unimolecular layer of egg-albumin was irreversibly adsorbed, although additional layers could be washed off with water; the adsorption of egg-albumin was observed to follow Langmuir's adsorption isotherm. From their adsorption data, Lindau and

Rhodius were able to calculate the size of the egg-albumin molecule at its isoelectric point and have reported their values to be in good agreement with those of Svedberg. They also observed that when the quartz surface is approximately 50% covered with egg-albumin, the adsorbate rapidly changes from the hydrophilic to the hydrophobic condition; and a theory is developed to explain why this change in wetting should take place at 50% surface covering.

When studying the adsorption of proteins on montmorillonite clays, Ensminger and Gieseking [18] observed by X-ray diffraction methods that the large protein molecules (gelatin and albumin) were adsorbed within the variable portion of the crystal lattice of the clay. The extent of adsorption of gelatin increased with decrease in pH of the solution but was considerably decreased when the gelatin was treated with nitrous acid to destroy the amino groups. From these observations it was concluded that the adsorption of proteins as cations was principally responsible for their combination with montmorillonite. Similar conclusions were deduced by Demolon and Brigando [19] when studying the adsorption of proteins by soil. Investigating the physico-chemical properties of colloids, Meyers [11] observed that a mixture of organic colloids and quartz showed a reduction in cation exchange capacity from the sum of the capacities of the two components. He also observed that maximum adsorption of the organic colloids on quartz was attained in acidic suspensions, and consequently postulated a polar mechanism of adsorption.

The adsorption of water vapour on amino acid crystals has been shown by Moore and Frey [20] to take place at localised sites, the polar

$-NH_3^+$ and $-COO^-$ groups, rather than by the formation of a mobile monolayer. The chromatographic separation of amino acids has been extensively studied; but the adsorbents used are in general charcoal, Norite, and similar substances, which are in no way similar to fine silica or quartz powders. Cheldelin and Williams[21] using charcoal as an adsorbent found that Langmuir isotherm equation of little use but deduced conclusions on the adsorption behaviour of each amino acid from the value of the factor 1/n in the corresponding Freundlich equation.

From the preceeding discussion we may conclude that the adsorption of proteins and organic colloids on quartz and montmorillonite clays were found by various workers to take place according to a polar mechanism.

EXPERIMENTAL METHODS.

1. Adsorbent.

The adsorbent used in these adsorption experiments in Parts 1 and 2 is a silica powder of very large surface area. This silica powder was kindly furnished by Messrs. Colin Stewart Ltd. This adsorbent was used because a large initial supply could be obtained and consequently the adsorption data of the different amino acids are comparative with respect to the same surface area of adsorbent. This silica powder is of extremely fine particle size, and has a very large surface area which is imperative since the extents of adsorption of these amino acids are very small, the lowest observed adsorption being 1×10^{-5} moles on 2 gms. of this silica powder. Further, a similar silica powder from the same source, has been used in pathological experiments by King[22] and others, who showed it to produce massive fibrosis in the lungs of rats when administered by the intra-tracheal route. Consequently, the present adsorbent is a typical industrially produced powder, which may be pathologically active. The surface area of the silica powder was found to be 20,700 sq.cms. per gm. as determined by the Lea and Nurse[23] air permeability method. Using this value of the specific surface, the average particle size of the powder was calculated to be ca. 1.1 μ.

Note. The air permeability method of measurement of surface area is not comparable in accuracy to methods based on adsorption on the surface of the powder.

The silica has as impurities iron and aluminium. The total impurity, estimated as oxides, was determined by evaporation of weighed samples of the powder with hydrofluoric acid and roasting the residues.

The percentage impurity calculated on this basis is 7.65%.

The silica powder was dried in an air oven at 110°C and stored until use in a vacuum desiccator over dry silica gel at room temperature.

For comparison purposes, adsorption isotherms of two typical amino acids were also obtained on very pure quartz and are reported in Part 3. It may be noted that the two dusts investigated showed no significant difference in adsorptive power.

2. Amino Acids.

The amino acids and related substances were purchased from commercial sources. The amino acid, its source, and calculated and observed % nitrogen values are shown in Table A of the Appendix, section 1.

N-acetylglycine and γ-aminobutyric acid were prepared as described in the Appendix, sections 2 and 3 respectively.

3. Adsorption Procedure.

3.1. Amino Acids.

3.1.1. Rates of adsorption.

The adsorption procedure followed during experiments to determine rates of adsorption of the amino acids is described below.

Standard 0.01 M unbuffered solutions of the amino acids in distilled water were prepared at their isoelectric pH values. Unless otherwise mentioned these experiments were carried out at the isoelectric pH values of the amino acids. All pH measurements in this work were made with a Marconi pH meter.

To 2 gm. samples of the dry silica powder, which had been

accurately weighed, in glass Quickfit tubes of 50 ml. capacity were added 25 ml. of the standard 0.01 M solution of the amino acid. The tubes were then sealed and their contents thoroughly mixed. The suspensions were allowed to rotate slowly in a water thermostat at a constant temperature for known time intervals, and then centrifuged for 10 minutes at 3,500 r.p.m. The clear supernatant liquids were decanted off and analysed to determine the adsorption. From a series of such tests, for different time intervals, graphs were constructed showing the rate of adsorption for each amino acid. Since these solutions were unbuffered, a pH change within the range $\pm$ 0.5 pH unit was observed in the tests after adsorption; the pH values recorded in graphs, tables and discussed in the text are the final pH after adsorption. Except where a range of temperatures was required to determine energies of activation, these rates of adsorption experiments were carried out at 37 $\pm$ 0.1°C., the normal temperature of the human body.

To express the adsorption data, an arbitrary standard surface area has been selected - namely, that of 2 gms. of the silica powder (41,400 sq.cms.) and is referred to as standard surface area 1. Consequently, the observed adsorption values are reported in moles x 10^{-5} / standard surface area 1. The experimental data for the rate of adsorption of the amino acids is expressed graphically, plotting adsorption (moles x 10^{-5}/ standard surface area 1) vs Time (hrs.).

3.1.2. Effect of pH on adsorption.

In experiments to determine the effect of pH on adsorption, the procedure is again as described above, the mass/liquid ratio having

the same value. The solutions of the amino acids used in these experiments were 0.01 M unbuffered aqueous solutions, their pH values having been adjusted to the desired figures by drops of N NaOH or HCL and the pH values of the solutions determined. The temperature at which these adsorption experiments were carried out was 25°C.

The experimental data for the effect of pH on adsorption are expressed graphically by plotting adsorption (moles x 10^{-5}/ standard surface area l.) vs pH.

3.1.3. Adsorption isotherms.

In the determination of adsorption isotherms of the amino acids, the procedure followed is essentially as described above. The mass/liquid ratio was kept constant and had the value already described. The temperature of the thermostat was altered to give three isotherms for each amino acid. The concentrations of the aqueous amino acid solutions used in the preparation of these isotherms ranged from 0.005 M to 0.05 M. Each isotherm for all the amino acids was determined at the isoelectric pH of the amino acid except for glycine (final pH 5.4), α-alanine (final pH 7.0), lysine (final pH 7.3), and proline (final pH 7.3). A pH change within the range ± 0.5 pH unit was observed in the tests after adsorption; the pH values recorded in graphs, tables and discussed in the text are the final pH after adsorption.

The adsorption isotherms are expressed graphically, plotting adsorption (moles x 10^{-5}/standard surface area l.) vs equilibrium concentration of the solution (moles x 10^{-2}/litre.)

3.2. Dipeptides.

3.2.1. Rates of Adsorption.

The extent of adsorption of these dipeptides was found to be very low and consequently the concentration of the dipeptide solutions had to be increased beyond that employed for the amino acids. For the same reason, the quantity of adsorbent used for each test had to be increased to 4 gms. of the silica powder (82,800 sq.cms.) and is referred to as standard surface area 2. The adsorption of these dipeptides was carried out from 0.02 M aqueous unbuffered solutions at their isoelectric pH values.

Other than these changes, the adsorption procedure is exactly as described for the amino acids. Except where a range of temperatures was required, to determine energies of activation these rates of adsorption experiments were carried out at 37 $\pm$ 0.1°C. A pH change within the range $\pm$ 0.5 pH unit was observed in the tests after adsorption.

The experimental data for the rate of adsorption of these dipeptides is expressed graphically, plotting adsorption (moles x 10^{-5}/ standard surface area 2.) vs Time (hrs.).

3.2.2. Effect of pH on adsorption.

The procedure is again as previously described. The solutions of the dipeptides used in these experiments were 0.02 M unbuffered aqueous solutions, their pH values being adjusted to the desired figures by drops of N NaOH or HCl and the pH values of the solutions determined. The quantity of adsorbent used was again 4 gms. of the silica powder, and the temperature at which these adsorption experiments were carried out was 25°C.

The experimental data for the effect of pH on adsorption are expressed graphically, plotting adsorption (moles x 10^{-5}/standard surface area 2.) vs pH.

3.2.3. Adsorption isotherms.

In the determination of isotherms of these dipeptides the procedure followed is essentially as described for the amino acids except that 4 gm. samples of the adsorbent were used. The concentrations of the aqueous dipeptide solutions used in the preparation of these isotherms ranged from 0.005 M to 0.06 M. Each isotherm for all the dipeptides was determined at the isoelectric pH of the dipeptide. The temperature of the thermostat was altered to give three isotherms for each dipeptide.

The adsorption isotherms are expressed graphically, plotting adsorption (moles x 10^{-5}/standard surface area 2.) vs equilibrium concentration of the solution (moles x 10^{-2}/litre).

4. Analysis.

To determine the adsorption of these amino acids and peptides, nitrogen analyses were carried out on the standard solutions before and after adsorption, by the semi-micro Kjeldall method described by Hitchcock and Belden[24].

Samples of the amino acid or peptide solutions containing approximately 5 mgms. of nitrogen are accurately measured into the Pyrex combustion tubes. To this solution is added 2 ml. of concentrated sulphuric acid and the reagents, potassium sulphate (of micro-analytical purity), selenium dioxide, and mercuric oxide, in the quantities stated by Hitchcock. An equivalent amount of selenium dioxide is used in place

of the selenium oxychloride used by Hitchcock. After the water has been gently boiled off, the concentrated sulphuric acid solution is heated strongly for approximately 20 minutes. After cooling, the solution is transferred to the steam-distillation apparatus, where the ammonia formed is liberated by the addition of 5 ml. of an NaOH solution and is steam distilled into 5 ml. of 0.1 N HCl. (The NaOH solution contains 500 gms. NaOH pellets (Analar) and 80 gms. of anhydrous sodium thiosulphate dissolved in 500 ml. of distilled water.) The excess 0.1 N HCl is back-titrated with 0.1 N NaOH, using methyl red as an indicator, from a micro-burette of 5 ml. capacity. Blank analyses were carried out frequently using only the reagents in distilled water.

The observed accuracy of analysis is $\pm$ 0.07%.

PART 1.

AMINO ACIDS.

Section 1.

Discussion of Results.

1.1. Mechanism of adsorption.

A glass U-tube was filled with a suspension of the silica powder in distilled water, and into each limb of the tube was placed a copper electrode dipping into the suspension. A 250 volt D.C. current was passed through the suspension and it was observed that the silica particles were repelled from one electrode and flowed toward the other. The silica suspension was then replaced by a similar suspension of a positively charged dye, Nacht Blau, in distilled water, and the current again passed through the system. In this instance, it was observed that the dye particles flowed toward the electrode which had repelled the silica particles; and it can consequently be concluded that the latter are negatively charged in aqueous suspension.

The Nacht Blau dye (Colour Index 731) is a Grubler microscopic stain, and from its formula is seen to be a basic dyestuff.

Since the amino acids exist in aqueous solution as zwitterions, an obvious mechanism of adsorption is the mutual attraction of the positively charged ammonium group ($-NH_3^+$) to the negatively charged silica surface.

It has been found that glycine is adsorbed by the silica, and to investigate the mechanism of this adsorption, the free ammonium group of glycine was protected by acetylation and adsorption experiments carried out with N-acetylglycine. No adsorption was observed. Further,

as will be discussed later, it was observed that no adsorption took place from an alkaline solution of glycine at a pH value where the positively charged ammonium group had been completely converted into the uncharged amino group.

However, in order to examine more fully this suggested mechanism of polar attraction, analogous adsorption experiments were carried out with 0.1 M unbuffered aqueous solutions of the following simple nitrogen compounds:-

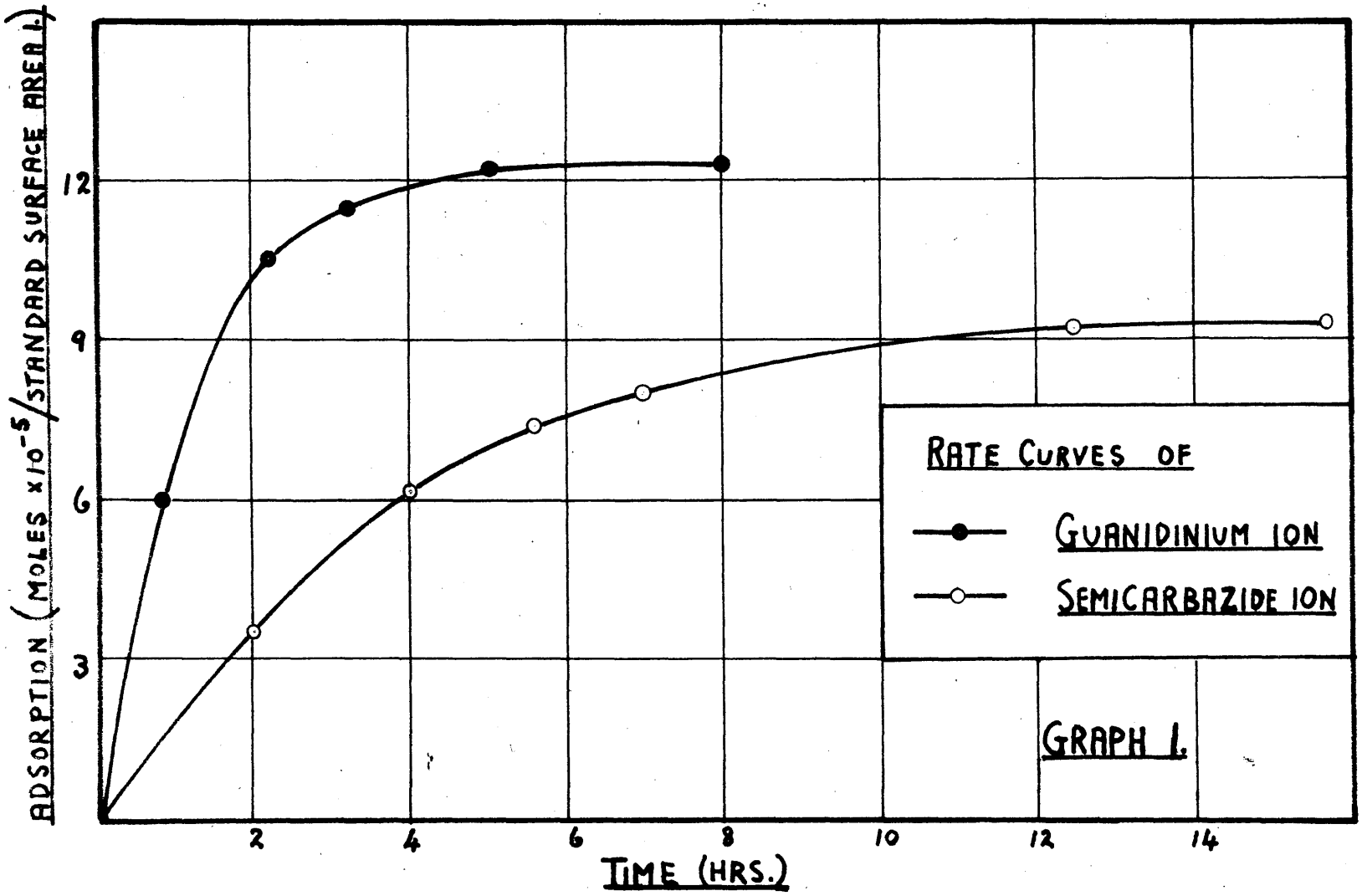
(a) urea (b) thiourea (c) guanidine (d) semicarbazide.

The aqueous guanidine solution was prepared by dissolving the calculated quantity of guanidine carbonate in distilled water so that the concentration of the free guanidinium ion was 0.1 M. The pH of this solution was approximately 11.

Similarly, the semicarbazide solution used in these adsorption experiments was prepared by dissolving the calculated amount of semicarbazide hydrochloride in distilled water, so that the concentration of the free semicarbazide ion ($NH_2 .CO.NH. NH_3^+$) was 0.1 M. The pH of this solution was 2.2.

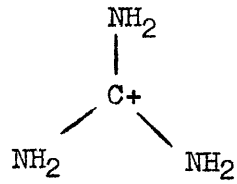
The adsorption and analytical procedures followed during adsorption experiments with these four nitrogen compounds are as described for the amino acids in the experimental section. The mass/liquid ratio is as for the amino acids and the temperature at which adsorption was studied was 37°C.

It has been found that urea and thiourea are not adsorbed by the silica, whereas the guanidinium and semicarbazide ions are readily adsorbed. Their rates of adsorption are recorded in graph 1.

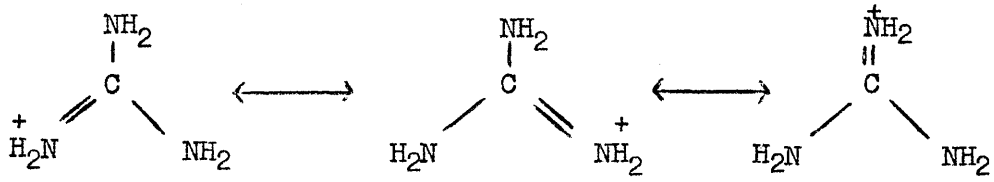

ADSORPTION (MOLES x10⁻⁵/STANDARD SURFACE AREA)
12
9
6
3
2
4
6
8
10
12
14
TIME (HRS.)
RATE CURVES OF
GUANIDINIUM ION
SEMICARBAZIDE ION
GRAPH I.

By a consideration of the structures of urea and the guanidinium ion in solution, the reason for their different adsorption affinities is disclosed.

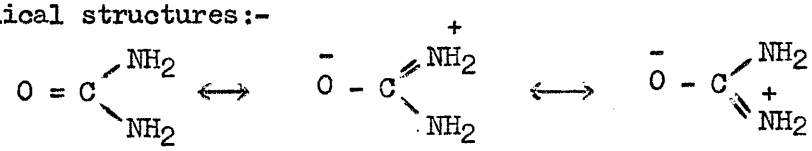
Guanidine is a very strong base, and readily acquires a proton at any pH below 14, being converted into the guanidinium ion



This ion is completely symmetrical, as has been shown by quantum mechanical reasoning [25], and experimentally proved by X-ray diffraction[26] data. It may be visualised as arising by resonance between the equivalent canonical structures:-



Owing to the complete equivalence of the three forms, the resonance energy is high and the ion very stable. On the other hand, the urea molecule may be considered as existing as a resonance hybrid of three non-equivalent canonical structures:-



It is very probable, therefore, that in solution the guanidinium ion is attracted and adsorbed on the silica by virtue of its strong positive charge. The urea molecule, although possessing a resonance structure in

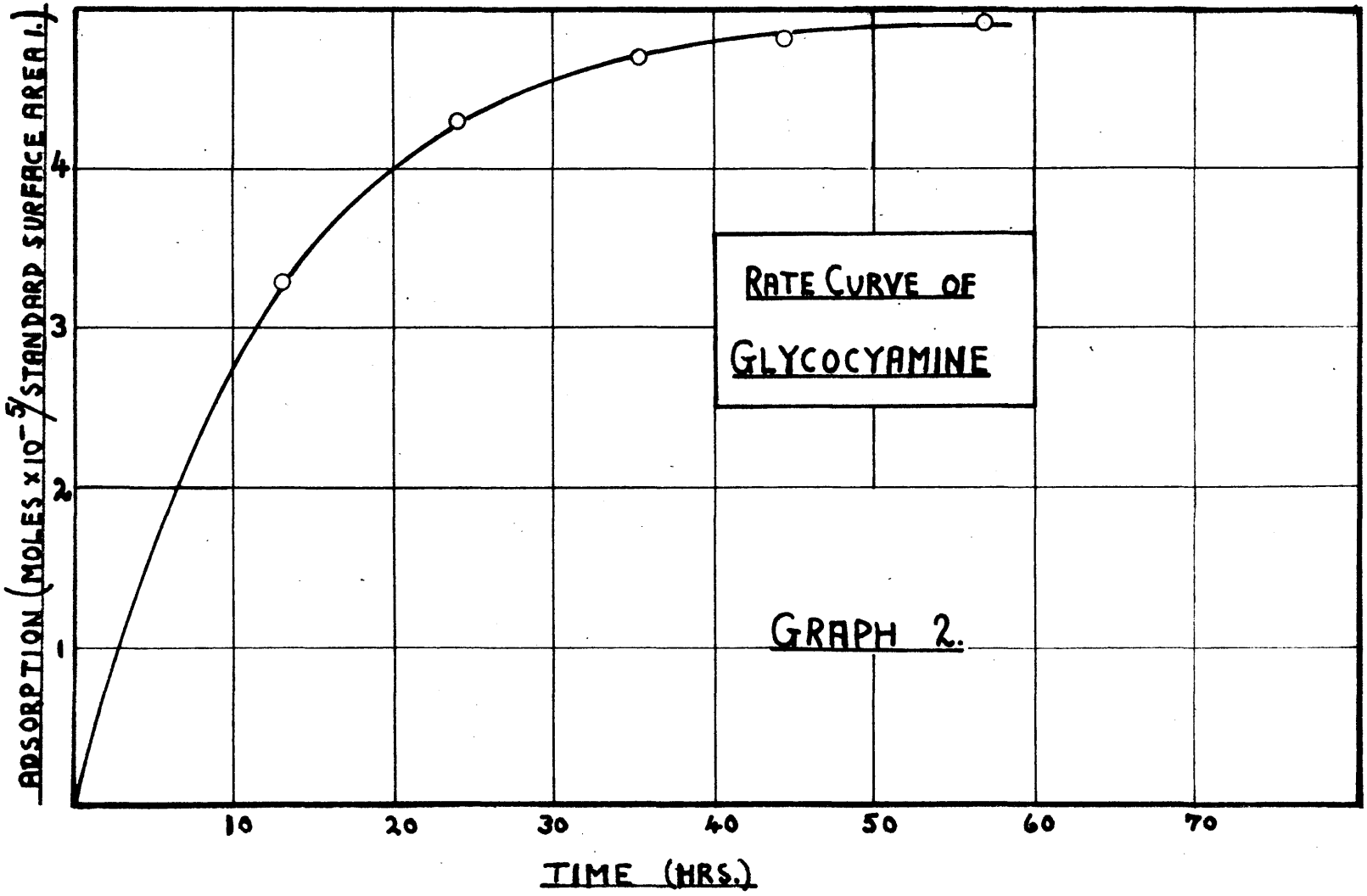
which there is a separation of charges, is not adsorbed. Since the three forms of urea are not equivalent, the polarity arising from the resonance contribution of polar canonical forms will be lower than that of the guanidinium ion, and consequently there is little possibility of adsorption by the positively charged amino group. Further, in these polar forms, the negative carbonyl group is in very close proximity to the positive amino group, and would be repelled by the silica possessing a like negative charge.

In semicarbazide hydrochloride solution, the semicarbazide will exist as an ion $H_2N.CO.NH.\overset{+}{N}H_3$ balanced by the chloride ions. It is again clear that the semicarbazide ion is adsorbed by virtue of its strong positive charge. The ion is asymmetrical, and the stability of resonance structures containing a negatively charged carbonyl group is low.

To test the application of the above suggested adsorption-mechanism to a molecule with a structure resembling a simple amino acid, adsorption experiments were carried out with glycocyamine. The rate of adsorption of this compound is recorded in graph 2, and is observed to bear a strong resemblance to that of glycine and of β-alanine, as will be discussed later, in section 1.5.

From previous conclusions, the adsorption of glycocyamine takes place by virtue of the guanidinium group in the molecule. Since there is a structural resemblance between glycocyamine and glycine it may be concluded that adsorption of glycine, and other amino acids, takes place by virtue of their positively charged ammonium group ($-\overset{+}{N}H_3$).

An important corollary arising from these adsorption experiments is that the pH value of the medium containing the positively charged ion,


ADSORPTION (MOLES x 10⁻⁵/STANDARD SURFACE AREA)
1
2
3
4
10
20
30
40
50
60
70
TIME (HRS.)
RATE CURVE OF
GLYCOCYAMINE
GRAPH 2.

which is adsorbed, does not greatly influence the adsorption, since the guanidinium and semicarbazide ions are adsorbed from media of pH 11 and 2.2 respectively. However, this situation must be clearly distinguished from one in which the pH of the medium controls the concentration of the positive ion to be adsorbed, as is the case in an amino acid solution.

1.2. Influence of other functional substituents in the carbon chain, on the adsorption of the amino acid.

1.2.1. The influence of the α-alkyl group.

Adsorption data for a number of α-amino acids of different structure are recorded in table 1. These amino acids may be conveniently regarded as substituted glycine molecules, of general formula $R.CH(NH_2).CO_2H$, where R is an alkyl radical. Using the data shown in table 1, conclusions may be drawn on the influence of the alkyl radical on the adsorption of the amino acid.

Comparison of the experimental data for glycine with those for α-alanine indicates the effect of the methyl group which has been introduced into the molecule. The effect is most clearly marked by the pronounced decrease in time required to attain equilibrium adsorption from 0.01 M aqueous solutions upon the same surface area of adsorbent. In general the effect of the alkyl group upon adsorption may be considered as being a function of:-

(a) its influence in the solution, and

(b) its influence in the adsorbed state.

(a) In solution, the electric moments of glycine and α-alanine are presumably identical(29), but the volume of the molecule is larger by

Table 1.

Temp. = 37°C.

General Formula = $R.CH(NH_2).CO_2H$

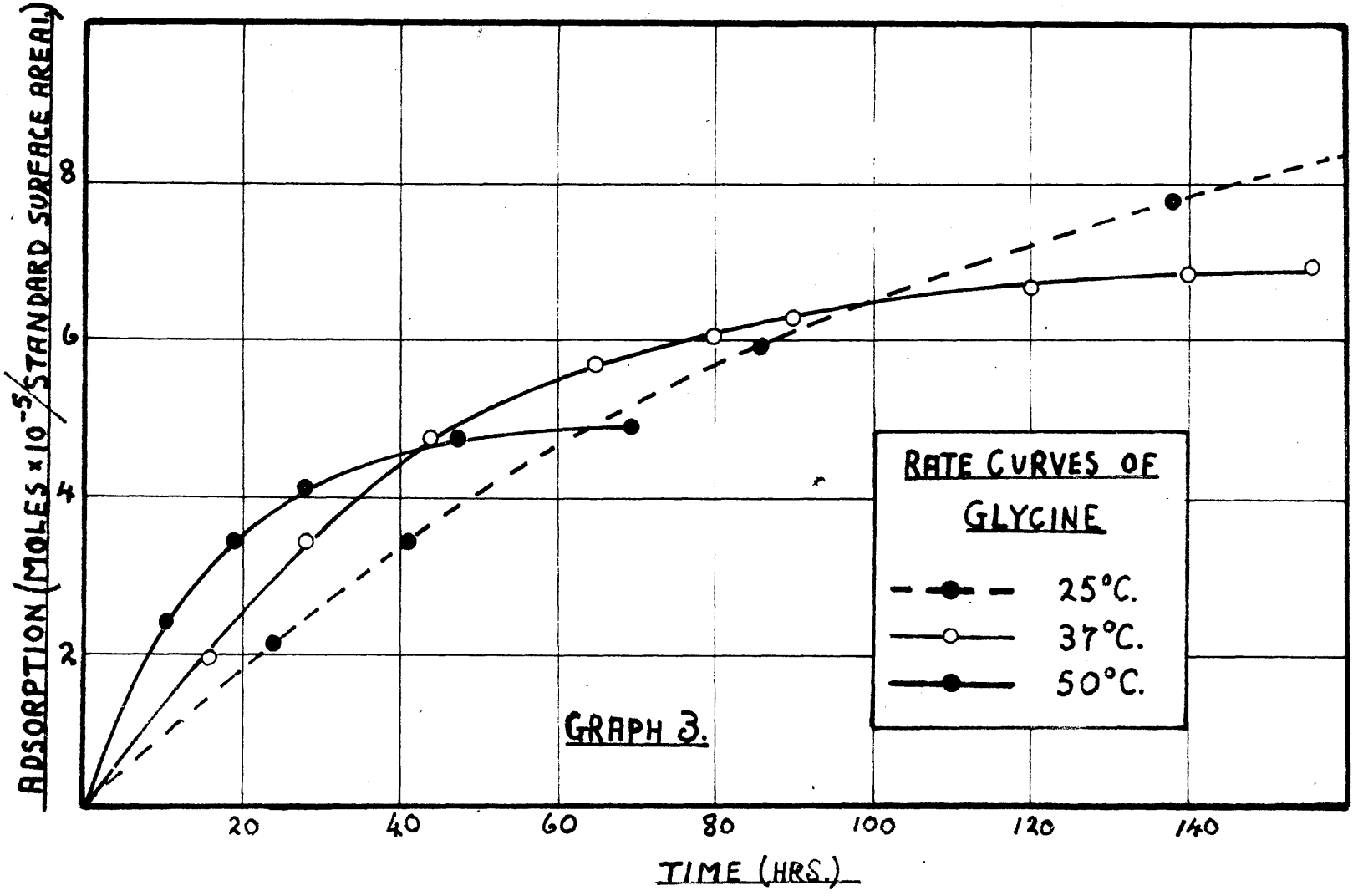
Amino Acid	Graph	R=	a_E	$(k)_{37°C}$
Glycine	3	-H	7.0	2.47×10^{-2}
+ ∝-Alanine	4	-Methyl	8.47	1.79
∝-Amino-n-Butyric Acid	5	-Ethyl	3.4	1.2
Norvaline	5	-n-Propyl	2.6	5.75×10^{-1}
Valine	5	-Isopropyl	2.35	5.65×10^{-1}
Norleucine	-	-n-Butyl	1.45	-
Leucine	-	-Isobutyl	1.45	-

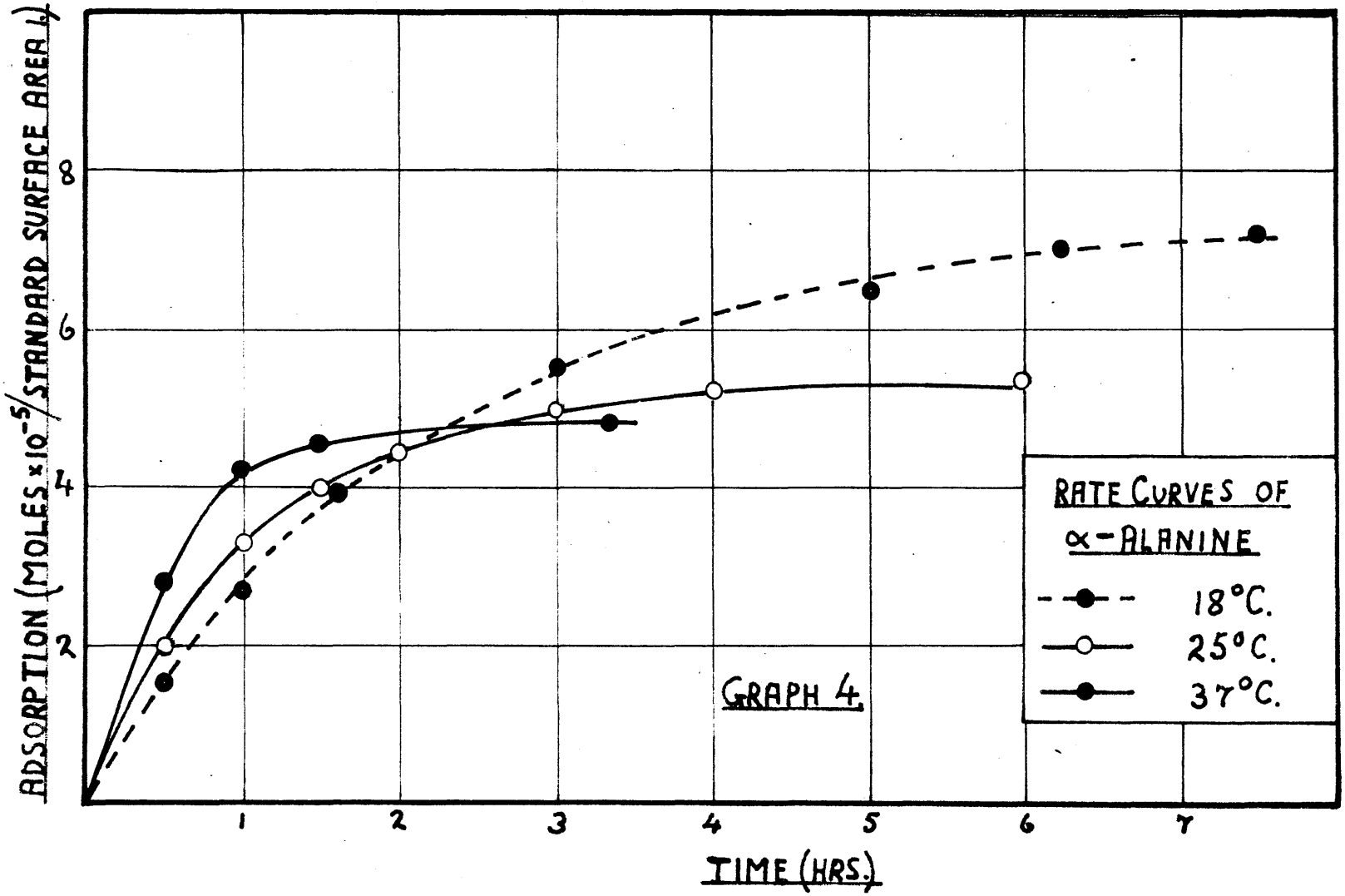
a_E = equilibrium adsorption in moles x 10^{-5}/standard surface area 1 from 0.01 M solution at its isoelectric pH.

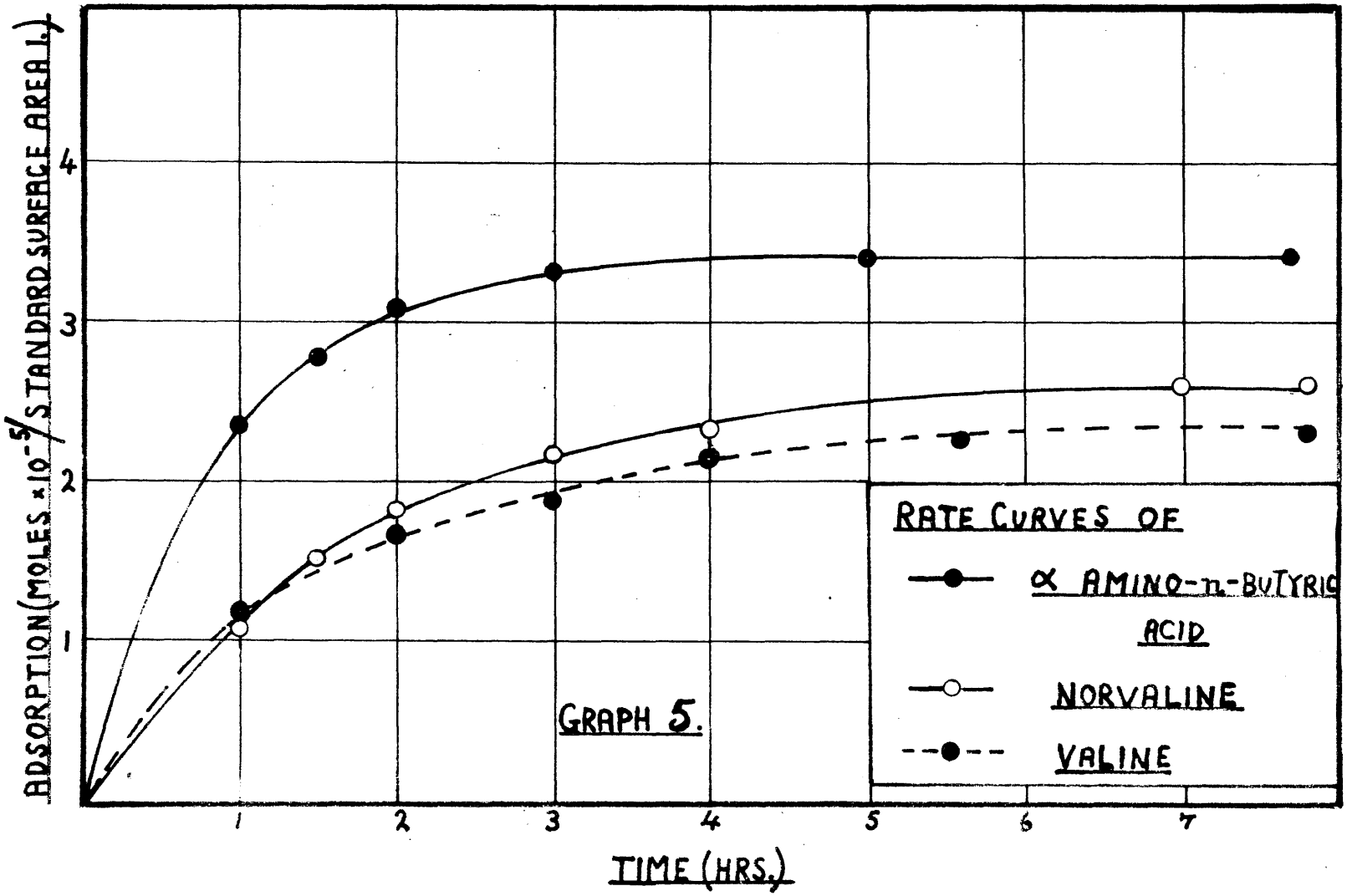
$(k)_{37°C}$ = Langmuir rate constant at 37°C.

+ The rate of adsorption of ∝-alanine and the value of $(k)_{37°C}$ were measured at pH 7.3.

NOTE: The data shown in tables are the basis of all discussion. Data deduced from graphs may be inaccurate due to method of graphing.


ADSORPTION (MOLES × 10⁻⁵/STANDARD SURFACE AREA)
2
4
6
8
20
40
60
80
100
120
140
TIME (HRS.)
RATE CURVES OF GLYCINE
25°C.
37°C.
50°C.
GRAPH 3.


ADSORPTION (MOLES x 10⁻⁵/STANDARD SURFACE AREA)
2
4
6
8
1
2
3
4
5
6
7
TIME (HRS.)
RATE CURVES OF
α-ALANINE
18°C.
25°C.
37°C.
GRAPH 4.


ADSORPTION (MOLES × 10⁻⁵/S. TANDARD SURFACE AREA I.)
1
2
3
4
1
2
3
4
5
6
7
TIME (HRS.)
RATE CURVES OF
α AMINO-n-BUTYRIC ACID
NORVALINE
VALINE

GRAPH 5.

one CH_2 group, or by 16.3 c.cs. per mole[27] and in ∝-alanine the centre of the dipole is presumably further from the centre of the molecule[28, 29, 30]. Interaction between glycine molecules [31, 32], and between ∝-alanine molecules[33, 29], in aqueous solution has been studied by measurements of both vapour pressure and freezing point, the results being expressed as activity coefficients. Cohn[29] discusses these results as follows: "Whereas the activity coefficients of glycine, like those of electrolytes, are less than unity for dilute solutions, those of ∝-alanine are greater, and, moreover, are opposite in sign. If the effect for glycine is ascribed to the dipole moment of its molecules, then that for ∝-alanine must be ascribed to repulsive forces in aqueous solution between the dipole of one molecule and the non-polar side chain of a second."

Therefore, the ease with which ∝-alanine is adsorbed is largely due to the weak interaction between the molecules in solution and conversely the stronger attraction between glycine molecules renders them more difficult to adsorb.

(b) After the initial adsorption, the negatively charged carboxylate groups ($-COO^{(-)}$) of the adsorbed glycine molecules protrude into the solution and attract other glycine molecules. However, with ∝-alanine there also protrudes from the adsorbed molecule the non-polar methyl group which does not attract other ∝-alanine molecules in the solution. The force of attraction exerted by a protruding carboxylate group for other molecules in solution cannot be great, since it has been found from isotherm data that these amino acids do not form multilayers.

Thus, the ease with which any amino acid molecule is adsorbed will be largely controlled by these factors which we have just discussed, and we may now examine more fully the experimental data for the individual amino acids shown in table 1.

The rates of adsorption of these amino acids are most clearly defined by considering their individual values of k, the Langmuir rate constant at 37°C. (Note. Although the Langmuir rate constant (k) is the sum of the velocity constants of the adsorption process (k_1) and the desorption process (k_2) for each amino acid, the values of k_1 and k_2 have been found to be virtually identical with one another for each of a number of amino acids and consequently the Langmuir rate constant (k) of each amino acid maybe used to compare their rates of adsorption.)

The rate of adsorption of each amino acid containing an alkyl group is much greater than that of glycine. As the length of the carbon chain increases, the rate of adsorption is found to decrease slightly although always considerably greater than that for glycine. A comparison of valine and norvaline shows a slight decrease in both rate and extent of adsorption; the decrease is small and may be of no great significance. The extent of adsorption of both leucine and norleucine is so small that their complete rate curves could not be obtained, and consequently the effect of branching in the carbon chain could not be confirmed.

Since α-alanine is so rapidly adsorbed, its rate of adsorption was determined at pH 7.3, instead of its isoelectric pH, in an endeavour to obtain more accurate results. The a_E value for α-alanine at its isoelectric pH, at 37°C, was determined, by separate experiments, to be 8.47×10^{-5} moles. This value for α-alanine is higher than a_E for

glycine at its isoelectric pH, namely 7.0×10^{-5} moles. In contrast to α-alanine, glycine does not attain its maximum adsorption from 0.01 M. solution at its isoelectric pH, but does so only at lower pH values. (This will be discussed in greater detail in section 2.1, p.43.) However, as the size of the alkyl group increases, a most pronounced decrease in extent of adsorption is observed.

1.2.2. Further substitution within the alkyl group.

Consideration of the influence of secondary functional groups on adsorption of the amino acid may be extended to include molecules where the alkyl group has itself been further substituted.

Dependent upon their nature, such secondary groups will determine the interaction between the molecules in solution and also exert an effect in the adsorbed state. In conjunction with these factors, the secondary groups alter the effective electropositive character of the molecule and consequently influence its adsorption.

Adsorption data for a number of amino acids containing different secondary groups are shown in table 2a, and using these data conclusions may be drawn on the influence of these groups on the adsorption of the amino acid. These amino acids may be conveniently regarded as substituted α-alanine molecules conforming to the general formula $R.CH_2.CH(NH_2).CO_2H$.

Cysteine.

The experimental data for cysteine indicate that adsorption, although slower than for α-alanine, is much more rapid than for glycine, and also takes place to a greater extent. The thiol group having a dissociation constant, (pK -SH) of 10.7[34] does not exist in a polar form at the isoelectric pH of the amino acid, and consequently there is little difference in polarity between the molecules of α-alanine and of cysteine. The effect of the thiol group may, therefore, be summarised as resembling that of the methyl group, since adsorption is rapid, the increase in extent being due to the slight change in polarity.

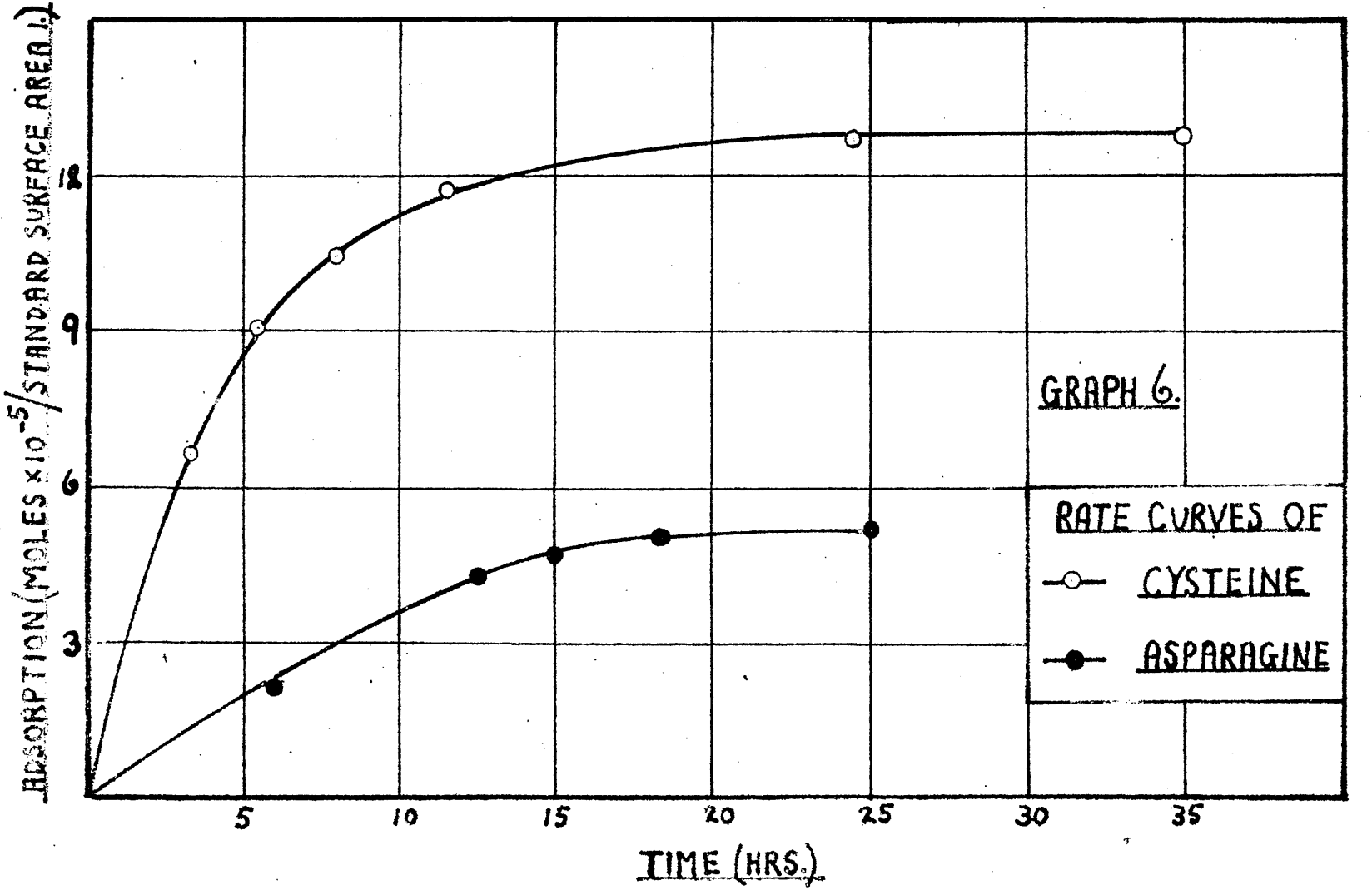
Table 2a.

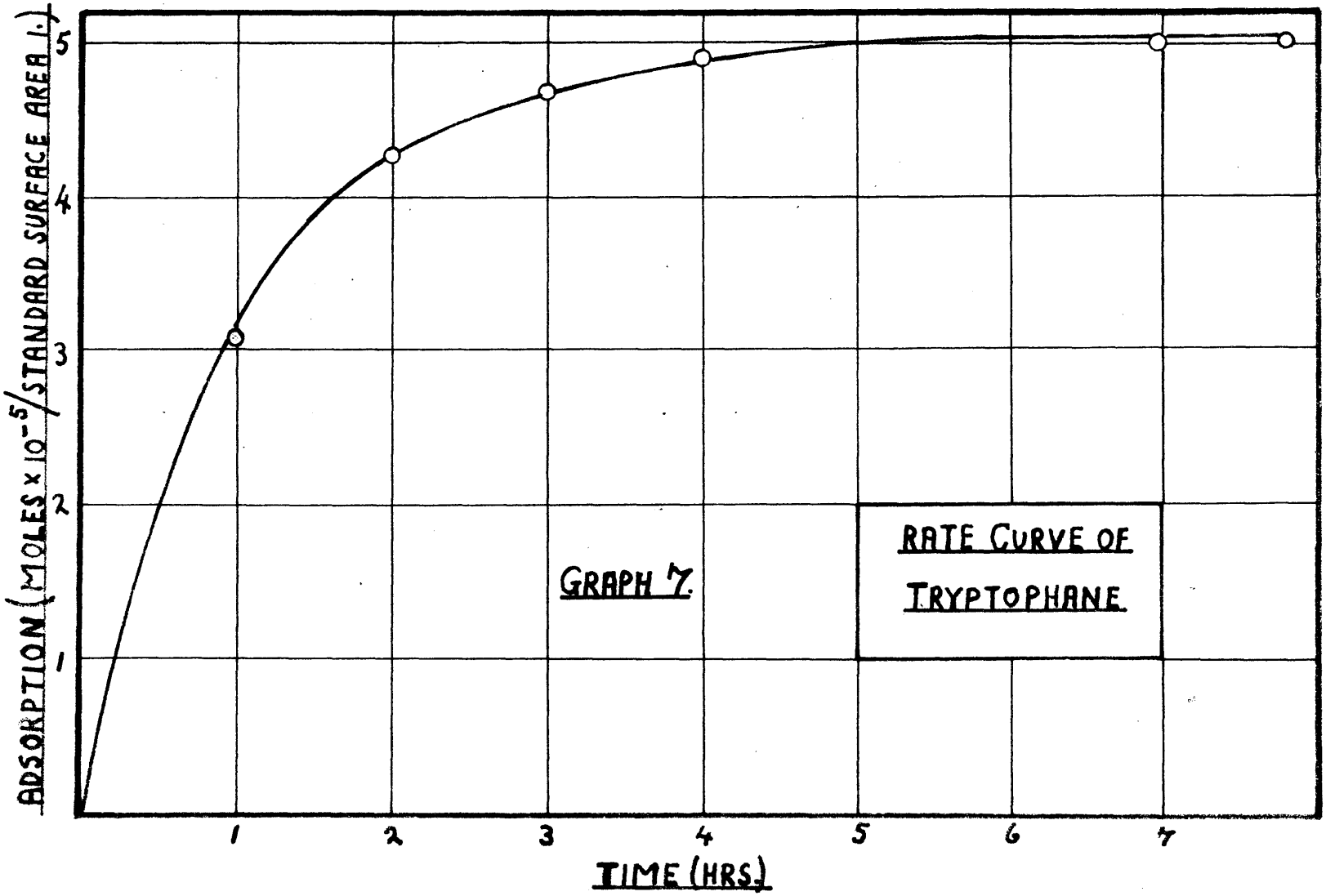
Temp. = 37°C.

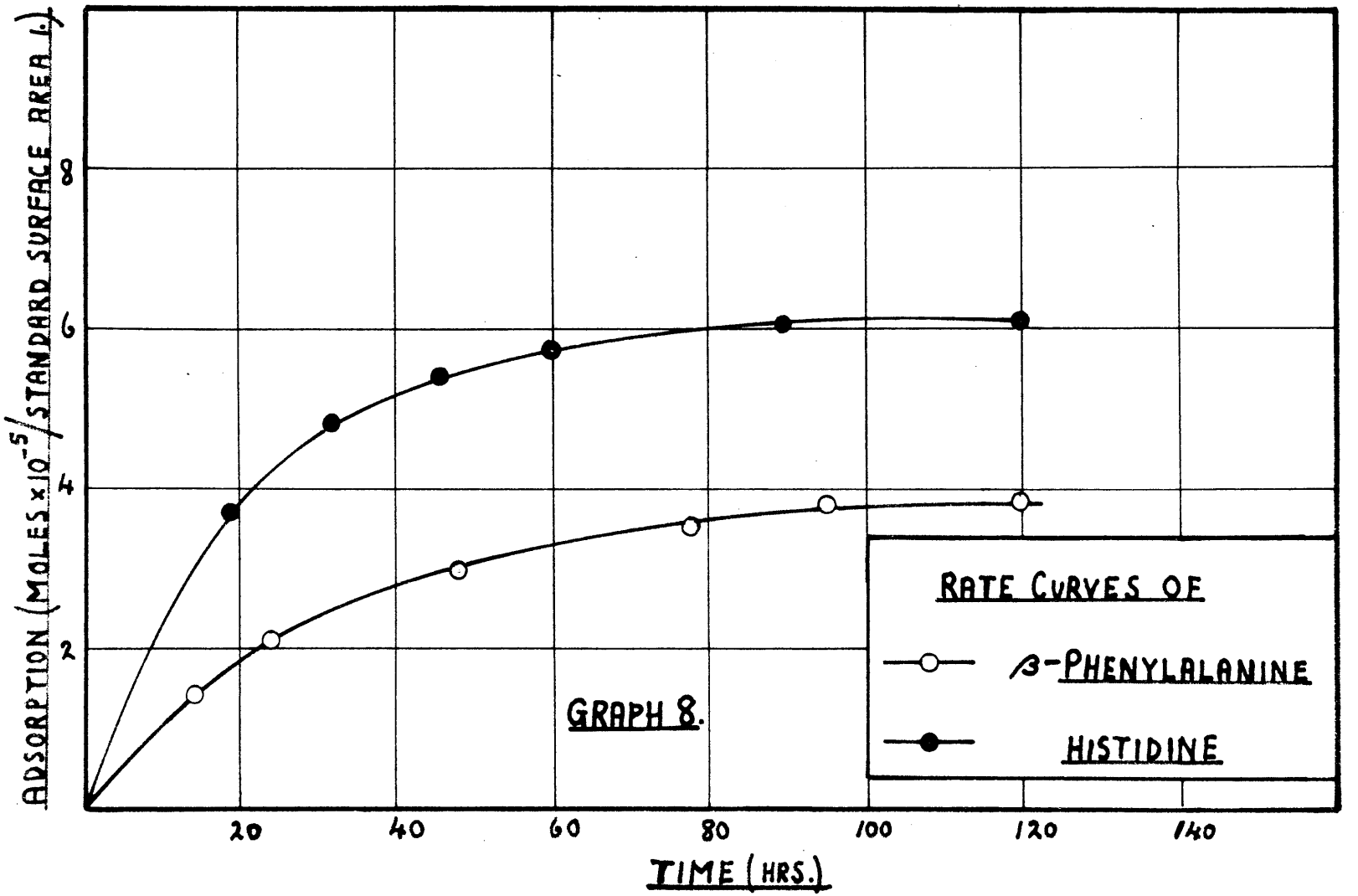
General Formula = $R.CH_2CH(NH_2).CO_2H.$

Amino Acid	Graph	R =	a_E	(k) 37°C.
α-alanine	4	-H	8.47	1.79
cysteine	6	-SH	12.75	2.13×10^{-1}
serine	-	-OH	2.86	$< 1 \times 10^{-2}$
asparagine	6	$-CONH_2$	5.3	1.45×10^{-1}
β-phenylalanine	8	$-C_6H_5$	3.8	3.275×10^{-2}
tryptophane	7	-indole radical	5.0	9.75×10^{-1}
histidine	8	-imidazole radical.	6.04	5.05×10^{-2}

* These figures do not represent equilibrium adsorption of serine, but are intermediate values, since the rate of adsorption of serine is so slow.


ABSORPTION (MOLES ×10⁻⁵/STANDARD SURFACE AREA)
12
9
6
3
5
10
15
20
25
30
35
TIME (HRS.)
GRAPH 6.
RATE CURVES OF
CYSTEINE
ASPARAGINE


ADSORPTION (MOLES x 10^{-5}/STANDARD SURFACE AREA)
1
2
3
4
5
1
2
3
4
5
6
7
TIME (HRS.)
GRAPH 7.
RATE CURVE OF TRYPTOPHANE


ADSORPTION (MOLES x10^{-5}/STANDARD SURFACE AREA)
8
6
4
2
20
40
60
80
100
120
140
TIME (HRS.)
GRAPH 8.
RATE CURVES OF
β-PHENYLALANINE
HISTIDINE

Serine.

However, when the highly polar hydroxyl group is introduced into the molecule, as in serine, the rate of adsorption is now even less than for glycine. The hydroxyl group has increased the polarity of the molecule[33] which now resembles glycine rather than α-alanine. In solution, the polar serine molecules interact with one another[33] and consequently resist adsorption. The serine molecule contains two electronegative centres, the carboxylate and hydroxyl groups, both of which will tend to be repelled by the negatively charged silica. This increase in electronegativity of the molecule is, therefore, an additional factor which tends to prevent or retard adsorption.

Asparagine.

In contrast to serine, the amino group of asparagine is observed to have increased the rate of adsorption which now resembles that of cysteine. The amino group is more polar than either the methyl or the thiol group, and the consequent interaction between the molecules of asparagine in solution will result in the observed decrease in extent of adsorption.

β-Phenylalanine.

On comparison with α-alanine, the introduction of the phenyl group is here observed to have decreased both the rate and extent of adsorption. The rate of adsorption of β-phenylalanine is not comparable with that for any other amino acid containing an aliphatic alkyl group, but is closely related to that of the polar glycine molecule. The phenyl group differs from the saturated alkyl groups in that it is considered to

be a hybrid of equivalent resonance forms and involves a fluctuation of electron density. Further, in the adsorbed state, the large benzene ring will conceal possible sites of adsorption on the surface of the silica and thus induce a low extent of adsorption.

Tryptophane.

The rate of adsorption for tryptophane is rapid and is comparable with that for α-aminobutyric acid and other amino acids, containing simple aliphatic alkyl groups. The contrast in adsorption behaviour between β-phenylalanine and tryptophane must be due to the new ring system containing a second nitrogen. However, the effect of pH on the adsorption of tryptophane, which is discussed in a later section, shows that the molecule is still adsorbed through the α-ammonium group and not through the indole -NH- group. The electropositive character of the molecule is increased by this second nitrogen-containing ring; and the stronger force of attraction between the molecule and the negatively charged silica results in the observed increase in rate, and extent of adsorption of tryptophane when compared with β-phenylalanine. Similar rapid adsorptions are observed with lysine and citrulline where the additional substituents have also increased the electropositive character of the molecule. In comparison with α-alanine, the low extent of adsorption is most probably due to the bulk of the large indole group.

Histidine.

The rate of adsorption for histidine, which is low, is comparable with that for glycine, and is characteristic of a highly polar molecule.

The ammonium group ($-NH_3^+$) is more electropositive than the imidazole group, as shown by comparison of their respective pK values, the negative logarithm of their acidity constants; pK for the ammonium group of histidine being 9.17 and for the imidazole group, 6.00[34]. Consequently, the adsorption of histidine takes place through the ammonium group and not the imidazole group. This is substantiated by experimental evidence which will be discussed when considering the effect of pH on adsorption. The extent of adsorption of histidine is lower than that of α-alanine, as would be expected of a highly polar molecule, but is greater than that of either β-phenylalanine or tryptophane. This observation supports the hypothesis that the bulky phenyl and indole groups decrease the extent of adsorption of the amino acid.

In table 2b are shown the experimental data for a number of additional amino acids in which alkyl groups of longer carbon chain length are further substituted.

Glutamic Acid.

Adsorption experiments were carried out with glutamic acid at pH5 and no adsorption was observed. From a study of the dissociation constants of the two carboxyl groups (table 3), it may be deduced that at pH5 one carboxyl group (-COOH) per molecule will be extensively dissociated into its negative carboxylate group ($-COO^-$), and the other partially dissociated. Consequently the molecule now tends to be electronegative in character, and will be repelled by the negative charge on the silica. A similar though less intense effect was shown by the hydroxyl group of serine, and thus these two amino acids clearly emphasise the

importance of the effect of the secondary group on the general polarity of the molecule, irrespective of the additional influence of the group in solution or in the adsorbed state.

Glutamine.

However, when the γ-carboxyl group of glutamic acid is replaced by the amide group, $-CONH_2$, the electronegative character of the molecule is no longer intense, and adsorption takes place fairly rapidly and to an appreciable extent. The adsorption behaviour of glutamine is similar to that of asparagine and the increased separation of the amide and ammonium groups, as in glutamine, has increased slightly both the rate and extent of adsorption.

Lysine.

The experimental data for Lysine show an interesting comparison with those for norleucine. The extent of adsorption of lysine at the isoelectric pH is very low, and is similar to that of norleucine (table 1). The Langmuir rate constant, (k) 37°C. for lysine, measured at pH 7.3, is slightly lower than that for norvaline, and has a value which would have been expected for norleucine.

Further, from the pK values of the two ammonium groups of lysine (table 4) it is observed that the ε-ammonium group is the more electro-positive, and also that at the isoelectric pH the α-ammonium group is completely dissociated into its uncharged state. Consequently, since adsorption still takes place at the isoelectric pH, it must do so through the ε-ammonium group and not through the α-group. This hypothesis is

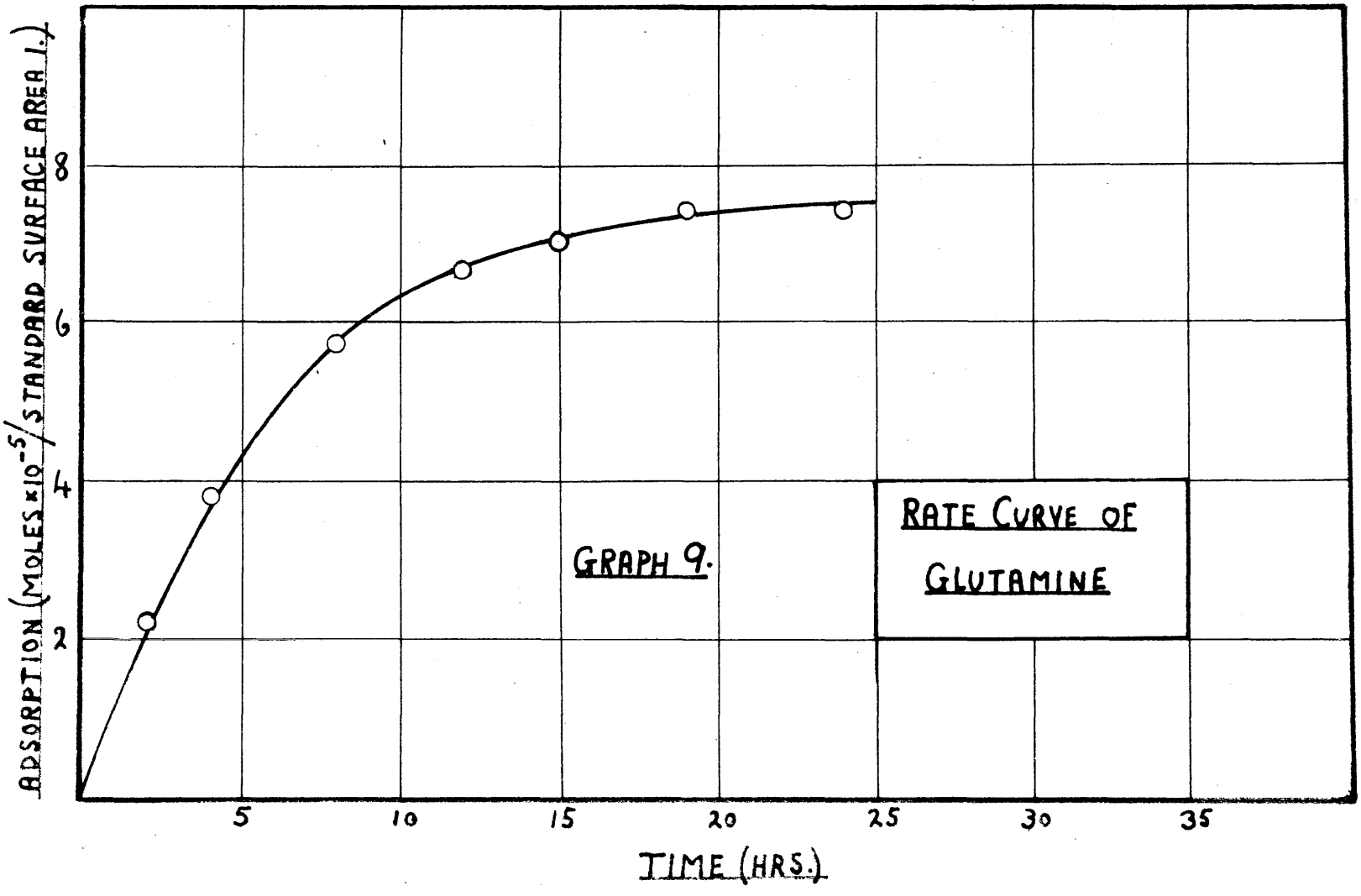
Table 2b.

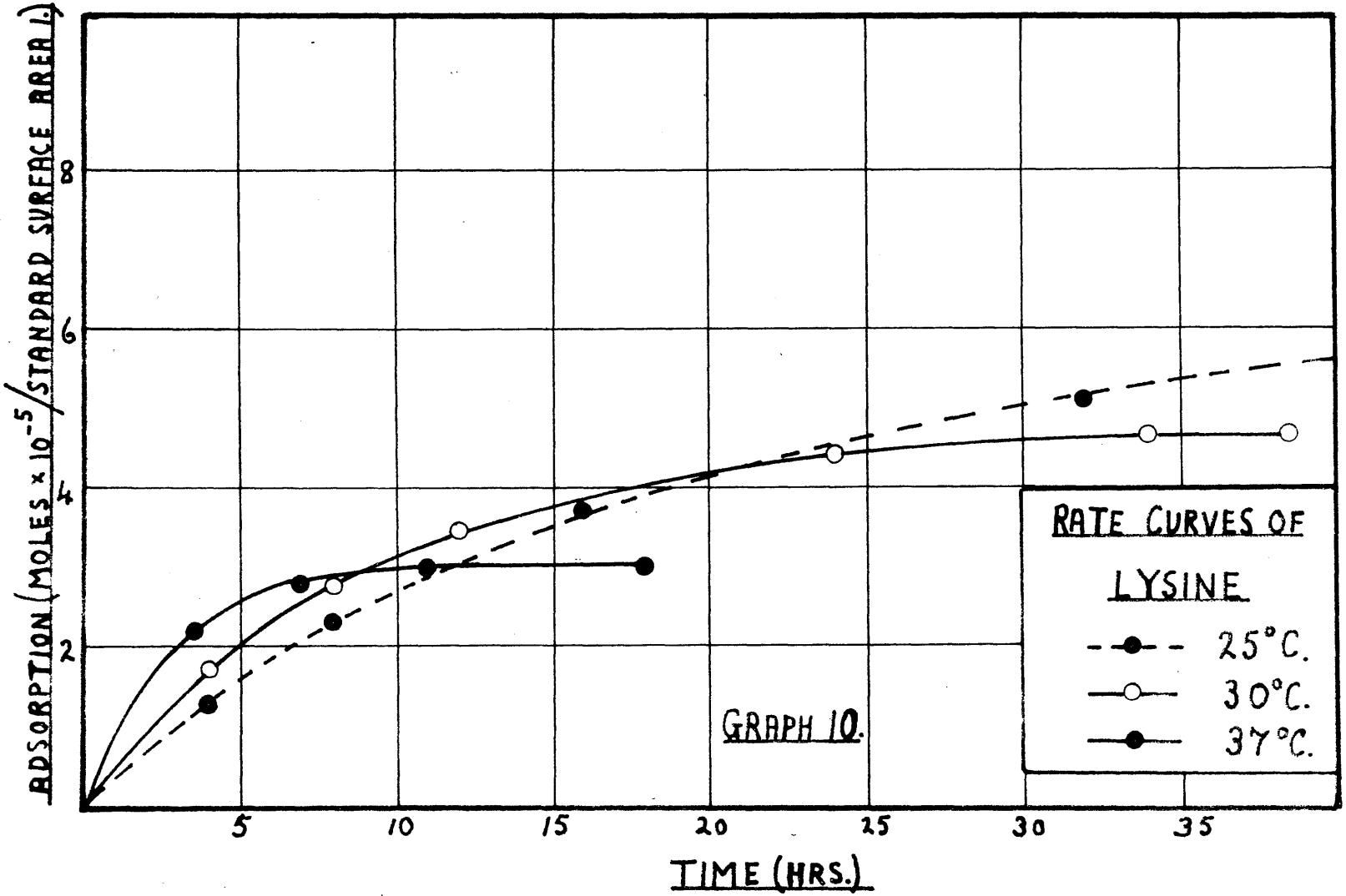
Tem. = 37°C.

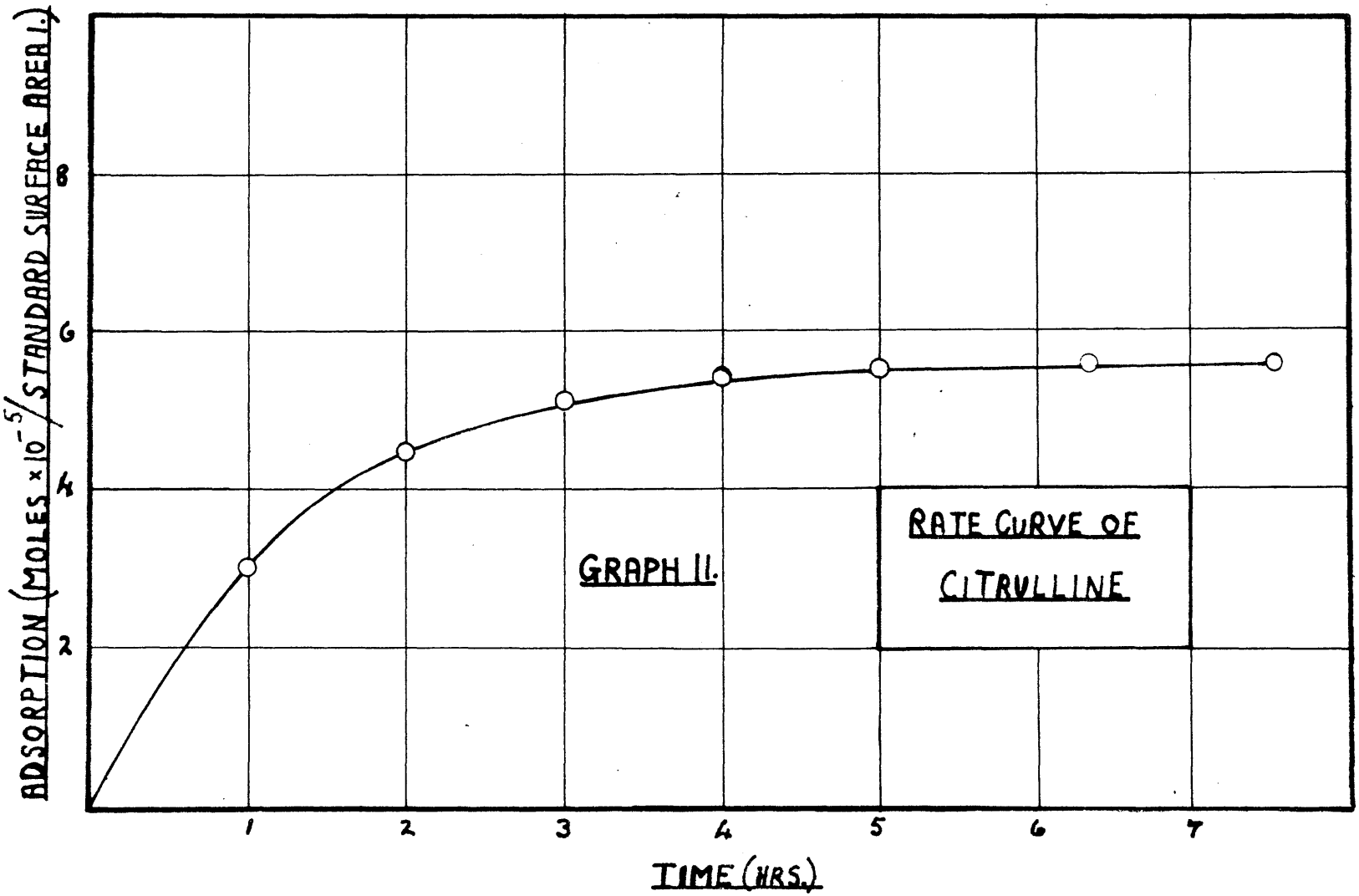
General Formula R. $(CH_2)_nCH(NH_2).CO_2H$.

Amino Acid	Graph	n	R =	a_E	(k) 37°C.
Glutamic Acid	-	2	-COOH	No Adsp^n.	-
Glutamine	9	2	$-CO.NH_2$	7.4	1.8×10^{-1}
✱ Lysine	10	4	$-NH_2$	1.0	3.77×10^{-1}
Citrulline	11	3	$-NH.CO.NH_2$	5.6	7.66×10^{-1}

✱ The rate of adsorption of lysine and the value of (k) 37°C. were measured at pH 7.3 since the adsorption of lysine is so small at the isoelectric pH.


ADSORPTION (MOLES x10⁻⁵/STANDARD SURFACE AREA I.)
2
4
6
8
5
10
15
20
25
30
35
TIME (HRS.)
GRAPH 9.
RATE CURVE OF GLUTAMINE


ADSORPTION (MOLES x 10^{-5}/STANDARD SURFACE AREA)
8
6
4
2
5
10
15
20
25
30
35
TIME (HRS.)
RATE CURVES OF
LYSINE
25°C.
30°C.
37°C.
GRAPH 10.


ADSORPTION (MOLES × 10^{-5}/STANDARD SURFACE AREA)
8
6
4
2
1
2
3
4
5
6
7
TIME (HRS.)
GRAPH II.
RATE CURVE OF CITRULLINE

also substantiated by data obtained from a study of the effect of pH on the absorption of lysine which will be discussed in a later section. An important corollary arising from the adsorption behaviour of lysine is therefore, that an amino acid containing two positively charged groups will be adsorbed through the more electropositive centre. This hypothesis has also been confirmed by the adsorption behaviour of arginine, which will be discussed in greater detail in a later section.

Citrulline.

The rate of adsorption for citrulline is high and its extent is slightly greater than for norleucine, although much lower than that for either α-alanine or cysteine. These results show the influence of the basic terminal ureido group, which increases slightly the electropositive character of the molecule. Because of its bulk, citrulline is adsorbed only to a small extent.

Experiments have shown that hydantoic acid (carbamyl-glycine) is not adsorbed at any pH, and it may therefore be deduced that citrulline cannot be adsorbed through the terminal ureido group. The reasons for the failure of hydantoic acid to adsorb are similar to those already discussed for urea itself.

1.3. The effect of separation of the ammonium and carboxylate groups in simple unsubstituted amino acids.

In Table 5 are shown the experimental data for a number of simple unsubstituted amino acids, conforming to the general formula $H_2N.(CH_2)_n.CO_2H$, in which the ammonium and carboxylate groups are separated by a straight

Table 3.[34]

Dissociation constants of glutamic acid at 25°C.

	pK
α- (COOH)	2.19
γ- (COOH)	4.25

Table 4.[34]

Dissociation constants of lysine at 25°C.

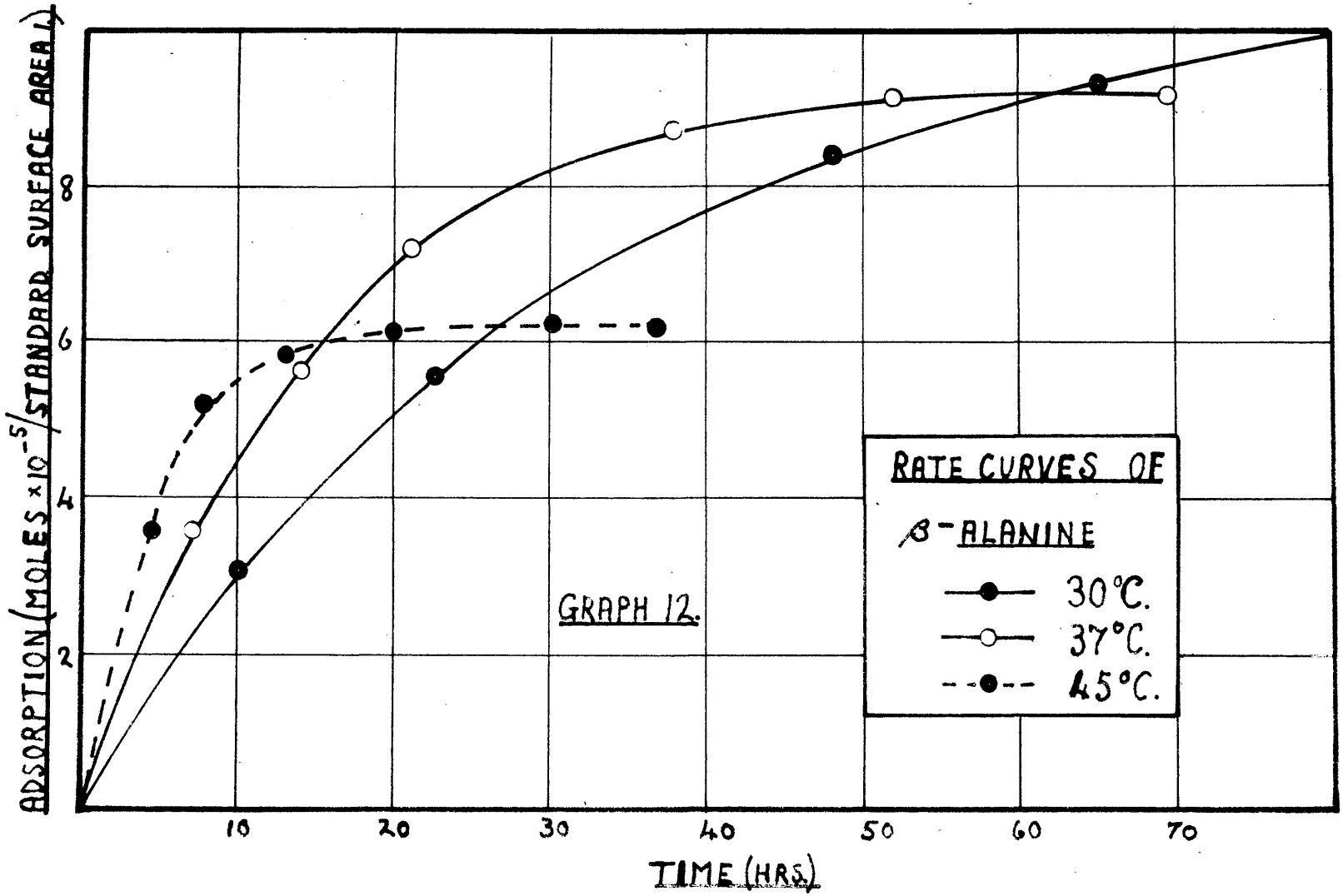
	pK	pI
α- (NH_3^+)	8.95	9.74
ε- (NH_3^+)	10.53	

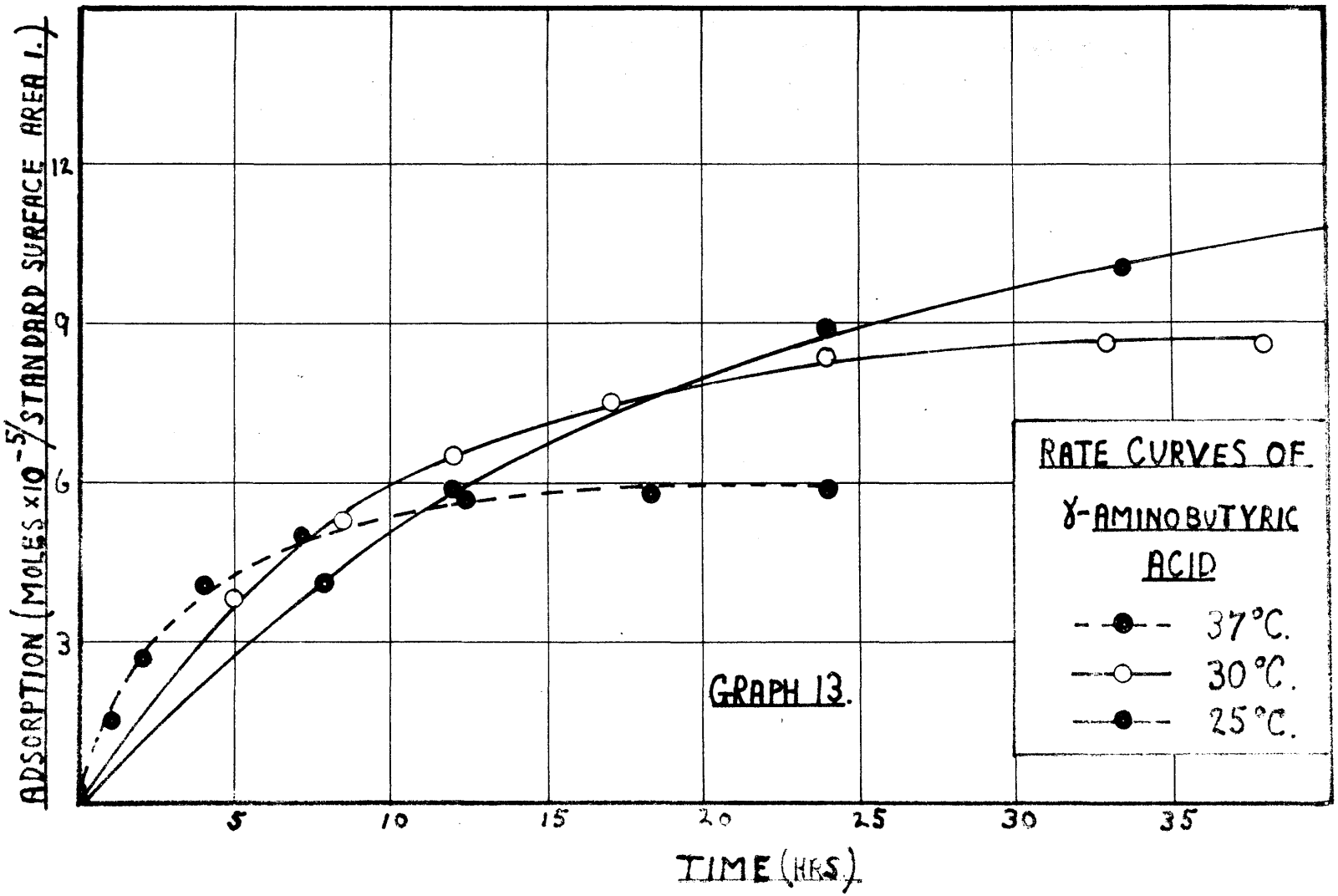
Table 5.

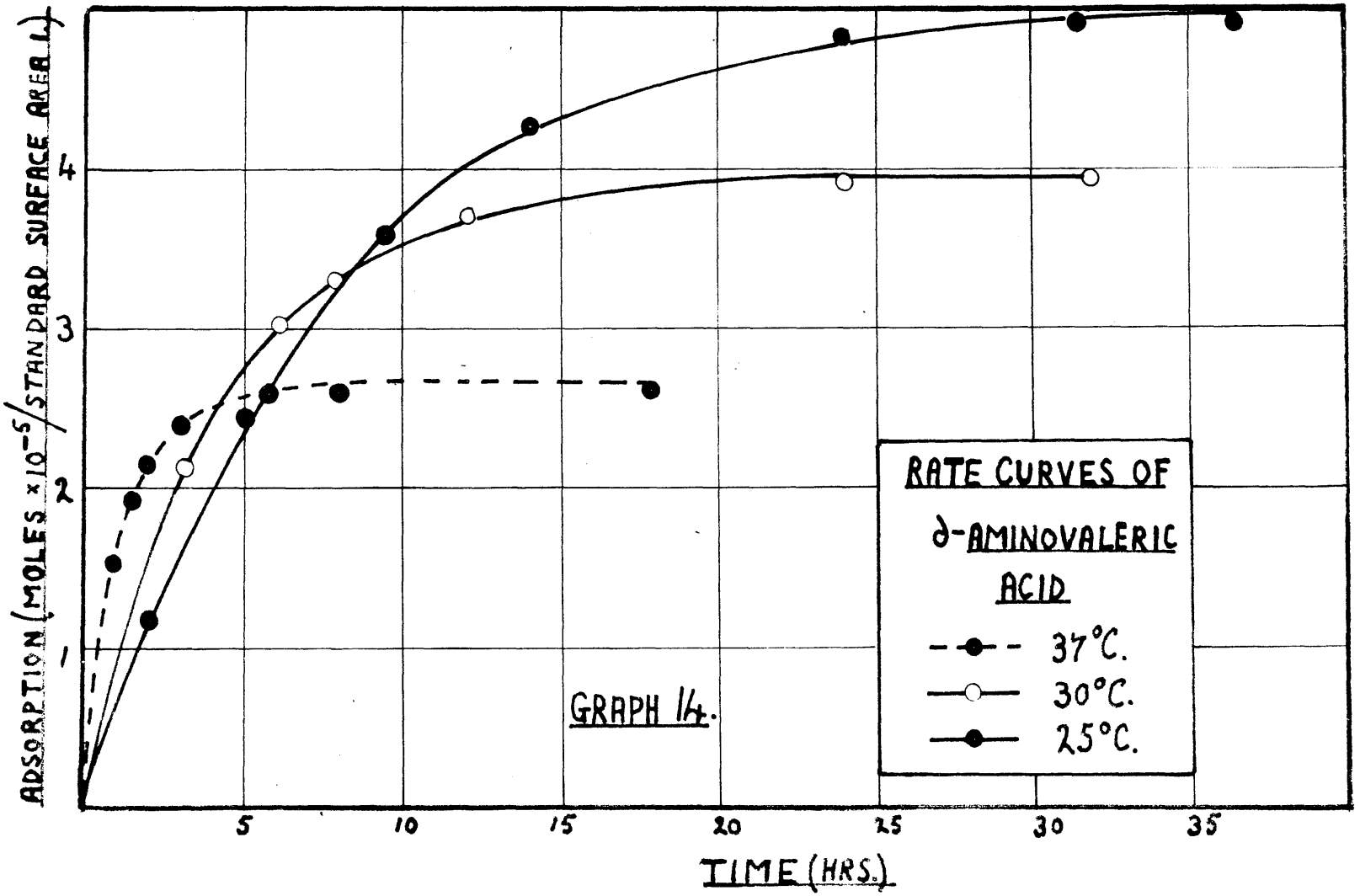
Temp. = 37°C.

General Formula = $H_2N.(CH_2)_n.CO_2H.$

Amino Acid	Graph	n	a_E	(k) 37°C.
Glycine	3	1	7.0	2.47×10^{-2}
β-Alanine	12	2	9.5	6.74×10^{-2}
γ-Aminobutyric Acid	13	3	5.8	3.1×10^{-1}
δ-Aminovaleric Acid	14	4	2.62	8.89×10^{-1}
ε-Aminocaproic Acid	-	5	0.71	1.2 approx.


ADSORPTION (MOLES × 10⁻⁵/STANDARD SURFACE AREA)
2
4
6
8
10
20
30
40
50
60
70
TIME (HRS.)
RATE CURVES OF
β-ALANINE
30°C.
37°C.
45°C.
GRAPH 12.


ADSORPTION (MOLES ×10⁻⁵/STANDARD SURFACE AREA L.)
12
9
6
3
5
10
15
20
25
30
35
TIME (HRS.)
RATE CURVES OF
γ-AMINOBUTYRIC
ACID
37°C.
30°C.
25°C.
GRAPH 13.


ADSORPTION (MOLES $\times 10^{-5}$/STANDARD SURFACE AREA)
1
2
3
4
5
10
15
20
25
30
35
TIME (HRS.)
RATE CURVES OF
δ-AMINOVALERIC
ACID
37°C.
30°C.
25°C.
GRAPH 14.

carbon chain of increasing length. From these data, conclusions may be drawn on the effect of increased separation of the two polar groups on the adsorption behaviour of the molecule.

Table 5 shows that both the rate and extent of adsorption of β-alanine are greater than for glycine; and it may consequently be deduced that the carboxylate group, which in glycine is in close proximity to the ammonium group, exerts an influence tending to retard the adsorption of the amino acid. This adverse influence is as to be expected of a highly electronegative group which will tend to be repelled by the negatively charged silica. Additional supporting evidence may be derived from the fact that the amino acid taurine is not adsorbed by the silica. Taurine is closely related to β-alanine, but contains the sulphonate group ($-SO_3^-$) in place of the carboxylate group. This sulphonate group is of greater volume than the carboxylate group, and is also much more strongly electronegative, as shown by comparison of their respective dissociation constants[34]. Consequently, the increase in intensity and area of influence of the electronegative group, as in taurine, will result in a stronger force of repulsion between the silica and the amino acid molecule, and no adsorption takes place. Thus, it may be deduced that in β-alanine the carboxylate group exerts a similar, although less intense, adverse influence on adsorption of the molecule. This reasoning may be carried further, to deduce that when the carboxylate group is in closer proximity to the ammonium group, as in glycine, its influence in retarding adsorption will be even greater than that shown in β-alanine.

The data in table 5 for the remaining amino acids show that, on

increasing the length of the carbon chain, there is a progressive increase in rate but a decrease in extent of adsorption of the amino acid. A comparison of the dissociation constants of the ammonium groups of the respective amino acids[34] shows little change in their electropositive strength; and it must be concluded, therefore, that these changes in adsorption behaviour of the amino acids are the direct result of the increased carbon chain lengths. It has already been shown in a previous section, that the adsorption characteristics of norvaline and noleucine, which contain long carbon chains, are small but rapid adsorption. It appears that in β-alanine the molecule is too small to have any significant steric effect in the adsorbed state, and that it is the decreasing influence of the carboxylate group which determines the adsorption behaviour of the molecule.

1.4. Substitution of the ammonium group.

In table 6 are reported the experimental data for the adsorption of a number of related substances in which there is substitution in the free ammonium group. Using these data conclusions may be drawn on the influence of the additional substituent on the adsorption of the molecule.

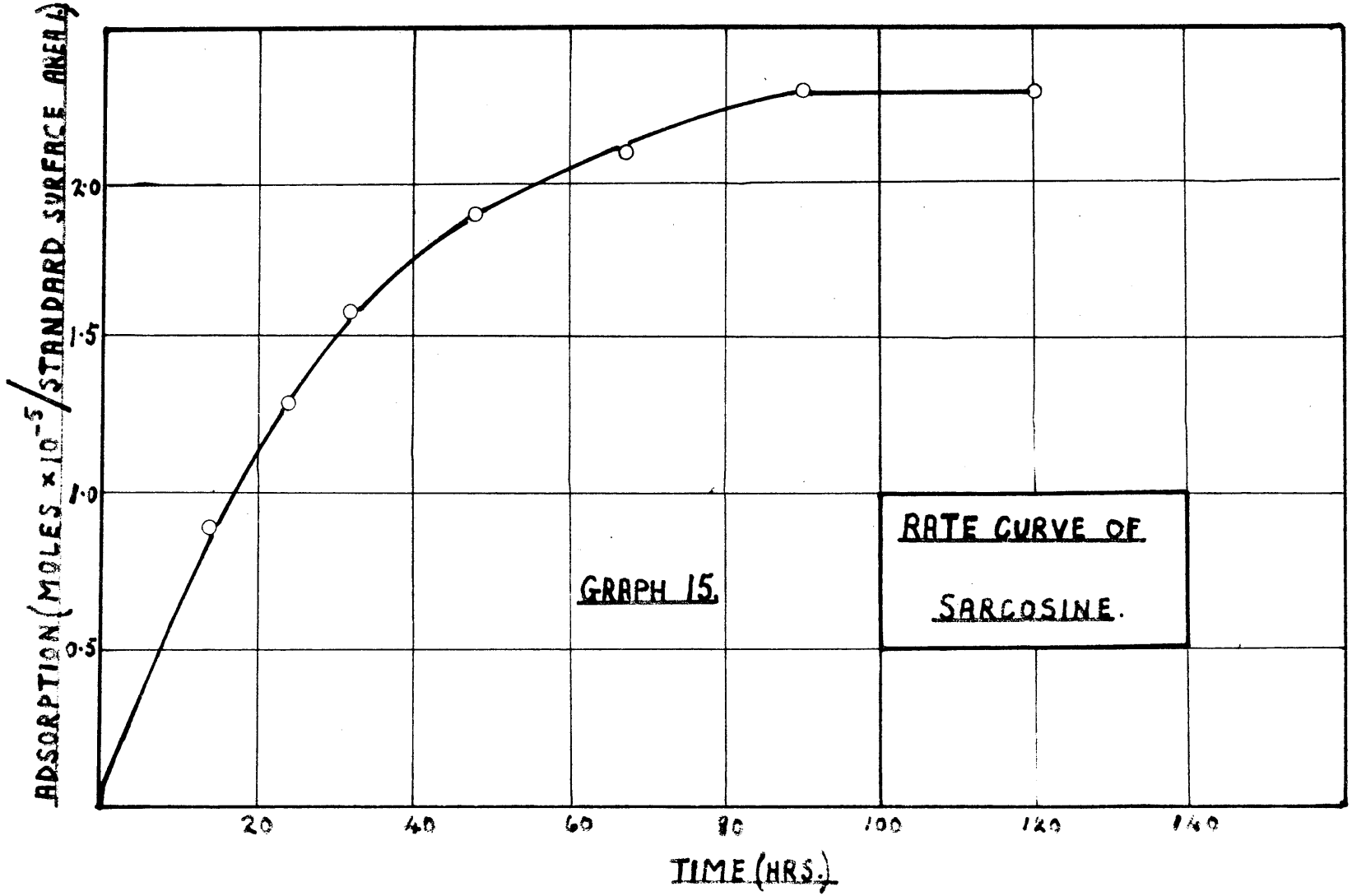
The acetylation of the free ammonium group of glycine is found to prevent completely the adsorption of the molecule. However, sarcosine, a related structure, is adsorbed by the silica, although to a small extent. Consequently, the complete inhibition of adsorption shown by N-acetylglycine must be due to the close proximity of the acetyl group to the imino group. The acetyl group, which is electronegative in character, will be repelled by the negatively charged silica and thus inhibit adsorption. A similar

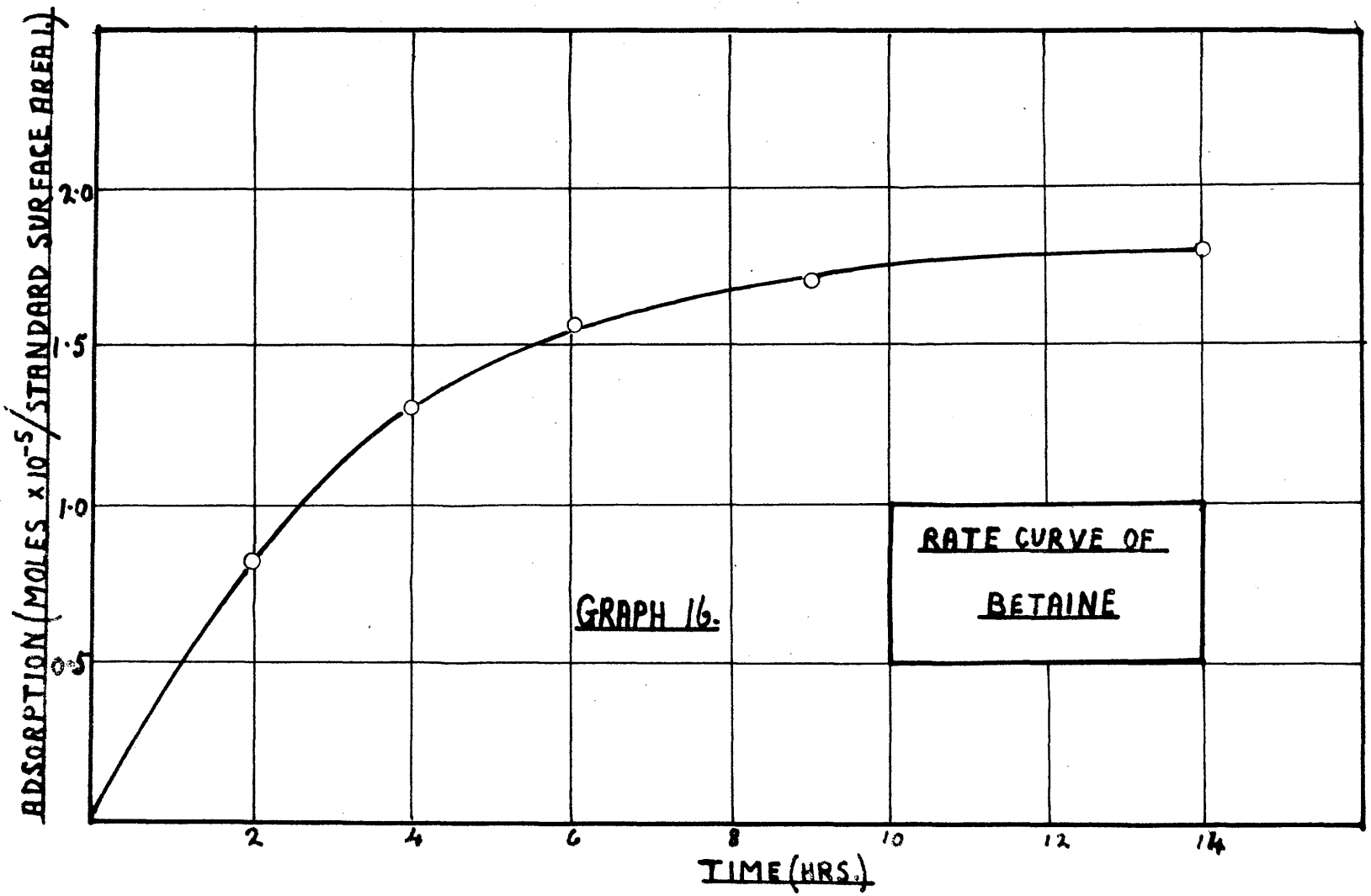
Table 6.

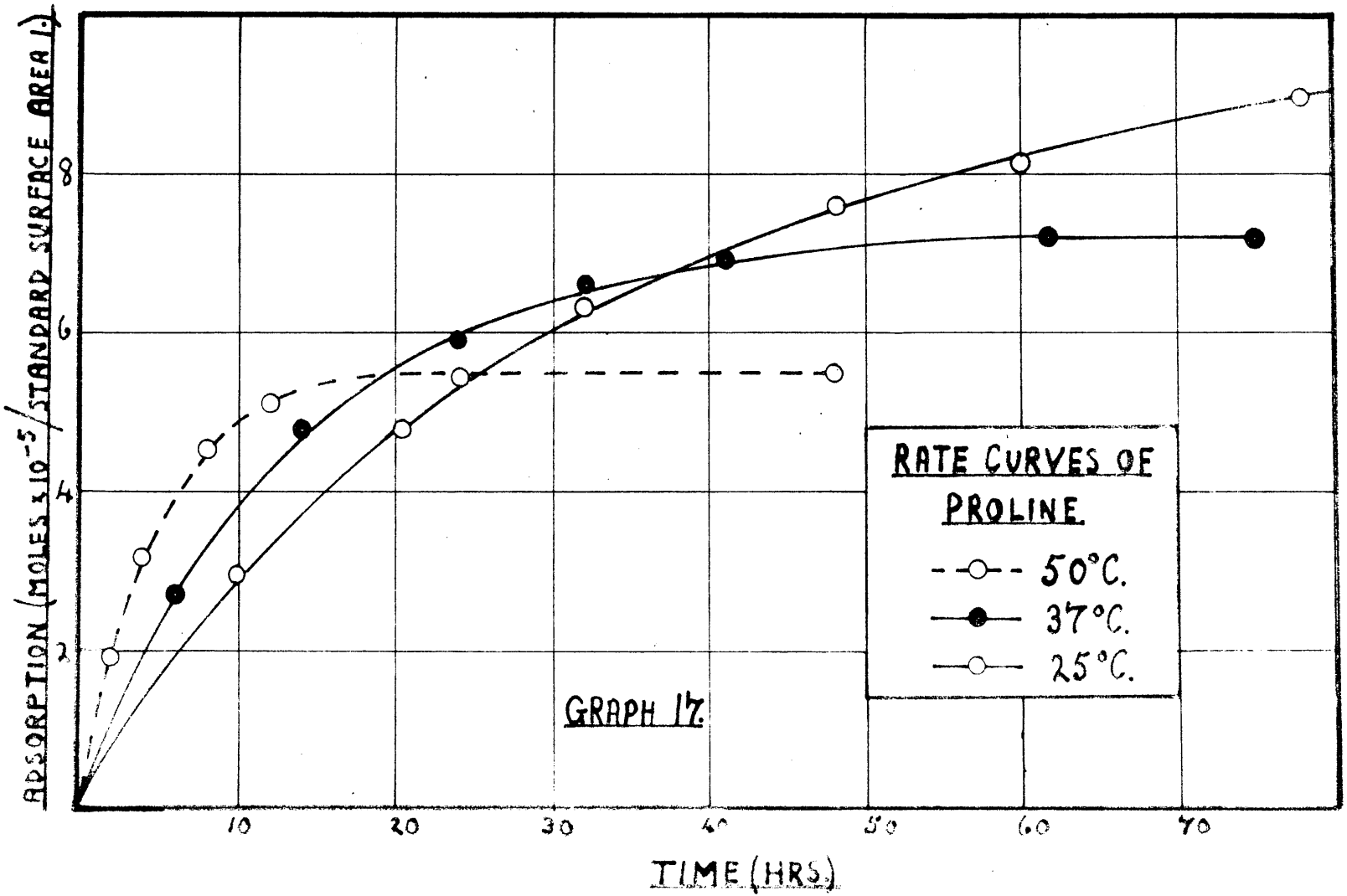
Temp. = 37°C.

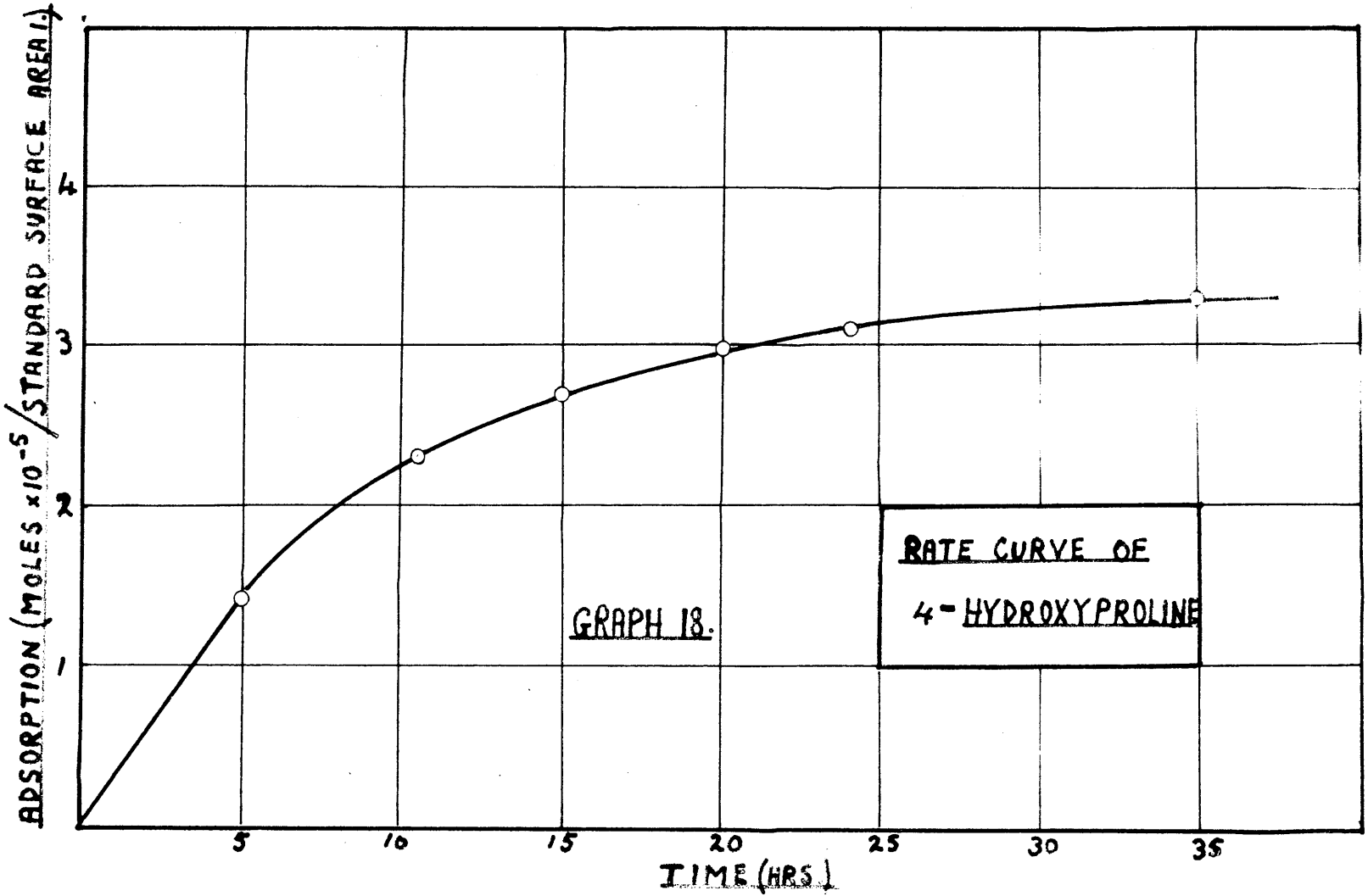
Amino Acid	Graph	a_E	(k) 37°C.
Glycine	3	7.0	2.47×10^{-2}
N-Acetylglycine	-	No Adsp$^{n.}$	-
Sarcosine	15	2.3	3.54×10^{-2}
+ Betaine	16	1.8	3.16×10^{-1}
Proline	17	7.2	7.84×10^{-2}
Hydroxyproline	18	3.3	1.1×10^{-1}

+ These figures shown for betaine were obtained from an 0.01 M aqueous solution of the hydrochloride at pH 2.14.


ADSORPTION (MOLES ×10⁻⁵/STANDARD SURFACE AREA)
2·0
1·5
1·0
0·5
20
40
60
80
100
120
140
TIME (HRS.)
GRAPH 15.
RATE CURVE OF
SARCOSINE.


ADSORPTION (MOLES x10⁻⁵/STANDARD SURFACE AREA)
2·0
1·5
1·0
0·5
2
4
6
8
10
12
14
TIME (HRS.)
GRAPH 16.
RATE CURVE OF
BETAINE


ADSORPTION (MOLES x 10⁻⁵ / STANDARD SURFACE AREA I)
2
4
6
8
10
20
30
40
50
60
70
TIME (HRS.)
RATE CURVES OF PROLINE
50°C.
37°C.
25°C.
GRAPH 17.


ADSORPTION (MOLES x10⁻⁵ /STANDARD SURFACE AREA)
1
2
3
4
5
10
15
20
25
30
35
TIME (HRS)
GRAPH 18.
RATE CURVE OF
4-HYDROXYPROLINE

influence was shown by the carbonyl group in both urea and hydantoic acid. However, the adsorption behaviour of N-acetylglycine is very significant, since it contains the important peptide linkage, free from the effects of other substituents, and it may, therefore, be deduced that the adsorption of any other amino acid, peptide or protein molecule does not take place through the imino group of the peptide linkage. Further, since N-acetylglycine is more acidic by 1.2 pH units than the fatty acid of comparable length, n-valeric acid[36], the peptide linkage is thus observed to increase the electronegativity of the molecule by influencing the dissociation of the carboxyl group.

On substitution of the ammonium group of glycine by an increasing number of methyl groups, there is a progressive increase in rate but decrease in extent of adsorption of the molecule, as shown by sarcosine and betaine. The adsorption of betaine was carried out from an 0.01 M aqueous solution of the hydrochloride at pH 2.14. In solution the betaine system may be represented as:-

$$(CH_3)_3 \cdot \overset{+}{N}.CH_2.\overset{-}{COO} \quad + HCl.$$

In the zwitterion the nitrogen atom bears a strong positive charge, but at pH 2.14 the adjacent carboxyl group (pK 1.84)[34] will be partially dissociated to its negatively charged carboxylate ion which will tend to retard the adsorption of the molecule.

Anomalous results have been obtained for the adsorption behaviour of proline and hydroxyproline. The experimental data for proline show no relationship with those for its straight chain analogues, α- and δ-

aminovaleric acids, the extent of adsorption of proline being greater and its rate less. However, as is to be expected, the rate of adsorption of proline is intermediate between those of the related structure, sarcosine and betaine, although its extent is considerably higher. Neither of these three amino acids contain an ammonium group, but sarcosine and proline both contain an imino group and betaine a completely substituted ammonium group.

The rate of adsorption for hydroxyproline is also very closely related to that for betaine. Comparison of the experimental data for proline and hydroxyproline shows that the hydroxyl group of the latter has slightly increased the rate of adsorption of the molecule and decreased the extent. This slight increase in rate is in direct contrast to the effect shown by the hydroxyl group in the straight chain amino acid, serine. However, Smith (33) has shown that the introduction of the hydroxyl group into the proline ring does not produce a similar strong interaction between the molecules in solution, as does the hydroxyl group of serine. Consequently, there should be no pronounced change in rate of adsorption between proline and hydroxyproline; the slight change observed is negligible when compared with that observed in the pair, α-alanine -- serine. Since there are two electronegative groups in the hydroxyproline molecule, both of which will be repelled by the negatively charged silica, the lower extent of adsorption, as compared with that for proline, is to be expected.

1.5. Miscellaneous simple nitrogenous compounds.

In table 7 are reported the experimental data for a number of

Table 7.

Temp. = 37°C.

Substance	Graph	a_E	(k) 37°C.
Glycine	3	7.0	2.47×10^{-2}
Glycocyamine	2	4.9	8.74×10^{-2}
Hydantoic Acid	-	No Adsp$^{n.}$	-
Semicarbazide Ion	1	9.6	2.56×10^{-1}
Guanidinium Ion	1	12.3	7.63×10^{-1}

simple nitrogenous compounds which are closely related in structure to glycine, and using our previous conclusions it may be possible to elucidate these data. Although these additional compounds are not true amino acids they contain polar groups similar to those of the amino acids.

The pH of an 0.01 M solution of glycocyamine (guanidinoacetic acid) in distilled water was 8.4 approx., which represents its isoelectric pH. A similar solution of glycine had a pH of 5.97, the isoelectric pH of glycine; and these data, therefore, show that the guanidinium group of glycocyamine is more electropositive than the ammonium group of glycine. Thus, the glycocyamine molecule will be more strongly attracted by the silica than the glycine molecule, and the greater rate of adsorption of glycocyamine is to be expected. However, because of the stronger electropositive nature of the guanidinium group, the force of interaction between individual molecules in the glycocyamine solution will be greater than that in the glycine solution. This effect tends to oppose the increased force of attraction for the silica and results in the lower extent of adsorption for glycocyamine.

It has already been reported that hydantoic acid is not adsorbed, for reasons similar to those given for urea itself. However, in citrulline (table 2b), the weakly basic character of the urea group appears to exert a slight influence on the adsorption behaviour of the molecule.

The adsorption data for the semicarbazide ion (table 7), $H_2N.CO.NH.NH_3^+$, were obtained from an aqueous solution of the hydrochloride of pH 2.2, in which the ion concentration was 0.1 M. Similarly, the data for the guanidinium ion were obtained from an aqueous solution of the

carbonate of pH 11 approx., where the ion concentration was 0.1 M. A comparison of the data for the respective ions shows that the guanidinium ion is more rapidly adsorbed and to a greater extent than the semicarbazide ion. These results show that the guanidinium ion is the more strongly electropositive and further the semicarbazide ion contains the electronegative carbonyl group which tends to be repelled by the silica. It should be noted, however, that these structures are true ions, as distinct from zwitterions, and consequently there is no interaction between individual ions in solution which would retard adsorption.

Summary of Section 1.

In the preceding section the adsorption characteristics of a number of amino acids and related substances have been discussed in detail. In addition to the ammonium and carboxylate groups, the influence of other secondary substituents contained in the molecule, on the adsorption behaviour of the amino acid have also been studied. It is now convenient to summarise the preceding data in the form of general conclusions.

(A) General Conclusions.

(1) In aqueous suspension, the silica adsorbent is negatively charged. Adsorption of the amino acids from aqueous solution occurs primarily by mutual attraction of the positively charged ammonium group of these molecules and the negatively charged silica. The semicarbazide and guanidinium ions are also adsorbed by virtue of their positive charge.

(2) The rate and extent of adsorption of the amino acid are determined by the following considerations:-

(a) the interaction of the molecules with one another in aqueous solution;

(b) the nature and position of secondary substituent groups within the molecule;

(c) the influence of these secondary substituents on the interaction between the amino acid molecules in solution;

(d) bulk of the secondary substituent in the adsorbed molecules, which, for large substituents, will hinder further adsorption.

(B) Influence of secondary substituent groups.

(1) Alkyl groups. (In $R.CH(NH_2).CO_2H$, where R = alkyl group.)

In general, rates of adsorption are high for amino acids conforming to the formula, $R.CH(NH_2).CO_2H$, where R is an alkyl group. As R increases in bulk, there is a slight decrease in the rate and a pronounced decrease in extent of adsorption.

(2) Further substitution in the alkyl group. ($R.(CH_2)_n.CH(NH_2).CO_2H$)

The polarity of R is very important. If strongly electronegative, as is the hydroxyl group of serine, the rate of adsorption of the amino acid is extremely low, and the extent very small. In the case of glutamic acid, where R is the strongly electronegative carboxyl group, adsorption is completely inhibited. When less strongly polar, R influences both the electropositive strength of the molecule and the extent of interaction between individual molecules in solution. For highly polar, electropositive amino acids (n = 1), this interaction in solution is considerable and results in rates and extents of adsorption which are both lower than for α-alanine. However, when R is strongly electropositive, as in histidine, this adverse effect in solution may be modified by the increased force of

attraction of the silica for the more strongly electropositive amino acid molecule. As the value of n increases, the extent of adsorption decreases owing to the bulk of the molecule in the adsorbed state.

(3) Separation of the ammonium and carboxylate groups.

$H_2N.(CH_2)_n.CO_2H.$

The rate and extent of adsorption of β-alanine (n = 2.) are both greater than for glycine, indicating that the carboxylate group exerts an influence on the adjacent ammonium group in glycine which tends to retard or prevent adsorption. On increasing separation (n > 2), the rate of adsorption of the molecule becomes increasingly rapid but the extent decreases sharply.

(4) Substitution in the ammonium group.

N-acetylglycine is not adsorbed, because of the electronegative acetyl group adjacent to the imino group. The result shows that amino acids and peptides are not adsorbed through the peptide linkage. Increasing substitution of the ammonium group of glycine by the methyl group gives rise to an increase in rate and decrease in extent of adsorption, from sarcosine to betaine.

Notes.

(1) The benzene ring of β-phenylalanine (table 2a) decreases in a pronounced manner the rate and extent of adsorption of the molecule when compared with α-alanine.

(2) The rate of adsorption of tryptophane, containing the indole group, is considerably greater than that of β-phenylalanine.

(3) Lysine is adsorbed through the ε-ammonium group, which is more electropositive than the α-ammonium group.

(4) Proline and hydroxyproline, which are both adsorbed through an imino group, show best comparison to sarcosine and betaine.

(5) Neither urea nor hydantoic acid is adsorbed, which suggests that neither glutamine nor asparagine can be adsorbed through their amide groups.

(6) Taurine is not adsorbed, which emphasises the adverse effect of the large electronegative group.

Section 2.

Effect of pH on adsorption.

2.1. The effect of pH in the series $R.CH(NH_2).CO_2H$

(where R = alkyl group).

All experiments to determine the effect of pH on adsorption were carried out from 0.01 M aqueous solutions of the amino acids at 25°C. These solutions were unbuffered since it is known that inorganic salts alter the solubilities of the amino acids in water[37] and also influence the interaction between the molecules in solution.

The fundamental factor involved when the pH of the amino acid solution is altered is the change in extent of dissociation of the amino and carboxyl groups; consequently the effect of pH on the adsorption of the amino acid may be discussed from this point of view.

The effect of pH on the extent of adsorption of glycine and the α-alkyl substituted amino acids is shown in graph 19, and the essential experimental data have been summarised and are reported in table 8.

Of these amino acids, only glycine is not adsorbed to the maximum extent at its isoelectric pH. However, as the pH of the solution decreases from the isoelectric pH of the amino acid, the extent of dissociation of the carboxyl group (-COOH) into the negatively charged carboxylate group ($-COO^-$) also decreases. Simultaneously, with decrease in pH of the solution, it is observed from graph 19 that the extent of adsorption of glycine increases to a maximum (table 8) which is attained over the pH range 4.3 - 5.5. Consequently, it may be concluded that the

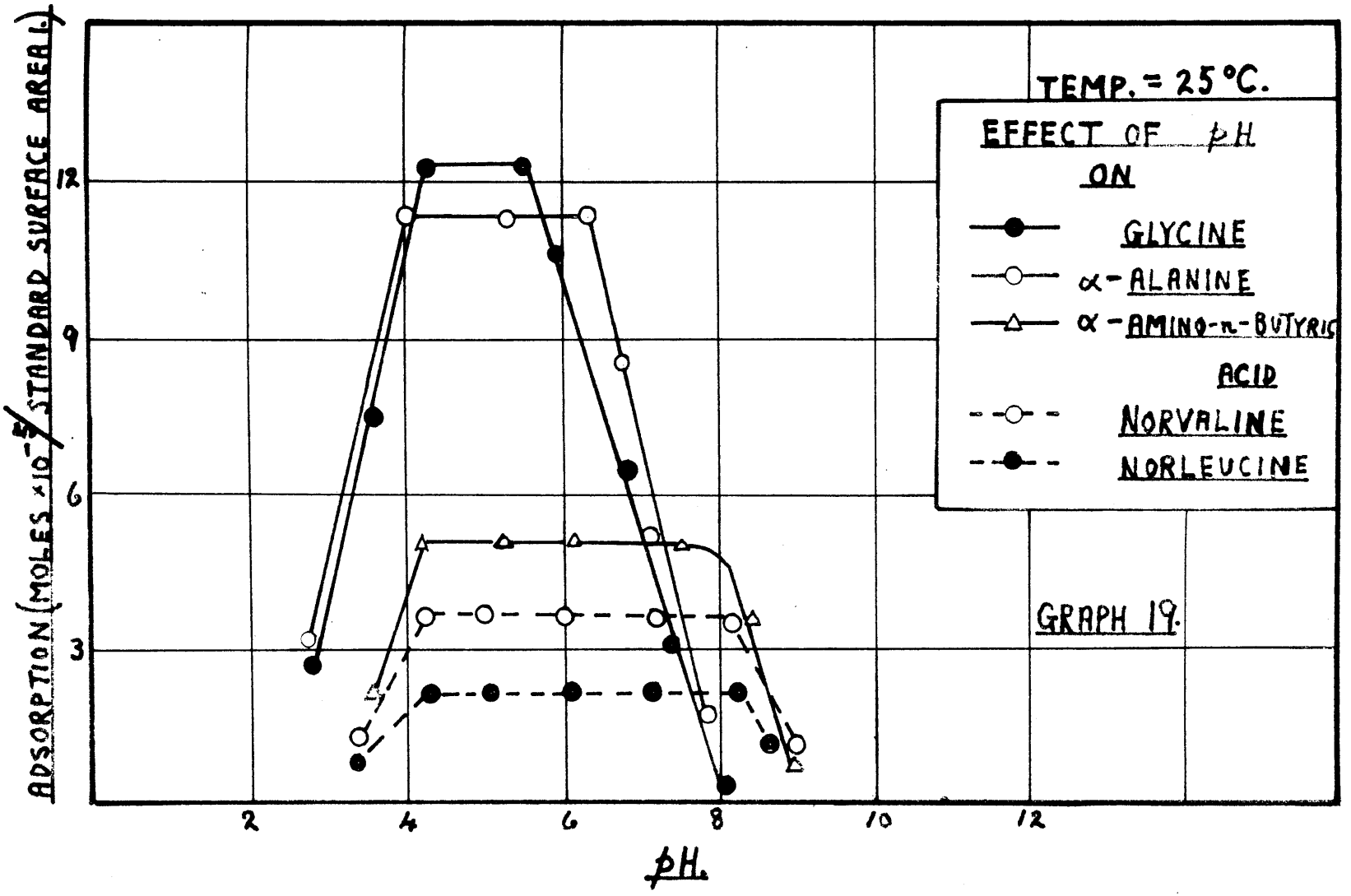
Table 8.

Temp. = 25°C.

Amino Acid	$a_{max.}$	$pH_{max.}$
Glycine	12.2	4.3 - 5.5
α-Alanine	11.3	4.0 - 6.3
α-Aminobutyric Acid	5.2	4.15- 7.5.
Norvaline	3.7	4.2 - 8.2
Norleucine	2.1	4.2 - 8.3

$a_{max.}$ = maximum adsorption in moles x 10^{-5}/standard surface area 1.

$pH_{max.}$ = pH range within which maximum adsorption is attained.


TEMP. = 25°C.
EFFECT OF pH
ON
GLYCINE
α-ALANINE
α-AMINO-n-BUTYRIC
ACID
NORVALINE
NORLEUCINE
GRAPH 19.
ADSORPTION (MOLES ×10⁻⁵/STANDARD SURFACE AREA)
3
6
9
12
2
4
6
8
10
12
pH.

negatively charged carboxylate group, exerts an influence tending to retard or prevent the adsorption of the amino acid. This conclusion is to be expected, since there will be a force of repulsion between the carboxylate group and the negatively charged silica.

In strongly acidic solutions, of pH 2 - 3 approx., low adsorptions are obtained for all amino acids, irrespective of structure. Verwey[38] has shown that the zeta-potential of silica particles in aqueous media of increasing (H^+) moves systematically in the positive direction. The following data is given by Verwey:-

Zeta-potential of silica in 10^{-4}n HCl (in millivolts)	= -56.
Zeta-potential of silica in 10^{-3}n HCl (" ")	= -37.
Zeta-potential of silica in 10^{-2}n HCl (" ")	= -20.
Zeta-potential of silica in 0.1n HCl (" ")	= weakly positive (Not to be measured).

Considering the above data, it becomes clear that the low adsorptions of the amino acids in strongly acidic solutions are due to the change in zeta-potential of the silica particles.

As the pH of the solution increases beyond the isoelectric pH of the amino acid, the extent of dissociation of the positively charged ammonium group, into its uncharged amino form, also increases. Simultaneously, the extent of adsorption of glycine, and of all amino acids studied, decreases rapidly and finally ceases at a pH where the ammonium group is virtually completely in its uncharged amino form. These observations show that adsorption of these amino acids takes place through the ammonium group

by virtue of its positive charge.

From table 8 it is observed that the pH range for maximum adsorption of α-alanine is greater than that for glycine. Further, α-alanine and the other α-alkyl amino acids attain maximum adsorption at their isoelectric points. These results indicate that the non-polar methyl group exerts an influence which modifies the adverse effects of the carboxylate group. The remaining α-alkyl amino acids in table 8 show that, as the alkyl group increases in size, there is a most pronounced decrease in extent of maximum adsorption, although there is an increase in the pH range within which it takes place. This increase in pH range for maximum adsorption indicates that the influence of the alkyl group increases with its length, and from table 8 is observed to attain its maximum effect with norvaline and remains constant with norleucine. The observed decrease in extent of adsorption of the amino acid, accompanying the increase in length of the alkyl group, has already been observed in section 1 when discussing the rates of adsorption of these same amino acids.

2.2. The effect of pH in the series $H_2N.(CH_2)_n.CO_2H$.

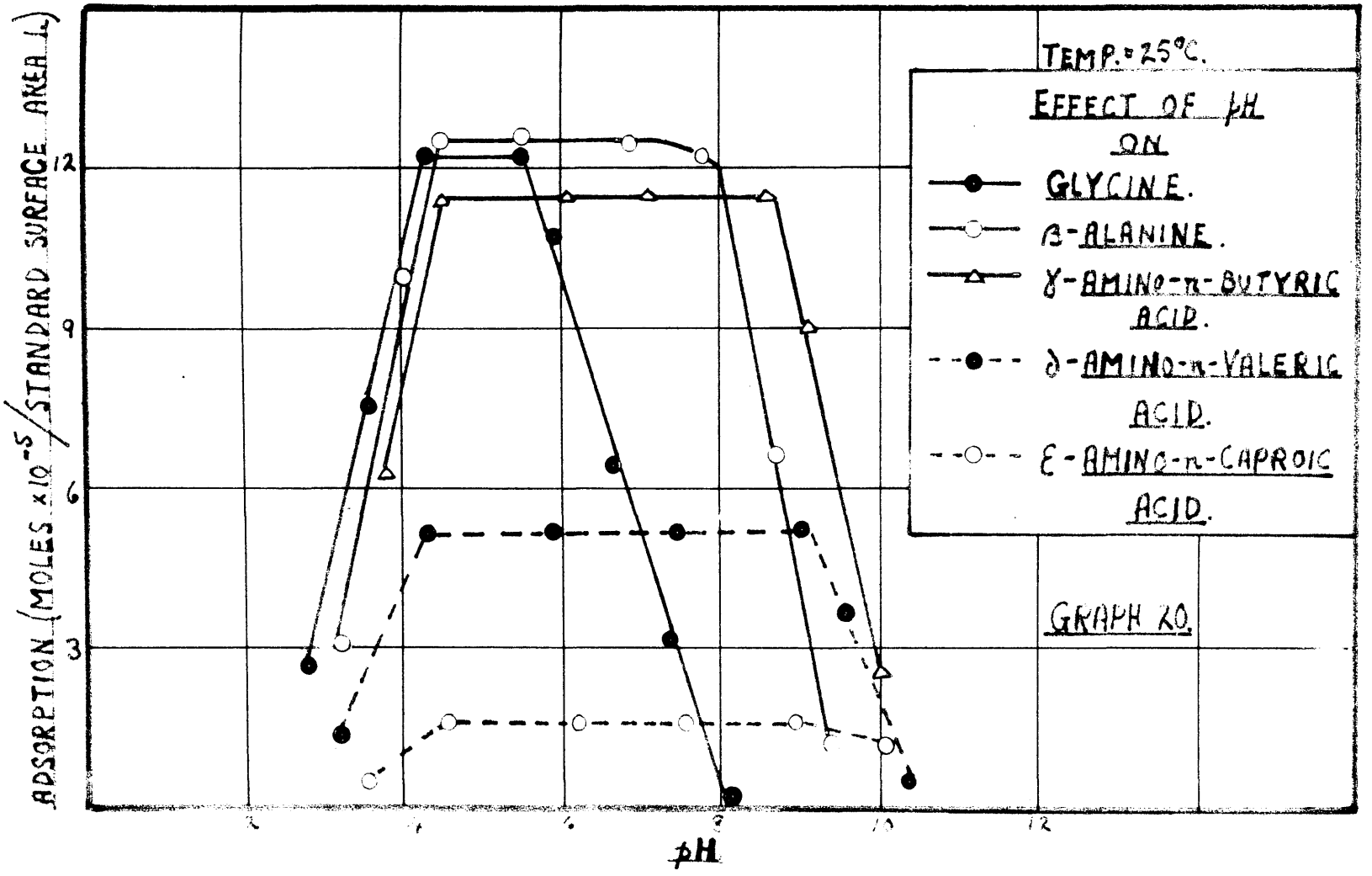
The effect of pH on the extent of adsorption of the amino acids in this series is shown in graph 20. The essential experimental data have been summarised and are shown in table 9.

The general behaviour of these amino acids resembles that shown by the α-alkyl amino acids of the preceding series. At alkaline pH values, the extent of adsorption of the present series again decreases rapidly with increasing dissociation of the ammonium group and finally ceases when the

Table 9.

Temp. = 25°C.

Amino Acid	$a_{max.}$	$pH_{max.}$
Glycine	12.2	4.3 - 5.5
β-Alanine	12.45	4.4 - 7.7
γ-Aminobutyric Acid	11.6	4.5 - 8.5
δ- Aminovaleric Acid	5.1	4.3 - 9.1
ε-Aminocaproic Acid	1.49	4.5 - 9.4


TEMP. = 25°C.
EFFECT OF pH ON
GLYCINE.
β-ALANINE.
γ-AMINO-n-BUTYRIC ACID.
δ-AMINO-n-VALERIC ACID.
ε-AMINO-n-CAPROIC ACID.
GRAPH 20.
ADSORPTION (MOLES x 10⁻⁵ / STANDARD SURFACE AREA L)
3
6
9
12
pH

ammonium group is completely in its uncharged amino form. In strongly acidic solutions low adsorptions are again obtained.

From table 9 it is observed that the pH range of maximum adsorption for glycine is less than that for β-alanine, in which there is a greater separation of the two polar groups. Further, in this series of amino acids only glycine is not adsorbed to the maximum extent at its isoelectric pH. These results emphasise the adverse influence of the negative carboxylate group, when adjacent to the ammonium group as in glycine or other α-amino acids.

The remaining amino acids of table 9 show that, on increasing separation of the two polar groups, there is an increase in the pH range for maximum adsorption and a pronounced decrease in extent. A comparison of the dissociation constants of the ammonium groups of the respective amino acids[34] shows little change in their electropositive strength, and consequently these changes in the adsorption behaviour of the amino acids are the direct result of the increased chain length separating the two polar groups. Since the pH range of maximum adsorption is the same for δ-aminovaleric acid as for ε-aminocaproic acid, it may be deduced that the adverse effect of the carboxylate group on the adsorption of these molecules is negligible. A progressive decrease in extent of maximum adsorption with increasing length of the molecule again takes place.

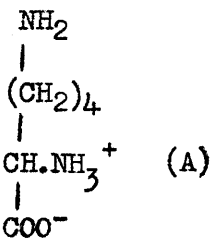
It is now clear that β-alanine is unique in this series (table 9), since the increase in pH range of maximum adsorption produced by the increased separation of the two polar groups is not accompanied by a decrease in extent owing to the bulk of the adsorbed molecules.

2.3. The effect of pH on the adsorption of lysine.

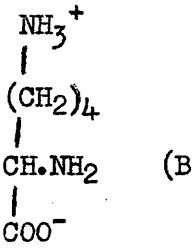
When the amino acid molecule contains two electropositive groups a study of the effect of pH on the adsorption of the molecule is of considerable assistance in determining the group through which adsorption takes place. Several examples involving this method of approach will be given in the remainder of this section.

Before examining the experimental adsorption data for lysine it is convenient to study first of all the nature of the molecule in solution. This has been done by Cohn[39], whose results and conclusions are briefly summarised in the following discussion.

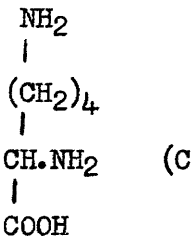
Lysine, as a dibasic amino acid, may exist in solution in the three isoelectric forms:-



(A)



(B)



(C)

Any one of these structures may lose a hydrogen ion to form the anion (D) $H_2N.(CH_2)_4.CH(NH_2).COO^-$.

The three dissociation constants involved are then,

$(H^+)\,(D)/(A) = K_A$; $(H^+)(D)/(B) = K_B$; $(H^+)(D)/(C) = K_C$

hence, $K_A\,(A) = K_B\,(B) = K_C\,(C)$ .....equation (1).

In estimating pK_A and pK_B it is probably best to ignore the effect of the distant $-NH_2$ group on the dissociation of the charged $-NH_3^+$ group; the effect is probably very small and will be about the same for

both the forms A and B. To obtain values for pK_A and pK_B we may employ Greenstein's equation[40, 41],

$$pK\ (NH_3^+) = 10.72 - (0.9 / d) \quad \text{..... equation (2)}$$

where d = number of carbon atoms separating the ammonium group from the carboxylate ($-COO^-$) group.

Hence from this equation (2) we calculate,

$$pK_A = 9.8 \quad \text{and} \quad pK_B = 10.54.$$

While pK_C may be taken as equal to pK_D, as has been deduced for the monoamino-monocarboxylic acids[39] $pK_C = 4.3$. From equation (1) the ratio of A:B:C in solution is then $32 \times 10^4 : 18 \times 10^5 : 1$.

Here, then, the very highly polar form (B) is predominant.

With this conclusion in mind the effect of pH on the adsorption of lysine, shown in graph 21, may now be studied. The essential experimental data are reported in table 10.

As the pH of the solution decreases from the isoelectric pH of lysine, the negative carboxylate group ($-COO^-$) will exist to an increasing extent as the uncharged carboxyl group (-COOH), and simultaneously there will be an increase in the number of molecules containing a positive charge on both the ε - and α-ammonium group. Consequently, the electro-positive character of the molecule as a whole becomes increasingly strong; and this results in the observed increase in extent of adsorption with decrease in pH of the solution.

In alkaline solutions, of pH greater than the isoelectric pH of lysine, the α-ammonium group is completely dissociated into its uncharged amino form and dissociation of the ε-ammonium group also takes place.

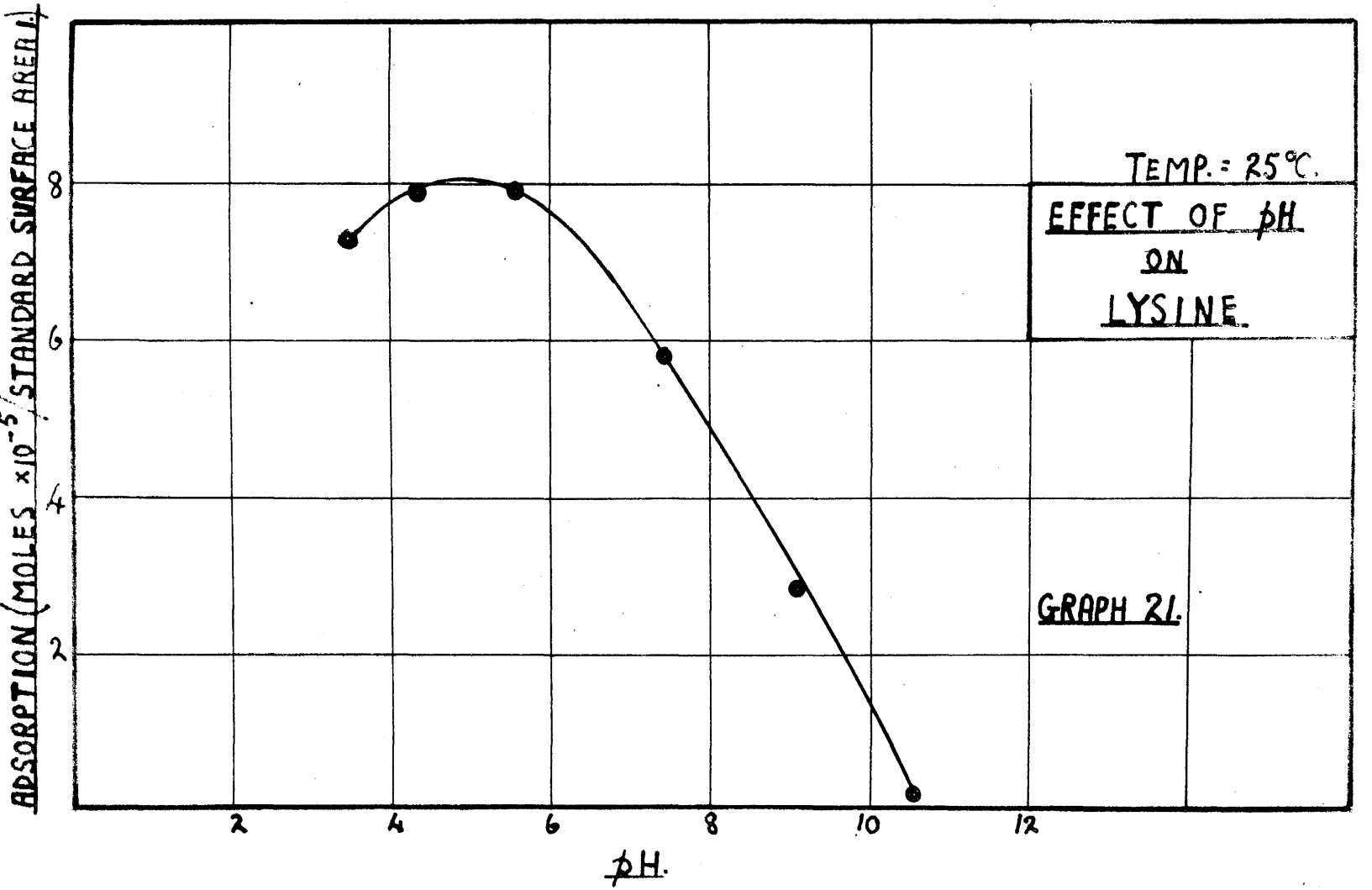

ADSORPTION (MOLES $\times 10^{-5}$ / STANDARD SURFACE AREA)
8
6
4
2
2
4
6
8
10
12
pH.
TEMP. = 25°C.
EFFECT OF pH
ON
LYSINE.
GRAPH 21.

Table 10.

Temp. = 25°C.

	$a_{max.}$	$pH_{max.}$
Lysine	9.1	4 - 6.2

Table 11a.

Arginine. Temp. = 25°C.

pH	3.1	4.3	5.0	5.4	6.9	9.2	11.1
$Adsp^{n}$.	4.02	4.71	4.0	2.41	0	4.02	7.12.

The adsorption is measured in moles x 10^{-5}/standard surface area 1.

Table 11b.

Glycocyamine. Temp. = 25°C.

pH	2.2	4.0	6.4	8.4	10.5
$Adsp^{n}$.	8.92	10.1	10.1	10.1	8.92

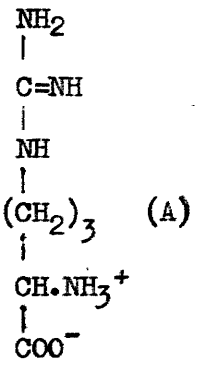
Adsorption in moles x 10^{-5}/standard surface area 1.

Simultaneously, uncharged carboxyl groups dissociate into the negative carboxylate groups. Under these conditions the extent of adsorption of lysine decreases rapidly and finally ceases at a pH where the ε-ammonium group is virtually completely in its uncharged amino form.

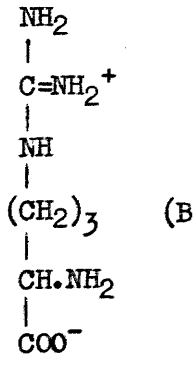
However, although the α-ammonium group is uncharged at the isoelectric pH of lysine, adsorption still takes place; and we may consequently deduce that the ε-ammonium group is the centre of adsorption, not the α-group. This conclusion also follows from the initial discussion which has shown that the polar form (B), $H_3^+N.(CH_2)_4.CH(NH_2).COO^-$, is predominant in solution.

2.4. The effect of pH on the adsorption of arginine and glycocyamine.

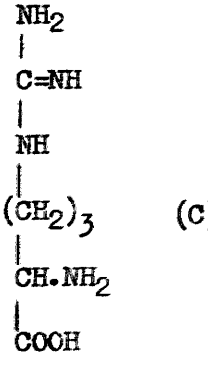
Before examining the experimental adsorption data it is again convenient to study first of all the nature of arginine in solution. As a dibasic amino acid, arginine may also exist in solution in three isoelectric forms to those shown for lysine.



(A)



(B)



(C)

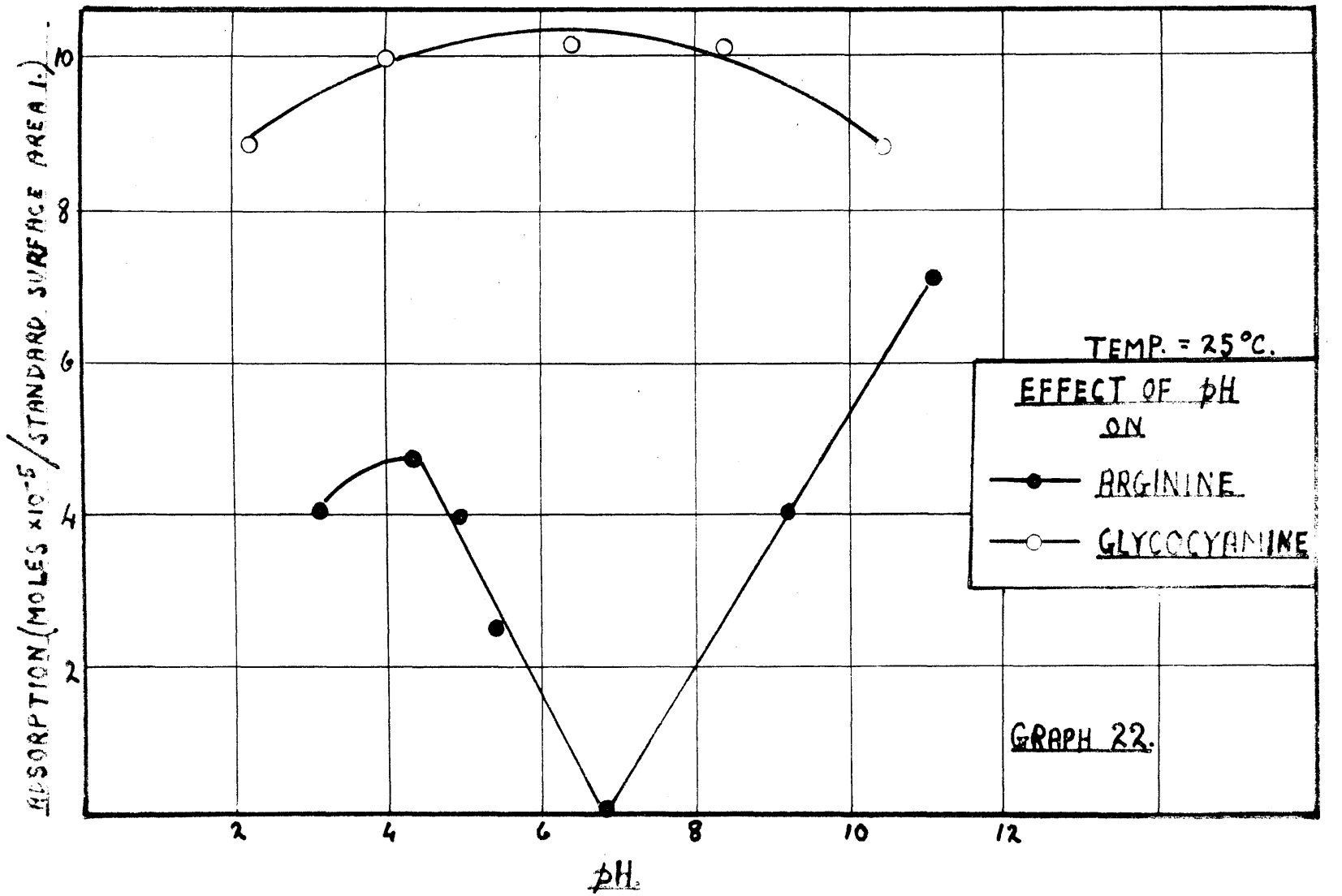
As for lysine, a similar calculation of the three dissociation constants may be made for arginine; and the same conclusion is obtained. Indeed, the extremely polar form, in which a positive charge resides on the

guanidine radical and a negative charge on the carboxylate group, predominates even more than in the corresponding form in lysine. This is to be expected since resonance can take place in the guanidine radical itself and the resonance energy will assist in stabilising form B.

The effect of pH on the adsorption of arginine from an 0.01M aqueous unbuffered solution at 25°C is shown in graph 22. The experimental data are also reported in table 11a (page 51).

The arginine was obtained as the mono-hydrochloride, and the pH of an o.o1 M aqueous solution of this material was 6.9. In this solution, there is a positive charge on the ammonium group of each molecule. However, no adsorption was observed at this pH. As the pH of the arginine solution becomes less than 6.9, the extent of adsorption increases in a manner previously encountered with glycine and the other amino acids. Further, as the pH of the arginine solution increases beyond 6.9, adsorption also increases and rises to a value greater than the maximum adsorption in acidic solutions. Concurrently with these experiments, the effect of pH on the adsorption of glycocyamine was studied. The experimental data for glycocyamine are shown in table 11b (page 51), and in graph 22. Glycocyamine is similar to arginine but possesses no α-amino group, and was found to be adsorbed to an extent which remained fairly constant over a very wide pH range, 2.2 - 10.5. The adsorption of glycocyamine has already been discussed and is known to take place by virtue of the positively charged guandinium radical.

These facts suggest, therefore, that at pH 6.9, corresponding to arginine mono-hydrochloride, the effective basicity of the α-ammonium


ABSORPTION (MOLES x10⁻⁵/STANDARD SURFACE AREA I.)
10
8
6
4
2
2
4
6
8
10
12
pH.
TEMP. = 25°C.
EFFECT OF pH
ON
ARGININE
GLYCOCYANINE
GRAPH 22.

group is equivalent to that of the terminal guanidinium radical. Thus the arginine molecule resembles a carbon chain with a positive pole of equivalent strength at each end, and it is possible that the interaction between these molecules in solution is such as to prevent adsorption taking place.

However, as the pH of the arginine solution becomes less than 6.9, the dissociation of the uncharged carboxyl group also decreases and consequently the electronegative influence adjacent to the α-ammonium group is decreased, resulting in an increase in the effective electropositive character of the α-ammonium group. The molecule has now one pole of greater positive strength than the other, adsorption taking place at the former, i.e., at the α-ammonium group. Similarly, as the pH increases beyond 6.9, a two-fold effect takes place. The dissociation of the carboxyl group increases, thereby increasing the electronegative influence adjacent to the α-ammonium group, and simultaneously the ammonium group itself dissociates into its uncharged amino form. Adsorption now takes place at the guanidinium group, which is now the more electropositive pole.

Since glycocyamine is adsorbed to a fairly constant extent over a wide pH range it may be deduced that the adjacent negative carboxylate group does not exert any appreciable influence on adsorption through the guanidinium group.

2.5. <u>The effect of pH on the adsorption of histidine and tryptophane.</u>

The effects of pH on the adsorption of histidine and tryptophane are shown in graph 23. The essential experimental data have been summarised

Table 12.

Temp. = 25°C.

Amino Acid	$a_{max.}$	$pH_{max.}$
Histidine	8.2	4.1 - 7.6
Tryptophane	7.4	4.2 - 7.7

Table 13.

Temp. = 25°C.

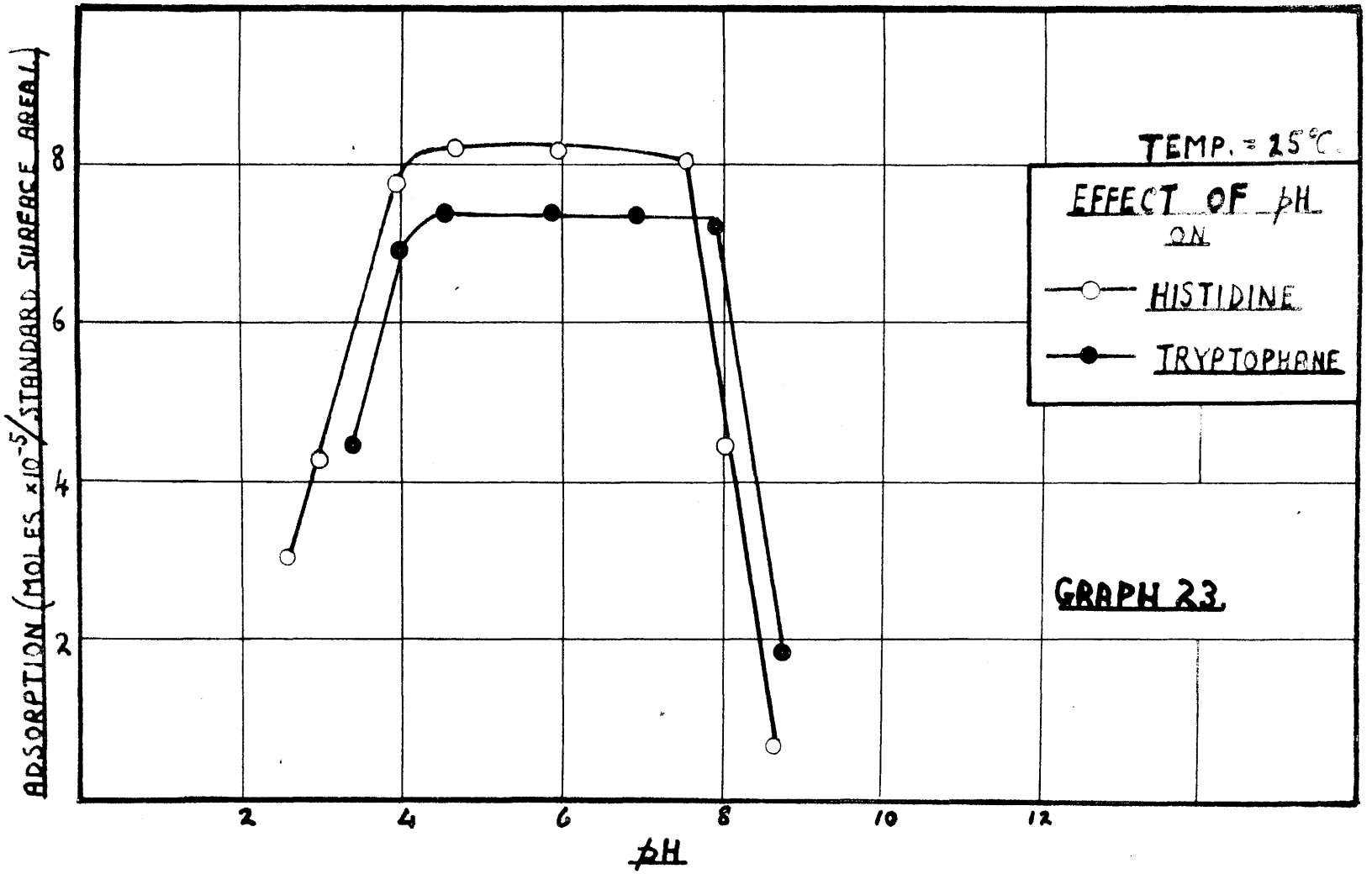
Amino Acid	$a_{max.}$	$pH_{max.}$
Proline	9.0	4.3 - 7.3
Hydroxyproline	5.5	4.3 - 5.8

Table 14.

Temp. = 25°C.

Betaine.

pH	2.14	5.1	8.0	10.2	11.9
Adsorption	1.8	4.74	6.72	7.6	7.9


TEMP. = 25°C.
EFFECT OF pH
ON
HISTIDINE
TRYPTOPHANE
GRAPH 23.
ADSORPTION (MOLES ×10⁻⁵/STANDARD SURFACE AREA)
pH
2
4
6
8
10
12

and are reported in table 12.

Maximum adsorption of histidine is attained over the pH range 4.1 - 7.6, which includes the isoelectric pH of the molecule. At the isoelectric pH of histidine the imidazole group is no longer positively charged; and it may be concluded that adsorption of histidine, at any pH, always takes place through the α-ammonium group. Further, as the pH of the solution increases beyond the isoelectric pH of histidine, the extent of adsorption decreases rapidly and finally ceases when the α-ammonium group has virtually completely dissociated into its uncharged amino form. This result also emphasises the preceding conclusion.

Maximum adsorption of tryptophane takes place over a similar pH range, 4.2 - 7.7, which again includes the isoelectric pH of the molecule. Consideration of their respective structures suggests that the indole group is less strongly electropositive than the imidazole group, and it is, therefore, unlikely that there is a positive charge on the indole -NH- group at the isoelectric pH of tryptophane. This conclusion is emphasized since maximum adsorption is still obtained at almost 2 pH units beyond the isoelectric pH of tryptophane, 5.89[34], but rapidly decreases beyond pH 7.7 and finally ceases when the ammonium group is in its uncharged amino form. The low extent of maximum adsorption is due to bulk of the large indole group in the adsorbed state.

2.6. <u>The effect of pH on the adsorption of proline, hydroxyproline, and betaine.</u>

The effect of pH on the adsorption of proline and hydroxyproline are shown in graph 24. The essential experimental data are reported in

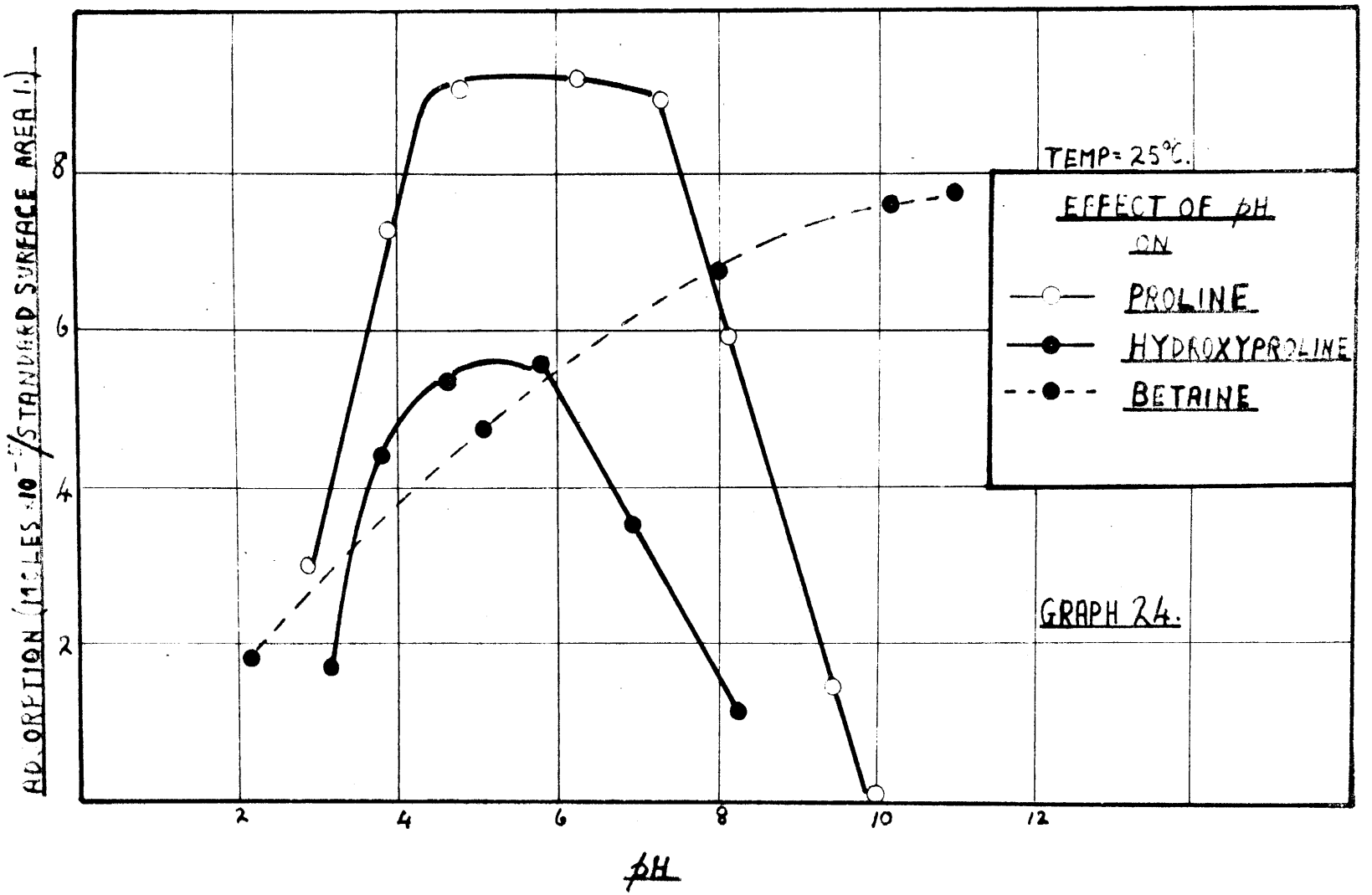

ADSORPTION (MOLES $\cdot 10^{-7}$/STANDARD SURFACE AREA I.)
8
6
4
2
2
4
6
8
10
12
pH
TEMP = 25°C.
EFFECT OF pH
ON
PROLINE
HYDROXYPROLINE
BETAINE
GRAPH 24.

table 13 (page 55).

Maximum adsorption of proline takes place over the pH range 4.3 - 7.3, which includes the isoelectric pH of the molecule, 6.3 [34]. Since glycine did not attain maximum adsorption at its isoelectric pH, it may be deduced that the heterocyclic ring of proline exerts an influence, similar to that of a straight carbon chain, which modifies the adverse effects of the negative carboxylate group adjacent to the imino group. As the pH of the proline solution increases beyond 7.3, the extent of adsorption decreases rapidly and finally ceases when the imino group is virtually uncharged. This result shows that the adsorption mechanism for proline is similar to that for glycine and the other α-amino acids, adsorption of proline taking place through the imino group by virtue of its positive charge.

Maximum adsorption of hydroxyproline takes place over the pH range 4.3 - 5.8, which also includes the isoelectric pH of the molecule. Again the heterocyclic ring appears to modify the adverse effects of the negative carboxylate group, as is shown by comparison with glycine. However, both the extent of maximum adsorption of hydroxyproline, and the pH range within which it takes place are considerably smaller than for proline. These results are to be expected since there are two electronegative groups in the hydroxyproline molecule, which will result in a stronger force of repulsion between the molecule and the negatively charged silica. In addition, the greater polarity of the molecule increases slightly the interaction between the molecules in solution, as was shown by Smith [33].

As the pH of the solution increases beyond the isoelectric pH of hydroxyproline, the extent of adsorption decreases rapidly and finally ceases when the imino group is virtually uncharged.

The effect of pH on the adsorption of betaine is shown in graph 24 and also in table 14. The experimental conditions are the same as for the preceding amino acids, the extent of adsorption also being measured in moles x 10^{-5} per standard surface area 1.

The betaine system in solution may be represented as follows:-

(in acidic solutions) $\overset{+}{N}Me_3 . CH_2 . \overset{-}{C}OO \quad \overset{+}{H}$

(in alkaline solutions) $\overset{-}{H}O \quad \overset{+}{N}Me_3 . CH_2 . \overset{-}{C}OO$

The existence of the dipolar ionic forms has been verified from dielectric constant measurements by Devoto[42] and by Edsall and Wyman[43].

From table 14 (page 55), it is observed that the effect of pH on the adsorption behaviour of betaine does not show a close relationship to that of any other amino acid. However, there is a distinct similarity to the pH data for glycocyamine, which is adsorbed to a fairly constant extent over a wide pH range, 2.2 - 10.5. The extent of adsorption of betaine is low in acidic solutions, but rises gradually to a maximum adsorption in the pH range 10.2 - 11.9. Further, there is also an appreciable interaction between the molecules themselves in solution, as has been shown by Smith[33], and it is probable that the extent of this interaction will also be influenced by the pH of the solution. These effects will combine to vary the extent of adsorption with pH of the solution. Since adsorption increases with increase in pH, it may be deduced that the influence of the negative

carboxylate group on the positively charged nitrogen is of minor importance in determining the adsorption behaviour of the molecule, and again this is in agreement with the preceding conclusions.

Summary of Section 2.

In this section, the effect of pH on the adsorption of a number of amino acids has been studied and it is now convenient to summarise the main conclusions deduced. Further, when the amino acid molecule contains two electropositive groups a study of the effect of pH on the adsorption of the molecule provides a means of identifying the group through which adsorption takes place.

(1) General Conclusions.

Adsorption of these amino acids and related substances takes place by virtue of the positive charge on the ammonium or similar group, and ceases when the group has dissociated into its uncharged form.

(2) The effect of pH in the series $R.CH(NH_2).CO_2H$ (where R = alkyl group).

In this series only glycine is not adsorbed to the maximum extent at its isoelectric pH and it may, therefore, be deduced that,

(a) the negative carboxylate group, especially when adjacent to the positive ammonium group, exerts an influence tending to retard or prevent adsorption, and

(b) that the alkyl group modifies this adverse effect of the carboxylate group.

As the alkyl group increases in length the pH range for maximum adsorption increases, but there is a pronounced decrease in extent because

of the bulk of the large alkyl groups in the adsorbed state.

(3) The effect of pH in the series $H_2N.(CH_2)_n.CO_2H$.

The extent of maximum adsorption of β-alanine and the pH range within which it takes place are both greater than for glycine. These facts emphasise the adverse effect of the carboxylate group. Increasing separation of the two polar groups results in an increase in the pH range for maximum adsorption, but there is a pronounced decrease in extent, due, as in the previous series, to the bulk of the large molecules in the adsorbed state.

Notes.

(1) Adsorption of lysine takes place through the ε-ammonium group.

(2) Adsorption of arginine takes place through the α-ammonium group at pH values less than 6.9, and through the guanidinium group at values greater than 6.9.

(3) Adsorption of histidine, at any pH, always takes place through the α-ammonium group and not through the imidazole group.

(4) Similarly, adsorption of tryptophane always takes place through the α-ammonium group and not through the indole nitrogen.

(5) The heterocyclic ring of proline and hydroxyproline modifies the adverse effect of the carboxylate group.

(6) Urea and hydantoic acid are not adsorbed at any pH and consequently citrulline, asparagine, and glutamine cannot be adsorbed through their terminal ureido or carbamyl groups respectively.

Section 3.

Kinetics of adsorption.

In Section 1 the rates of adsorption of the amino acids have been discussed in a general manner and conclusions derived from the experimental data on the influence of various substituents on the rate of adsorption of the molecule in which they are contained. Further, in this discussion we have utilised the Langmuir rate constant, k, at 37°C and the validity of its use will now be shown. Other fundamental processes involved in many adsorption reactions will also be discussed and the possibility of their application to the present study of the adsorption of these amino acids will be examined.

According to Hill[44], when a semi-infinite solid is brought into contact with a large quantity of a well-mixed liquid containing a diffusible substance in concentration C_o, the total amount which diffuses across unit area of the boundary in time t is:-

$$A = 2\, C_o \sqrt{(kt/\pi)}$$ where k = diffusion coefficient.

A similar equation was derived by Washburn[45], for the rate of capillary condensation and he tested this equation from data obtained by Cude and Hulett[46] for the penetration of water into charcoal.

The application of the above equations to the experimental data obtained for the adsorption of these amino acids on silica, was tested by a plot of adsorption vs. time, and in no instance were the equations satisfied. Consequently, it would appear that the adsorption of these amino acids on silica is not fundamentally a process of diffusion of the

molecules in solution to the surface of the adsorbent, or of capillary condensation.

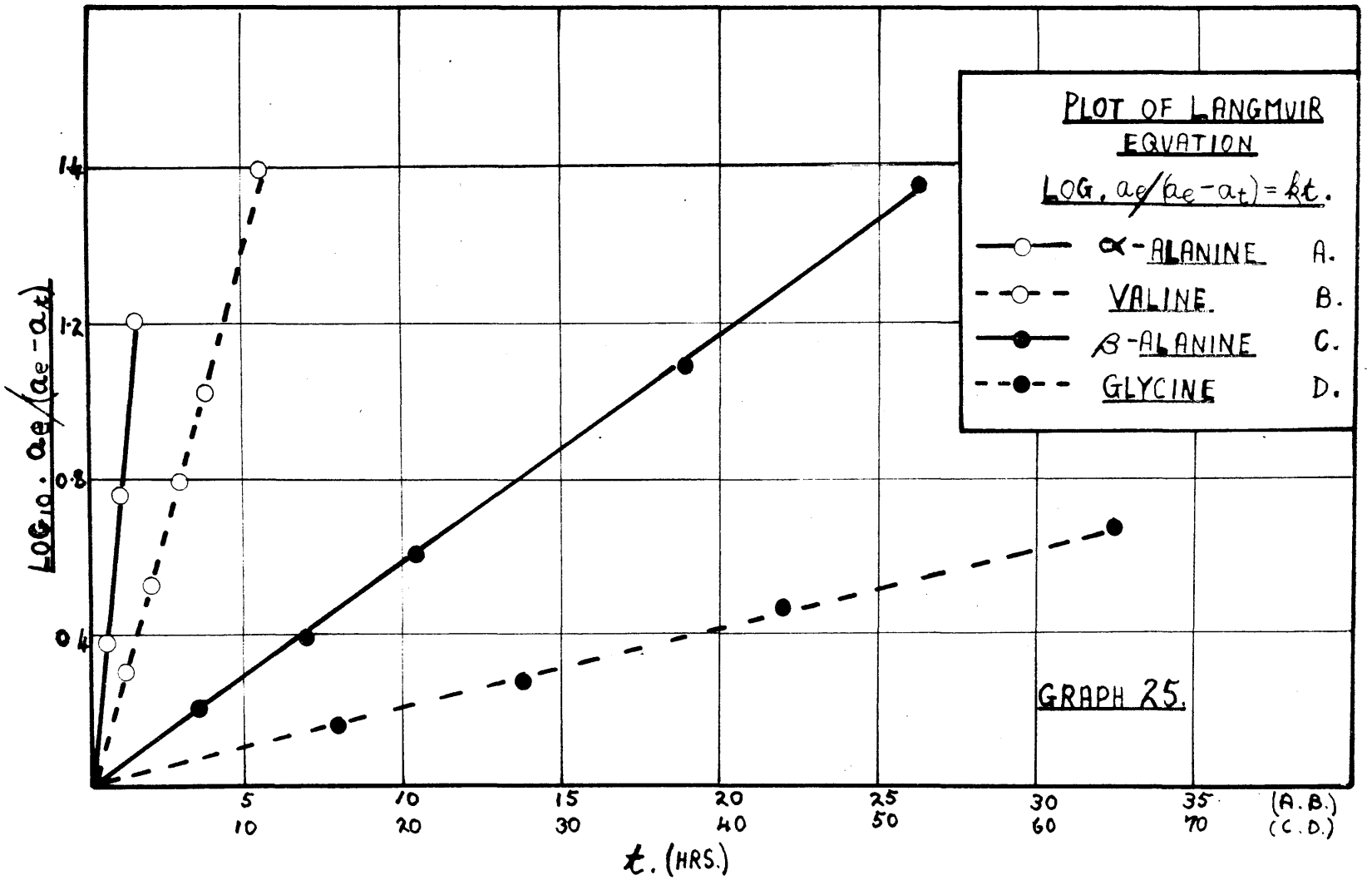
The theory of the rate of adsorption on a free surface was first derived by Langmuir[47], and his theoretical equation may be expressed in the form:-

$$\text{ln. } a_E/(a_E - a_t) = kt$$ where a_E = adsorption at equilibrium, a_t = adsorption at time t.

The application of the Langmuir theory of adsorption to the experimental data for the adsorption of each amino acid on silica at 37°C, was tested by a plot of ln. $a_E/(a_E - a_t)$ vs. t.

Typical plots are shown in graph 25, for glycine, α-alanine, valine and β-alanine. It has been found that the experimental data for each amino acid, irrespective of its molecular structure, are in good agreement with the Langmuir equation, and values of k, the rate constant, at 37°C have been determined and discussed in Section 1. It is interesting to note that the adsorption data for the semicarbazide and guanidinium ions also agree with the Langmuir equation. The rates of adsorption of several amino acids have also been studied at different temperatures and the experimental data at each temperature also agree with the Langmuir equation.

Consequently, the empirical assumptions inherent in the Langmuir theory may possibly be applied to the adsorption of these amino acids by silica. Thus, it is probable that the amino acid molecules are adsorbed to definite points of attachment on the surface of the silica and that each point of attachment can accommodate only one adsorbed molecule.


PLOT OF LANGMUIR EQUATION
LOG. ae/(ae - at) = kt.
α-ALANINE A.
VALINE B.
β-ALANINE C.
GLYCINE D.
GRAPH 25.
LOG10. ae/(ae - at)
1·4
1·2
0·8
0·4
5
10
10
20
15
30
20
40
25
50
30
60
35
70
(A.B.)
(C.D.)
t. (HRS.)

Further, the Langmuir theory of adsorption precludes a mechanism involving diffusion or capillary condensation.

These results, therefore, suggest that the ammonium group, the active centre of the amino acid molecule, is attached to the oxygen atom of the silica, and the primary force of adsorption is the mutual attraction of the positively charged ammonium group and the negatively charged oxygen atom of the silica. However, to examine in greater detail the nature of the adsorption mechanism, some thermodynamic data must be obtained. In the following section the energy of activation and heat of adsorption will be studied and from these data a more complete understanding of the adsorption bond may be derived.

Section 4.

Thermodynamic data.

4.1. Energy of Activation.

The rates of adsorption of several amino acids have been studied at three different temperatures and in each case it is observed that as the temperature rises there is an increase in the rate of adsorption, although the extent decreases. These facts indicate a distinct temperature coefficient, adsorption taking place with a characteristic velocity. Taylor[48] has called adsorption occurring under these conditions 'activated adsorption'. Consequently, a definite energy of activation is necessary for the formation of the adsorbed system and this energy may be calculated in the following manner.

According to the Langmuir theory of the rate of adsorption on a free surface, the experimentally measured rate is the difference between the rate at which the molecules are bound on the surface and the rate at which they leave the surface. The experimental rate determined at constant solution concentration is given by

$$(1) - \qquad d\theta/dt = k_1 (1-\theta) - k_2\theta \ .$$

where θ is the fraction of the surface covered with adsorbed molecules, and k_1 and k_2 are constants. Integrating with the boundary condition that $\theta = 0$ when $t = 0$, we obtain

$$(2) - \qquad \theta = (k_1/k_2+k_2) \left(1 - e^{-(k_1+k_2)t} \right)$$

when $t = \infty$, $\theta = \theta_E$, where θ_E is the fraction of the surface covered when equilibrium is reached. This gives the relation

(3) - $$\theta_E = k_1 / (k_1+k_2)$$

from which it follows that

(4) - $$\theta = \theta_E (1 - e^{-kt})$$ where $k = k_1+k_2$.

Since $\theta = a/a_m$, equation (4) can be written in the form

(5) $$a = a_E (1 - e^{-kt})$$

where a and a_E are the amounts adsorbed after time t and at equilibrium respectively and a_m is the amount adsorbed when the surface is completely covered. Equation (5) may be re-written

(6) - $$\text{ln. } a_E/(a_E - a) = kt.$$

By applying equation (6) to the experimental data for each amino acid, values of k at the three temperatures may be obtained.

Since $\theta_E = a_E/a_m$, and values of a_m may be obtained from the isotherms of each amino acid, then values of θ_E may be calculated at each temperature.

From equation (3),

$$k_1 = \theta_E k. \quad \text{and}$$

$$k_2 = (1- \theta_E)k.$$

Consequently values of k_1 and k_2, the velocity constants of the adsorption and desorption process respectively, may be calculated for the three temperatures.

Using the Arrhenius equation which relates the variation of the velocity constant with temperature, the energy of activation of the adsorption process (E_1) and of the desorption process (E_2) may be calculated and are shown in table 15.

Since the Arrhenius equation may be expressed in the form

$$\ln k_1 = \ln B_1 - E_1/RT \qquad \text{(Adsorption)}$$

or

$$\ln k_2 = \ln B_2 - E_2/RT \qquad \text{(Desorption)}$$

then values of B_1 and B_2, the frequency factors of the adsorption and desorption process respectively, may be calculated and are also shown in table 15.

Conclusions from Section 4.1.

(1) Group 1.

Glycine, α-alanine, and proline show a close relationship between their respective values of E_1, B_1, E_2, and B_2.

(2) Group 2.

A relationship is also observed between the respective values for β-alanine, γ-aminobutyric acid, δ-aminovaleric acid and lysine.

It is significant that the amino acids in group 1 are all α-amino acids whereas those in group 2 are adsorbed through an ammonium group which is not adjacent to the carboxylate group. However, the data in table 15 may be more conveniently discussed in a later section (section 4.4) after more conclusive evidence on the nature of the adsorption bond has been obtained from heat of adsorption data.

Note.

Values of E_1 and E_2 for ε-aminocaproic acid could not be determined experimentally since the extents of adsorption from an 0.01 M aqueous solution over the temperature range, 25°C - 50°C, were so small as to prevent the determination of complete and accurate rate curves. Values of E_1 and E_2 for betaine could not be determined since no appreciable

Table 15.

Amino Acid	Graph	E_1	B_1	E_2	B_2
Group 1.					
Glycine	26	8.7	2.82×10^2	15.3	2.45×10^7
α-Alanine	27	9.4	4.22×10^4	15.5	1.49×10^9
Proline	28	8.59	1.40×10^3	17.8	2.78×10^9
Group 2.					
β-Alanine	29	15.6	6.34×10^7	27.1	4.18×10^{17}
γ-Aminobutyric Acid	30	15.25	1.65×10^8	-	-
δ-Aminovaleric Acid	31	16.0	7.92×10^8	29.0	4.43×10^{18}
Lysine	33	16.4	8.27×10^8	30.3	1.41×10^{19}

E_1 and E_2 = kg. cals./mole.

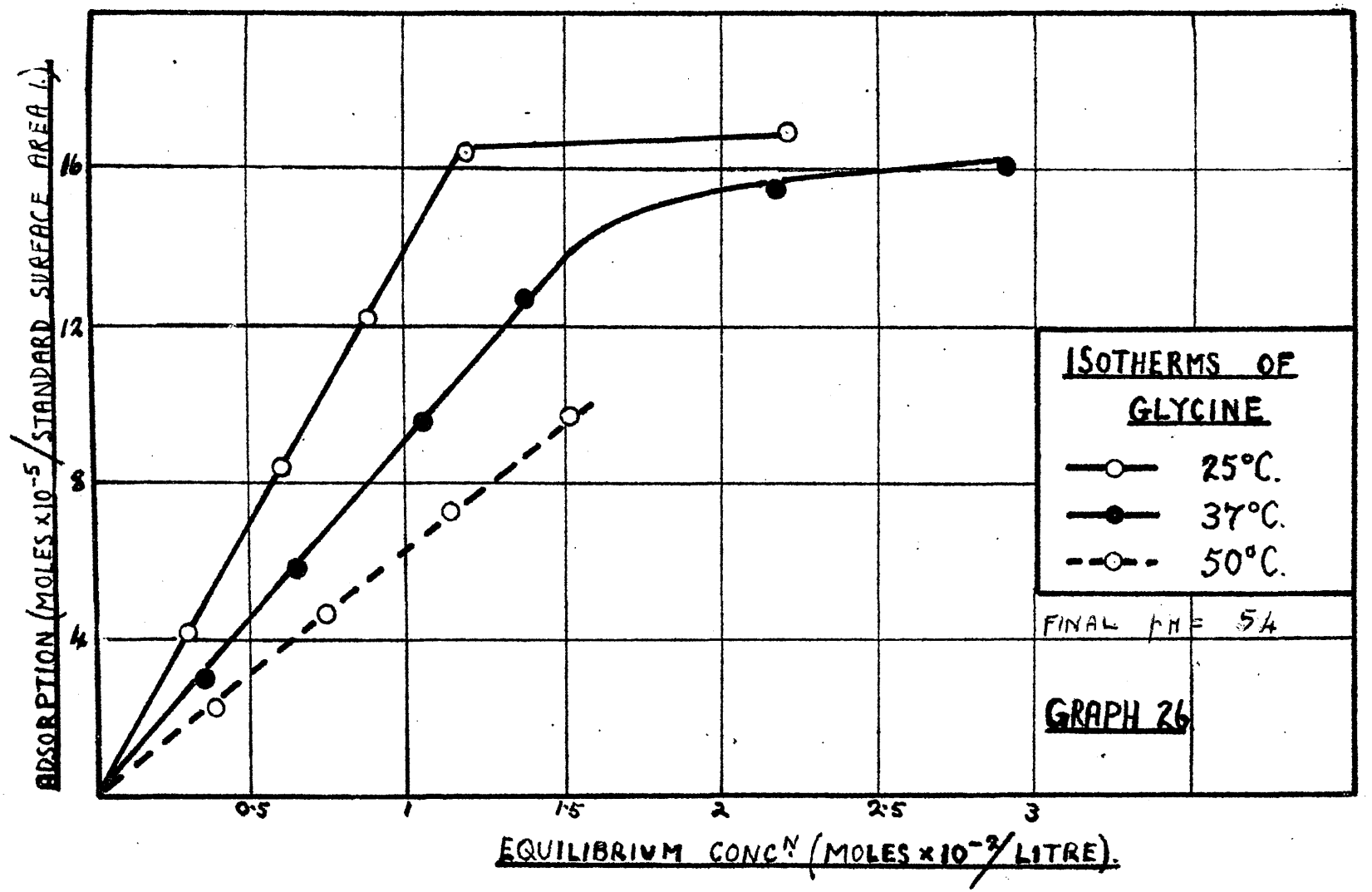
B_1 and B_2 = moles/litre/sec.

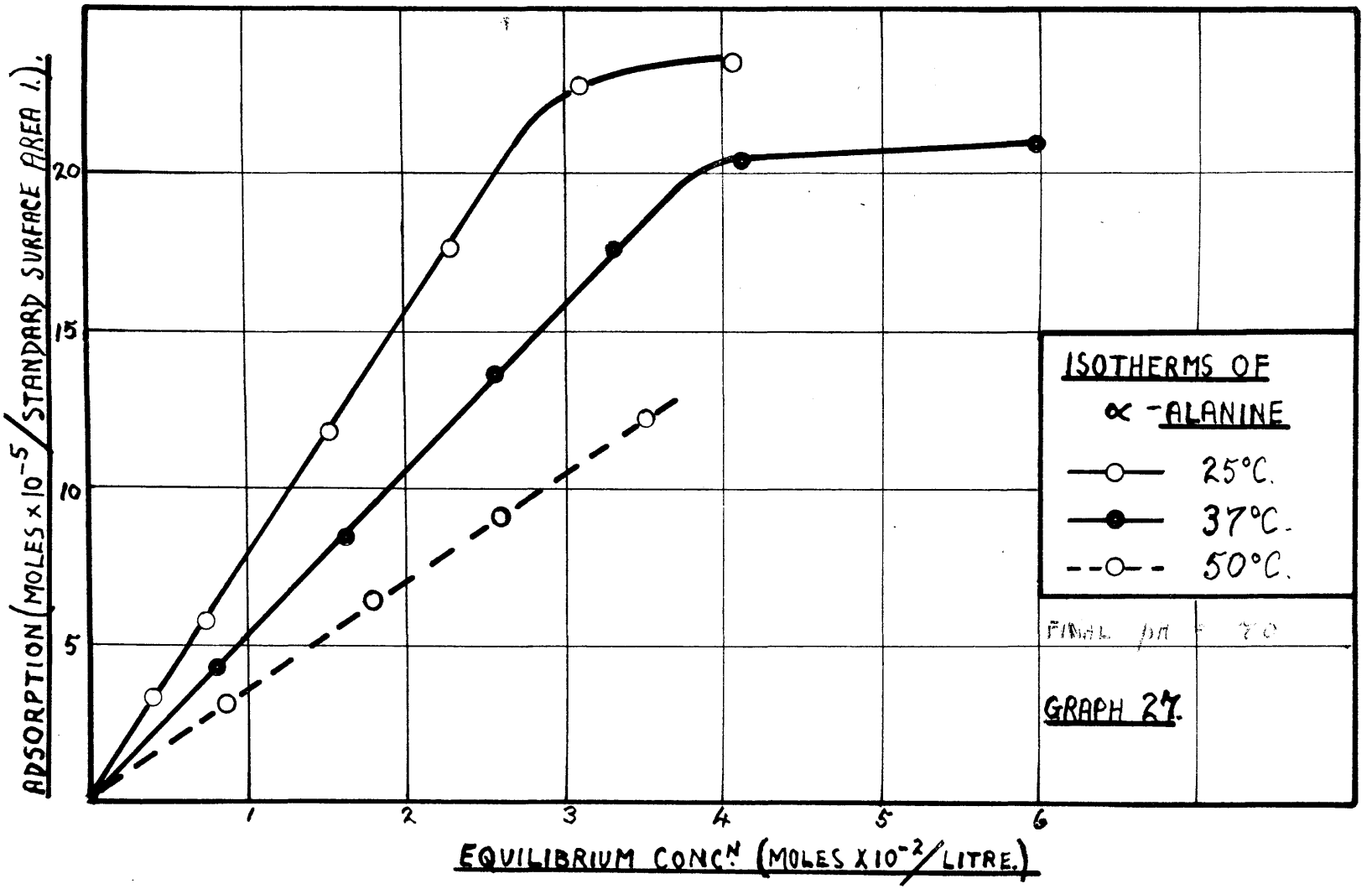
difference in extent of adsorption from the 0.01 M aqueous solution could be detected at the three temperatures, 25°C, 37°C and 50°C. Accurate values of E_2 for γ-aminobutyric acid could not be calculated. In this case, θ_E is greater than $(1-\theta_E)$ and since the error in determination of θ_E is magnified in the calculation of $(1-\theta_E)$, the resulting value of k_2 is only approximate.

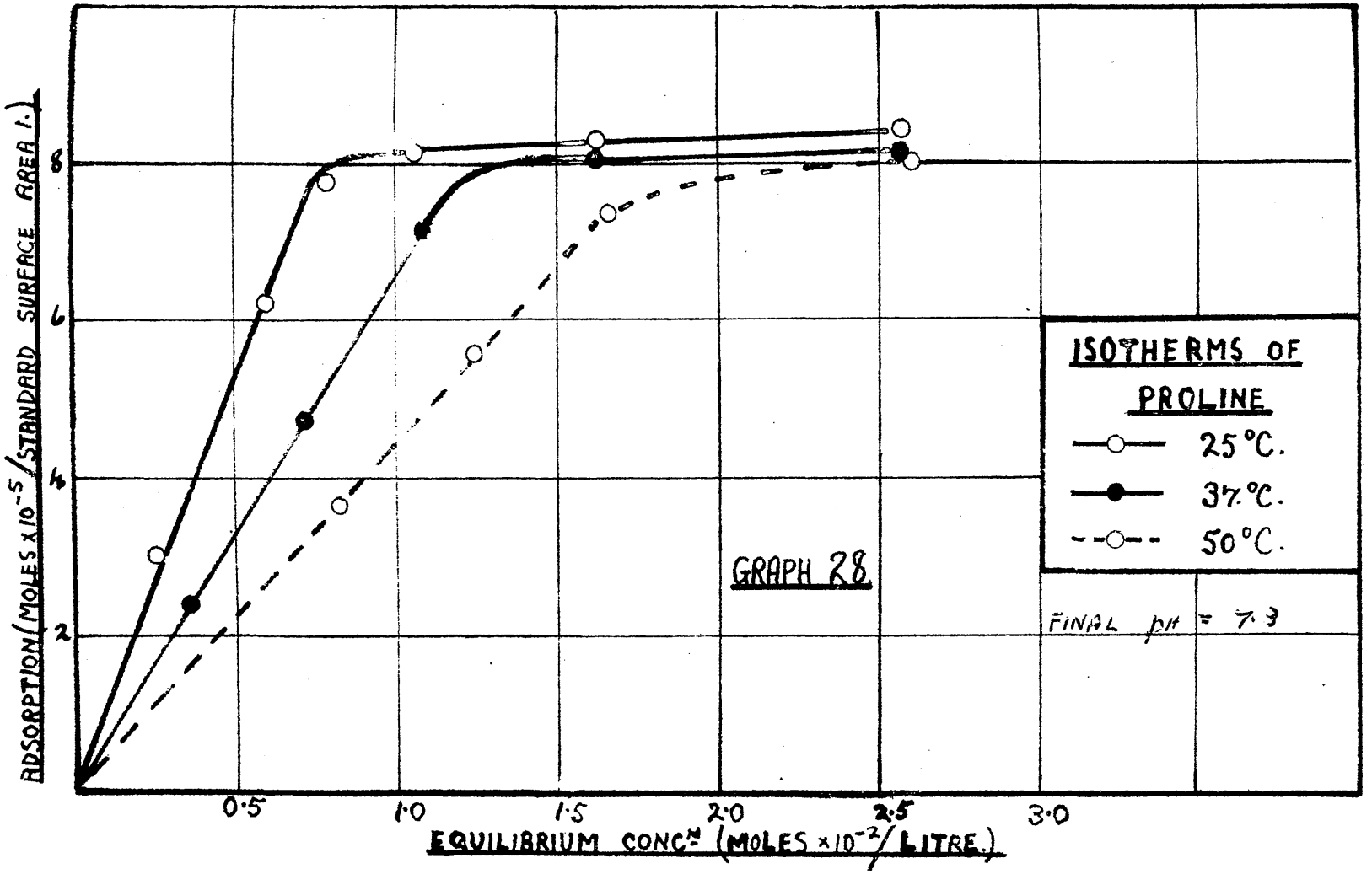
4.2. Isotherms.

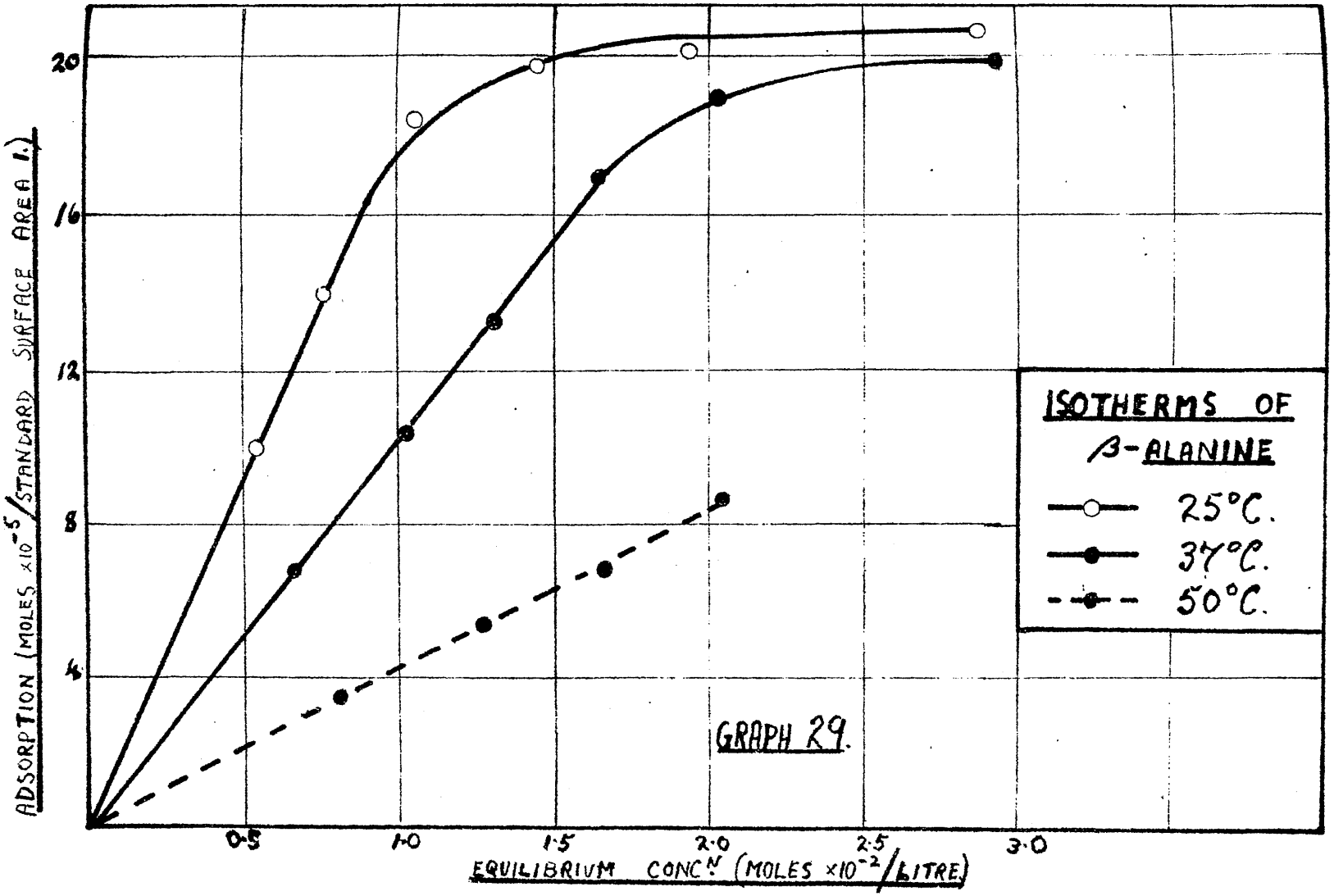
Isotherms for three temperatures have been obtained for glycine, α-alanine, proline, β-alanine, γ-aminobutyric acid, δ-aminovaleric acid, ε-aminocaproic acid and lysine, and are shown in graphs 26-33 respectively.

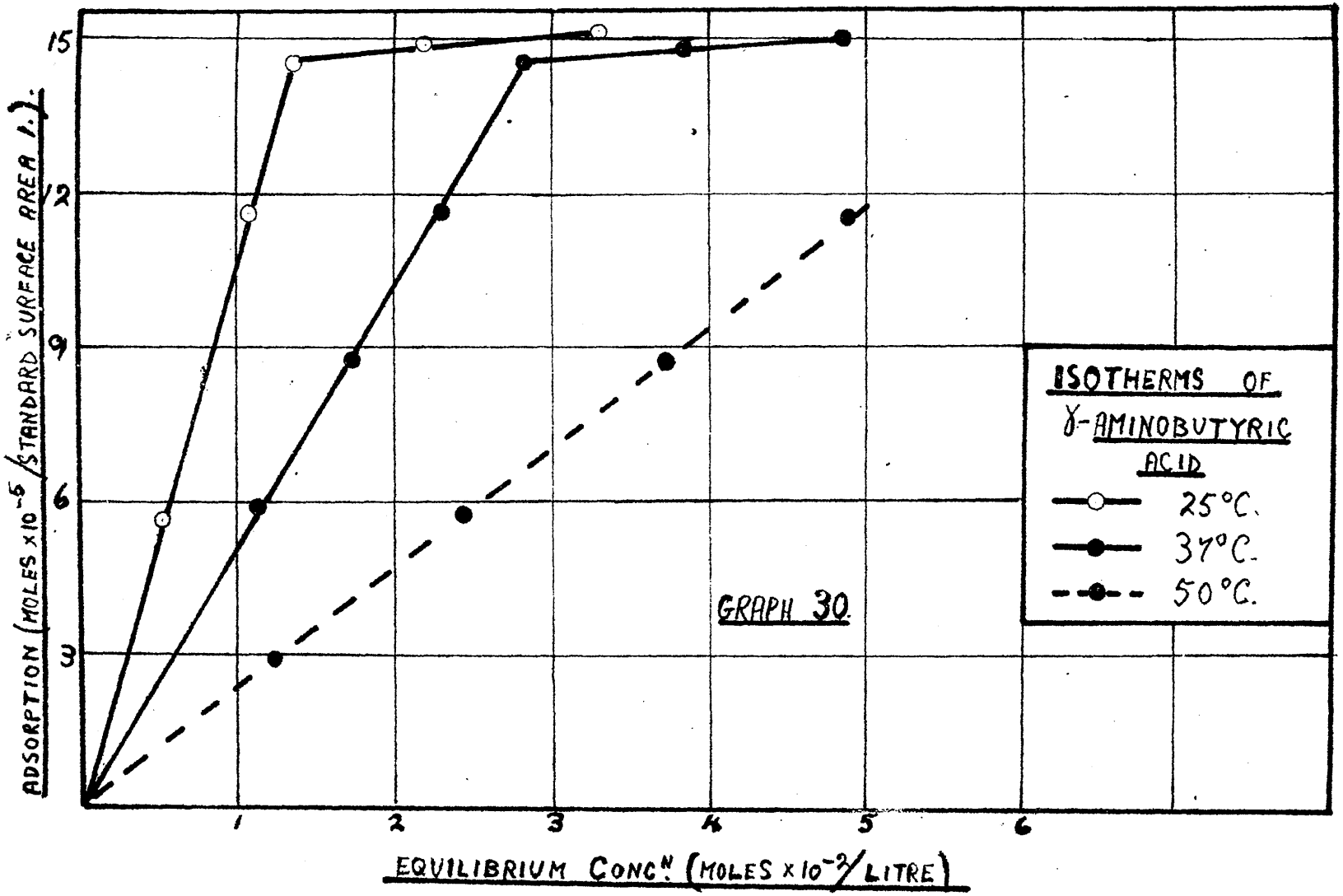
The isotherms of each amino acid show that a constant maximum adsorption is attained representing complete covering of the total available surface and also that the value of this maximum adsorption is identical at each temperature. These facts suggest that there is a finite number of adsorption sites for the standard surface area of adsorbent. These isotherms possess a linear portion, in which the adsorption is proportional to the equilibrium concentration. Sheppard[49], when studying the adsorption of dyes on AgBr, (the adsorption of the dye is attributed primarily to the electrostatic attraction of the dye cation for the halide Br^- ion in the AgBr lattice), also obtained isotherms in which the adsorption rises linearly with equilibrium concentration and finally attains a constant maximum value equivalent to a monomolecular adsorbed layer. Sheppard reports the adsorption of the dye cations to be irreversible and remarks, "It appears probable that the agreement of

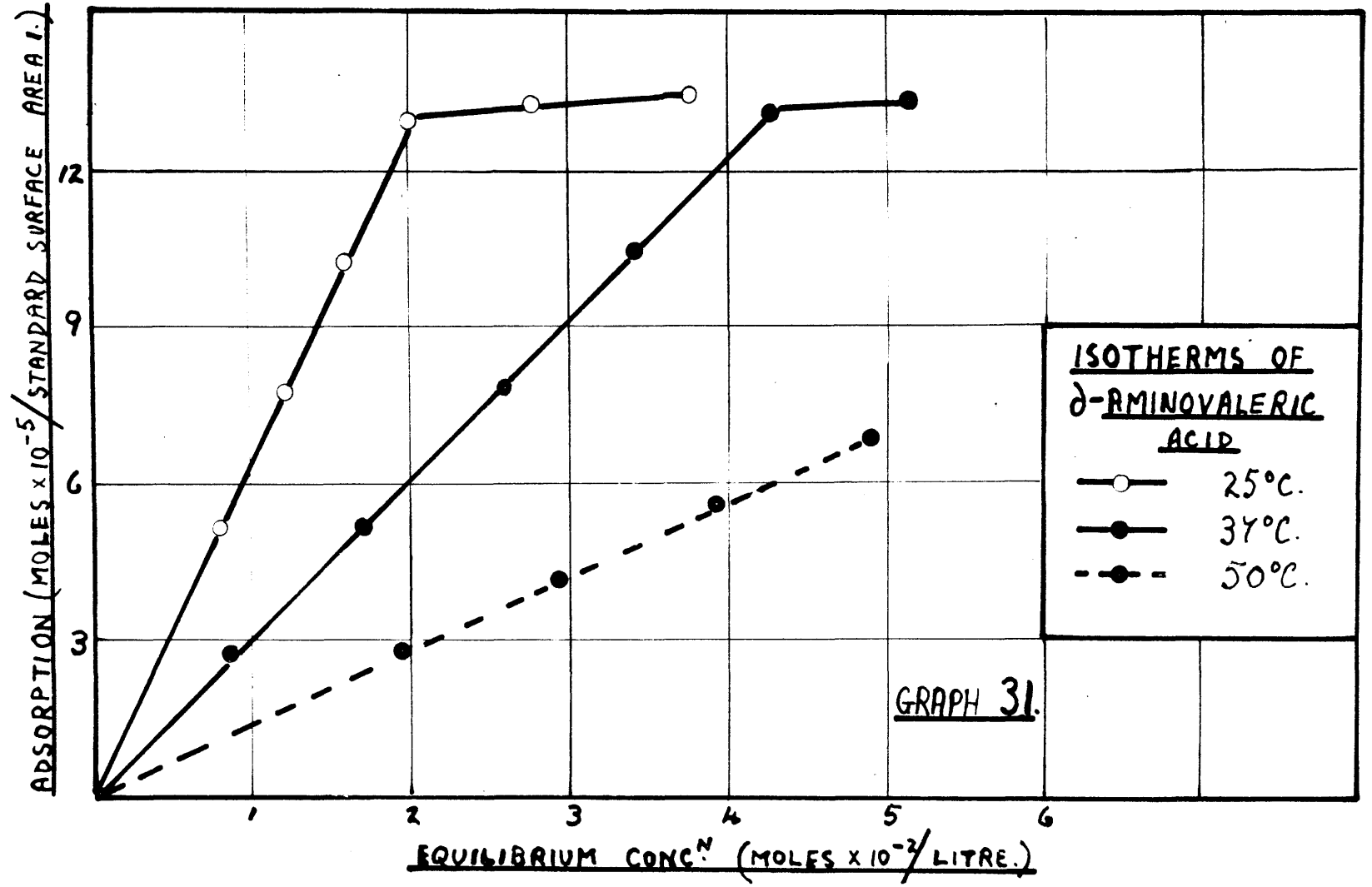

ADSORPTION (MOLES x10⁻⁵ / STANDARD SURFACE AREA)
16
12
8
4
0·5
1
1·5
2
2·5
3
EQUILIBRIUM CONCⁿ (MOLES x10⁻³ / LITRE).
ISOTHERMS OF GLYCINE
25°C.
37°C.
50°C.
FINAL pH = 5·4
GRAPH 26

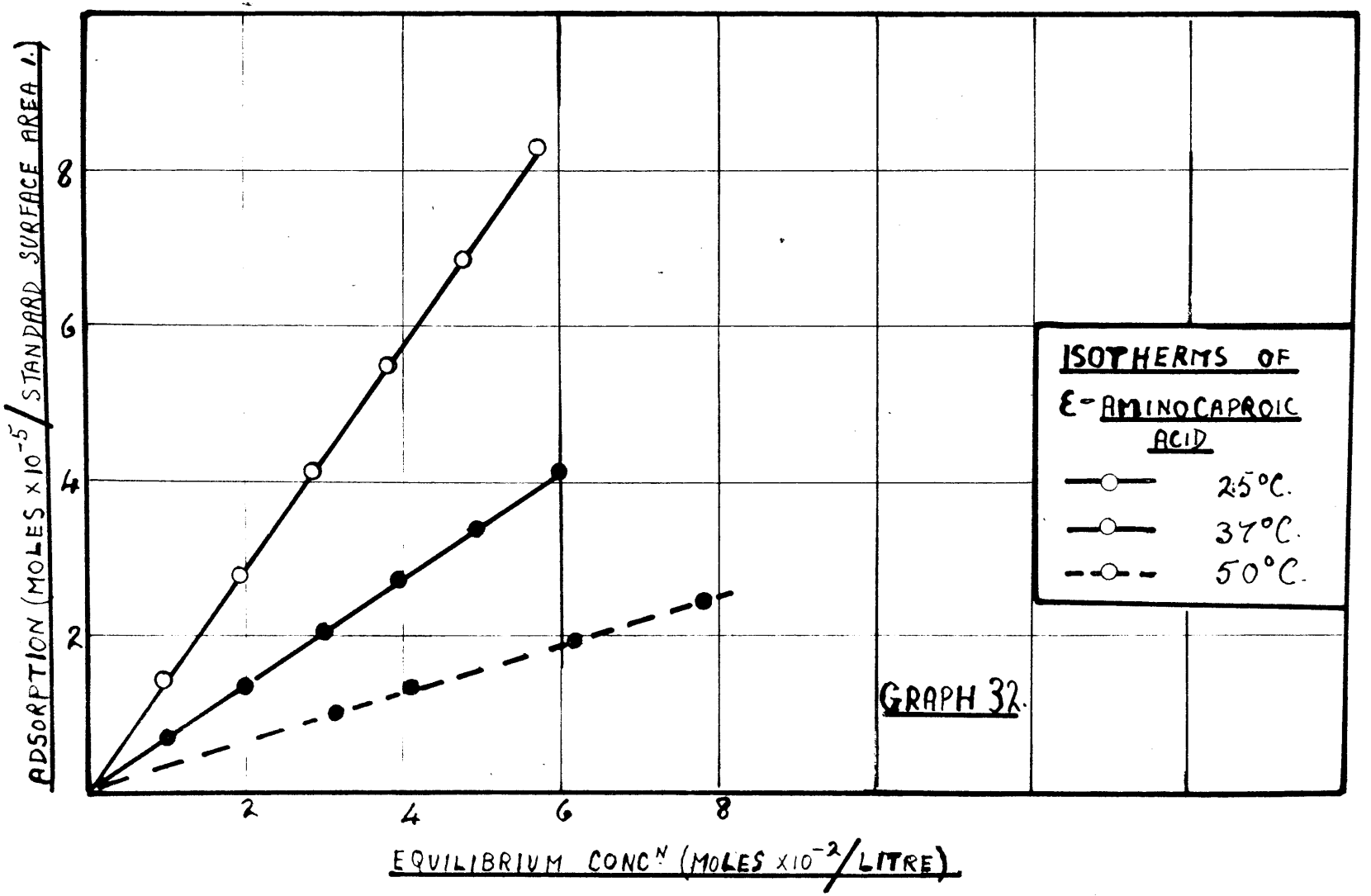

ADSORPTION (MOLES × 10⁻⁵ / STANDARD SURFACE AREA I.)
EQUILIBRIUM CONCⁿ (MOLES X 10⁻² / LITRE.)
5
10
15
20
1
2
3
4
5
6
ISOTHERMS OF
∝ - ALANINE
25°C.
37°C.
50°C.
GRAPH 27.

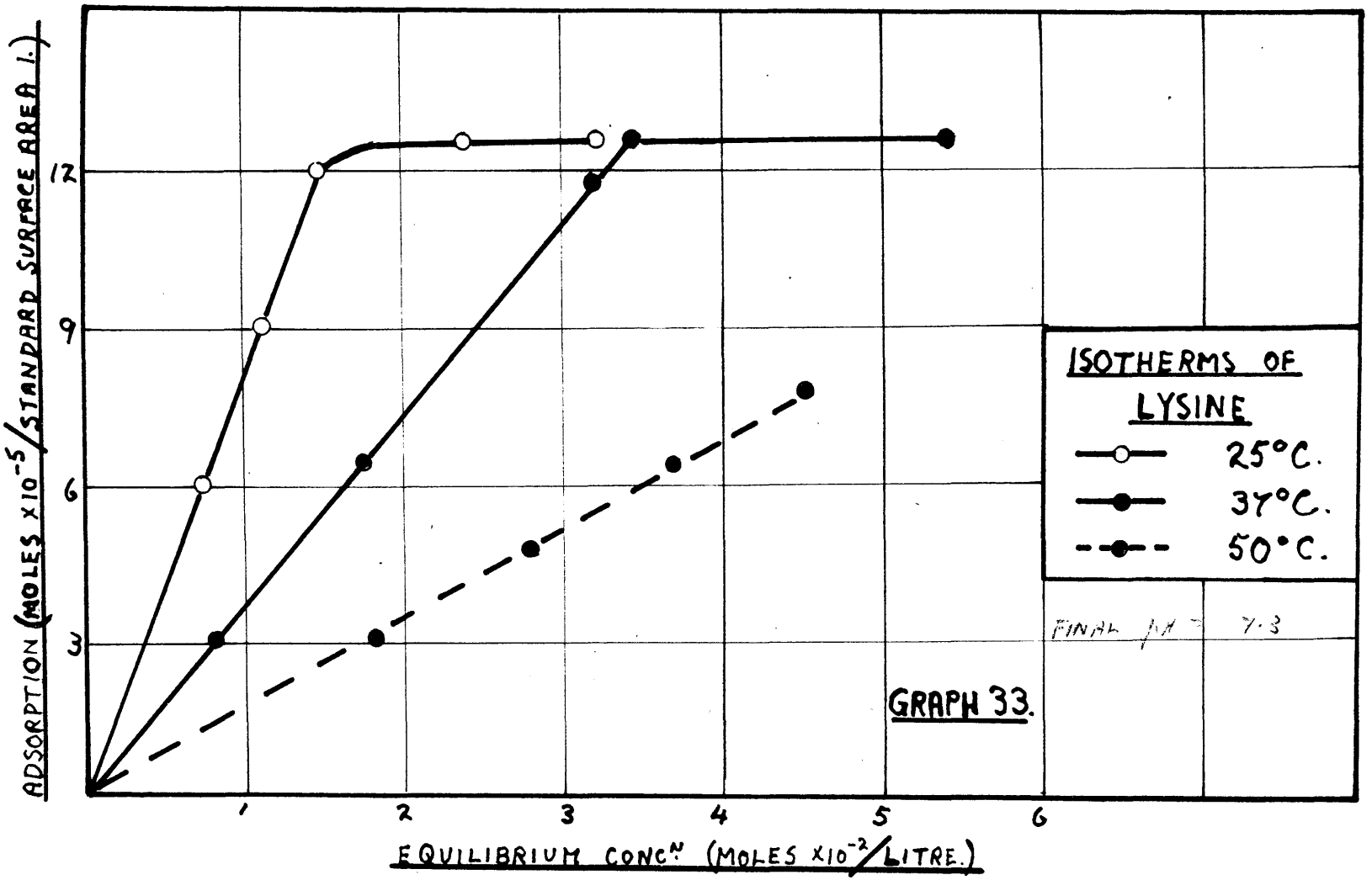

ADSORPTION (MOLES x 10⁻⁵ / STANDARD SURFACE AREA I.)
8
6
4
2
0·5
1·0
1·5
2·0
2·5
3·0
EQUILIBRIUM CONCⁿ (MOLES x 10⁻² / LITRE.)
ISOTHERMS OF PROLINE
25 °C.
37 °C.
50 °C.
GRAPH 28
FINAL pH = 7·3


ISOTHERMS OF
β-ALANINE
25°C.
37°C.
50°C.
GRAPH 29.
ADSORPTION (MOLES ×10⁻⁵/STANDARD SURFACE AREA I.)
EQUILIBRIUM CONCⁿ (MOLES ×10⁻²/LITRE)
20
16
12
8
4
0·5
1·0
1·5
2·0
2·5
3·0


ADSORPTION (MOLES x 10⁻⁵ / STANDARD SURFACE AREA 1.).
15
12
9
6
3
1
2
3
4
5
6
EQUILIBRIUM CONCⁿ (MOLES x 10⁻² / LITRE)
ISOTHERMS OF γ-AMINOBUTYRIC ACID
25°C.
37°C.
50°C.
GRAPH 30.


ADSORPTION (MOLES x 10⁻⁵/STANDARD SURFACE AREA I.)
12
9
6
3
1
2
3
4
5
6
EQUILIBRIUM CONCⁿ (MOLES x 10⁻²/LITRE.)
ISOTHERMS OF
δ-RMINOVALERIC
ACID
25°C.
37°C.
50°C.
GRAPH 31.


ADSORPTION (MOLES x 10⁻⁵ / STANDARD SURFACE AREA)
8
6
4
2
2
4
6
8
EQUILIBRIUM CONCⁿ (MOLES x 10⁻² / LITRE)
ISOTHERMS OF
ε-AMINOCAPROIC ACID
25°C.
37°C.
50°C.
GRAPH 32.


ADSORPTION (MOLES X10⁻⁵/STANDARD SURFACE AREA I.)
12
9
6
3
1
2
3
4
5
6
EQUILIBRIUM CONCⁿ (MOLES X10⁻²/LITRE.)
ISOTHERMS OF LYSINE
25°C.
37°C.
50°C.
FINAL pH 7·3
GRAPH 33.

CALCULATION OF AREA OCCUPIED PER MOLECULE FROM ISOTHERM DATA.

The surface area of the silica used in the preparation of these isotherms, as determined by the Lea and Nurse method, is 41,400 sq.cms. Assuming that the maximum adsorption of each amino acid, shown in graphs 26-33 represents complete covering of the silica surface, then the area occupied by each amino acid molecule on the surface of the silica may be calculated. The area per molecule so calculated for the amino acids in graphs 26-33 are within the range 3-5 sq.A.^{o} (for proline, 8 sq.A.^{o})

These values are obviously too small to represent a monolayer, and indeed would suggest approximately 4-5 monolayers on the surface of the silica. However, a study of each isotherm, for each amino acid, shows a continuous rise in adsorption to a constant saturation value. This smooth rise in adsorption is in contradiction to the possibility of 4-5 layers on the silica surface. Consequently, the value for the surface area of the silica determined by the Lea and Nurse method must be grossly in error. The air-permeability method is by nature much less sensitive and accurate than a method based on adsorption on the surface of the powder.

apparent adsorption isotherms with a simple Langmuir equation is coincidental and not of real significance.

Lindau and Rhodius[7], when studying the adsorption of gelatin and egg-albumin on quartz, also obtained isotherms which show a fairly linear rise in adsorption with equilibrium concentration, and report the monomolecular layer of protein to be irreversibly adsorbed.

The possibility that the amino acids may be apparently irreversibly adsorbed on the silica, or at least difficult to desorb, is partially supported by the very high values calculated for E_2, the energy required to desorb the amino acid molecule.

It should be noted here that the rate curves for the adsorption of these amino acids appear to obey the Langmuir adsorption equation, but this behaviour may be coincidental and of no real significance.

4.3. Heat of adsorption.

From the experimental data of these isotherms, values of the heat of adsorption (ΔH°) were calculated in the following manner, derived and applied by Vickerstaff[50] to determine heats of dyeing.

The absolute temperature, the standard affinity, and the standard heat of adsorption, are related by the equation

(9) $$d(\Delta u^{\circ}/T)/d(1/T) = \Delta H^{\circ}$$

The standard heat of adsorption so calculated may be defined as the heat adsorbed per mole of amino acid, when a small quantity of the amino acid is transferred from a large volume of the solution in its standard state. The change of ΔH° with temperature is comparatively small, especially over the narrow temperature range involved in the adsorption experiments,

so that as a first approximation is may be regarded as constant.

On integration, equation (9) may be expressed in the form

(10) $\Delta u^{o}/T = \Delta H^{o}/T + C$ c = a constant,

which can be re-written as

(11) $\Delta H^{o} = (\Delta u^{o}_{1}/T_{1} - \Delta u^{o}_{2}/T_{2})/(1/T_{1} - 1/T_{2})$

where Δu^{o}_{1} and Δu^{o}_{2} are values of Δu^{o} at the two temperatures T_1 and T_2 respectively. In calculating affinities there is difficulty in finding a satisfactory expression for the activity or effective concentration of the amino acid on the adsorbent in terms of the measurable concentration $(a)_a$. If the activity be represented as an unknown function of the concentration of the amino acid on the adsorbent, independent of temperature to a first approximation, and written fn.$(a)_a$, then, neglecting the activity coefficients in solution,

(12) $- \Delta u^{o} = RT \ln. \text{ fn.}(a)_a/(c)_s$

where $(c)_s$ = concentration of amino acid in solution in equilibrium with $(a)_a$ adsorbed.

From the affinity at two temperatures T_1 and T_2

(13) $\Delta H^{o} = -R.T_1.T_2./(T_2-T_1)\left\{\ln.(\text{fn.}(a)_a.(c)_s 1./\text{fn.}\ (a)_a 1.(c)_s)\right\}$

If the conditions are so adjusted that the concentration of amino acid on the adsorbent is the same at both temperatures, fn. $(a)_a$ = fn. $(a)_a^1$, so that

(14) $\Delta H^{o} = -R.T_1.T_2./(T_2-T_1)\left\{\ln.(c)_s 1/(c)_s\right\}$

Consequently, it is possible to calculate the heat of adsorption from a knowledge of the concentrations of amino acid in solution which are in equilibrium with the same amount of amino acid on the adsorbent at two

different temperatures.

In table 16 are shown the experimental data, for each amino acid, from which mean values of ΔH^{o} (observed) over the temperature range studied (25°C - 50°C) were obtained by using equation (14).

An independent calculation of the values of ΔH^{o} may be made from the relationship,

$$E_1 - E_2 = \Delta H^{o}$$

and the values so obtained are shown in table 16 as ΔH^{o} (calc.).

Conclusions from Section 4.3.

(1) The values of ΔH^{o} (calc.) are in good agreement with those of ΔH^{o} (obs.).

(2) For each amino acid in group 1, the values of ΔH^{o} is 6.00 kg. cals./mole approximately.

(3) For each amino acid in group 2, the value of ΔH^{o} is 11.6 kg. cals./mole approximately.

(4) All values of ΔH^{o} are negative in sign indicating that the adsorption process is exothermic.

It is now desirable to examine in detail the values of E_1 and E_2 (table 15) and those of ΔH^{o} and to derive from them some conclusions on the nature of the adsorption bonds between the amino acids and silica.

4.4. Nature of the adsorption bonds.

The significance of hydrogen bridging in amino acid and protein structures is now well known. Albrecht and Corey[51] have established the structure of the glycine crystal by X-ray methods, and have concluded that

Table 16.

Amino Acid	$(c)_s$	$1/Tx10^3$ (°K)	$-\Delta H^o$ (obs.)	$-\Delta H^o$ (calc.)	
Group 1.					
Glycine	0.57	3.36			
	0.86	3.23	5.93	6.60	
	1.24	3.10			
α-Alanine	1.30	3.36			
	1.90	3.23	5.87	6.10	
	2.80	3.10			
Proline	0.68	3.36			
	1.00	3.23	6.00	9.20	
	1.49	3.10			
Group 2.					
β-Alanine	0.40	3.36			
	0.83	3.23	11.57	11.50	
	1.82	3.10			
γ-Aminobutyric acid.	0.46	3.36			
	0.99	3.23	11.61	-	¥
	2.13	3.10			
δ-Aminovaleric acid.	0.93	3.36			
	1.98	3.23	11.55	13.00	
	4.22	3.10			
ε-Aminocaproic acid.	1.40	3.36			
	2.98	3.23	11.54	-	¥
	6.19	3.10			
Lysine	0.77	3.36			
	1.68	3.23	11.60	13.90	
	3.53	3.10			

$(c)_s$ = concentration of amino acid in solution in moles x 10^{-2}/litre which is in equilibrium with the same amount of amino acid adsorbed at the three temperatures.

ΔH^o (obs.) = kg. cals./mole

ΔH^o (calc.) = E_1-E_2 = kg. cals./mole.

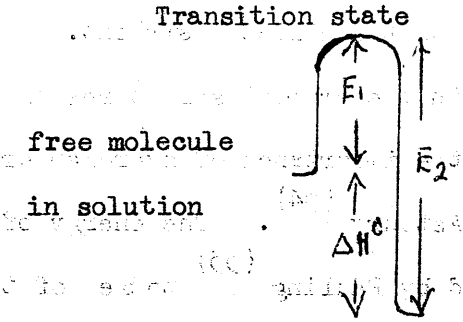
¥ See note in conclusions of Section 4.1.

in the crystal the molecule has the zwitterion structure with two of the three hydrogen atoms attached to the nitrogen, forming strong hydrogen bonds to oxygen atoms in the same layer and the third sharing its bond-forming capacity nearly equally between two nearest oxygen atoms in the adjacent layer. In view of these facts, it is possible that similar hydrogen bonds may be formed between the ammonium group of the adsorbed amino acid and the oxygen atoms of the silica adsorbent. Similar hydrogen bonds have been found in the crystal structures of α-alanine and diketopiperazine [53], and the importance of hydrogen bridging in proteins has been discussed by Astbury [54]. The energy of formation of the hydrogen bond is reported by Pauling [55] to be of the order of 5 kg. cals./mole.

From a study of the numerical values of ΔH^{o} obtained for the amino acids, the following discussion may be advanced on the possible nature of adsorption bonds formed. The agreement between the ΔH^{o} values for these amino acids and the values of Pauling for hydrogen bonds may be coincidental. It is noted, however, that for betaine ΔH^{o} is approximately 1-2 kg. Cals./mole. (see p.76.) It is significant that betaine has no hydrogen atoms attached to the quaternary nitrogen and can thus form no hydrogen bonds with the oxygen atoms of the silica. The mechanism of adsorption deduced in the following discussion is not considered as superseding the earlier theory of electrostatic attraction between the silica and the ammonium group of the amino and molecule, but is thought of as a secondary bonding mechanism operating in conjunction with the electrostatic attraction. Adsorption takes place primarily by electrostatic attraction and when the hydrogen atoms of the ammonium

group come within the sphere of influence of the oxygen atoms of the silica, then hydrogen bonds may be formed thus constituting secondary adsorption bonds.

The relationship between E_1, E_2, and ΔH^o may be expressed diagrammatically as follows:-



The values of ΔH° reflect the difference between the number of bonds formed in the adsorbed state (indicated by E_2) and the number broken in solution (indicated by E_1).

For Group 1, the values of ΔH°, 6 kg. cals./mole, (which is comparable to the bond strength of one hydrogen bond) indicate that the number of bonds formed in the adsorbed state is greater by one than the number which may be broken in solution. It is, therefore, possible that each amino acid molecule of group 1 forms one hydrogen bond in the adsorbed state.

Comparison of the E_1 values of groups 1 and 2 show than an additional quantity of energy, 6 kg. cals./mole approx., is required to activate the molecules of group 2 and consequently these latter molecules may form one hydrogen bond in solution. The ΔH° values of group 2 indicate that in the adsorbed state the number of bonds formed is greater by two (each of strength 6 kg. cals./mole approx.) than the number broken in solution. Since in the formation of the transition state in solution one hydrogen bond is broken, it is, therefore, possible that group 2 molecules form three hydrogen bonds in the adsorbed state.

If, in group 1, one hydrogen bond is formed in the adsorbed state, then there remain $(E_2 - 6) = 10$ kg. cals./mole approx. to be evolved in the formation of other adsorption bonds. Similarly, in group 2, if three hydrogen bonds are formed in the adsorbed state, then $(E_2-18) = 10-11$ kg. cals./mole approx. remain which also reflect the formation of other adsorption bonds. A similarity is thus observed between groups 1 and 2.

This energy, 10 - 11 kg. cals./mole, is probably evolved in the formation of the polar bond between the positively charged ammonium group of the amino acid molecule and the negatively charged silica, which is the fundamental mechanism of the adsorption process as has been shown in previous sections. Additional data supporting this hypothesis may be derived from the values of B_1 and B_2 (table 15), the frequency factors, of both groups 1 and 2.

The interpretation of the frequency factor in reaction kinetics in solution has been discussed in detail by Bell[56], who reports that values of B of 10^{13} - 10^{19} are only observed in reactions between two oppositely charged polar molecules. The very high values of B_2 of 10^{17} - 10^{19} for group 2 molecules must mean partial charge neutralisation in the reverse process from the adsorbed to the transition state. Conversely, in the forward adsorption process the general trend of B_1 values of both groups 1 and 2 is one of continuous separation of charges in the amino acid molecule from the free to the adsorbed state. These facts suggest, therefore, that in solution at its isoelectric pH, the dipolar amino acid molecule, which has a net charge of zero, absorbs energy, the effect being to increase the separation of its opposite charges. Finally, beyond the transition state adsorption takes place, and the charge separation is greatest in the adsorbed state. Thus the values of B_1 and B_2 of both groups 1 and 2 support the polar mechanism of adsorption between the amino acid and silica.

In group 1 the values of E_1, the energy of activation, are 8-9 kg. cals./mole. If, in group 2, 6 kg. cals./mole are required to break

one hydrogen bond in solution, then a further 9 kg. cals./mole approx. are also required to activate the molecule. This quantity of energy, common to both groups 1 and 2, arises owing to the following factors.

(1) Energy is adsorbed by the amino acid molecules the effect being to increase the separation of their opposite charges.

(2) The adsorption site on the surface of the silica will be solvated and energy is, therefore, required to desolvate the site before adsorption will take place.

(3) Similarly, the positive ammonium group of the amino acid molecule in the transition state must be desolvated before adsorption takes place.

Since each amino acid in group 1 forms only one hydrogen bond in the adsorbed state, and those in group 2 form three such bonds, it is evident that the carboxylate group, when adjacent to the ammonium group, as in group 1, exerts a steric "blocking" effect in the adsorbed state. This steric effect is reflected in the very low values of B_1 of group 1. Since the geometrical requirements for adsorption are highly specific for molecules of group 1, their values of E_1 and E_2 must be correspondingly smaller than those for molecules of group 2, in order that their rates of adsorption and desorption shall be comparable.

As has been reported (page 66), no appreciable change in extent of adsorption of betaine could be detected at the temperatures 25°C, 37°C and 50°C. Consequently the heat of adsorption for betaine must be very low, and is probably 1-2 kg. cals./mole. Betaine possesses no hydrogen atoms directly attached to the positively charged nitrogen and thus cannot

form hydrogen bonds with the silica. The adsorption bond between betaine and silica is probably purely electrostatic and thus the bond between silica and glycine (ΔH° = 6 kg. cals./mole.) is stronger than a purely electrostatic bond and is therefore possibly a hydrogen bond as has been suggested.

Thus the mechanism of adsorption of the amino acids on silica is primarily one of electrostatic attraction and when the ammonium group approaches the oxygen atom of the silica, then hydrogen bonds may be formed which constitute secondary bonding forces.

Tiselius[57, 58], has investigated the adsorption of water and ammonia on the zeolite crystals, heulandite and analcite, and considers that such adsorption, which takes place between molecules with strong permanent dipoles and ionic crystals, constitutes the borderline between physical and chemical adsorption. The heat of adsorption of water on heulandite was 14.1 kg. cals./mole, and that of ammonia on analcite was 16.6 kg. cals./mole. Thus, these adsorption studies by Tiselius are similar in certain respects to the present study of the adsorption of amino acids on silica.

Conclusions from Section 4.4.

From the preceding discussion we may derive the following general conclusions concerning the mechanism of adsorption of these amino acids on silica.

(1) The primary mechanism of adsorption is the mutual attraction of the positively charged ammonium or imino group of the amino acid molecule and the negatively charged oxygen atom of the silica.

(2) In the adsorbed state, the ammonium group may also form hydrogen bonds with the electronegative oxygen atoms of the silica.

(3) α-Amino acids, in contrast to β-, γ-, and higher acids, form only one hydrogen bond in the adsorbed state due to a steric "blocking" effect of the carboxylate group.

(4) The values of E_1, E_2 and ΔH° for lysine confirm the conclusion derived in section 2 that lysine is adsorbed through the ϵ-ammonium group. Consequently, we may observe that if the amino acid molecule contains two reactive ammonium groups, the determination of the energy of activation and heat of adsorption of the molecule may identify the group through which adsorption takes place.

(5) The values of B_1 and B_2, the frequency factors of the adsorption and desorption process respectively, are of fundamental importance in elucidating the mechanism of adsorption and distinguish between a polar and a non-polar mechanism.

Part 2.

PEPTIDES.

Section 1.

The influence of the composition of the peptide on its adsorption.

Note. The extent of adsorption of the dipeptides examined is extremely small and it was found necessary to carry out these adsorption experiments from 0.02 M aqueous solutions of the dipeptides on a surface area of adsorbent of 82,800 sq. cms., referred to as standard surface area 2. (see Experimental section). This concentration of solution is twice that used in the amino acid experiments; similarly, the new surface area is also twice that used for the amino acids. These experimental conditions were kept constant for all dipeptides; the extent of adsorption is now reported as moles x 10^{-5}/standard surface area 2.

Since urea, hydantoic acid, and N-acetylglycine are not adsorbed, it may be deduced that peptides are not adsorbed on the silica through the peptide link.

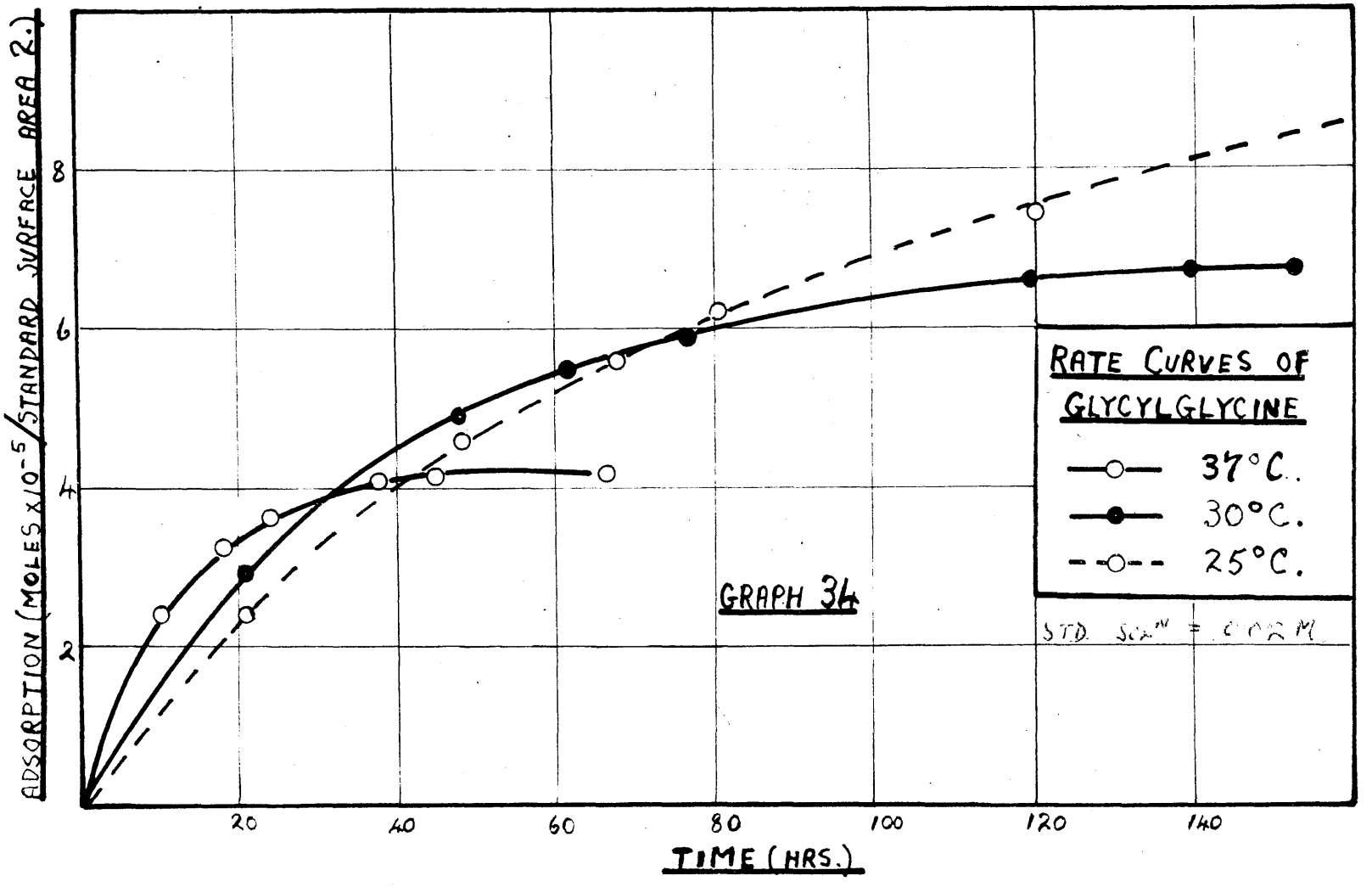
The experimental data for the rates of adsorption of four dipeptides, of closely related structures, are summarised in table 17. The dipeptides are compared at 25°C, since the adsorptions of alanylglycine and leucylglycine at 37°C are so rapid that accurate rate curves could not be obtained. The adsorption characteristics of the simple amino acids glycine, α-alanine, and leucine have been discussed in Part 1. The rate of adsorption of glycylglycine is considerably lower than that of alanylglycine, glycylleucine, or leucylglycine. The behaviour of the simple α-amino acids and their related dipeptides are in this instance

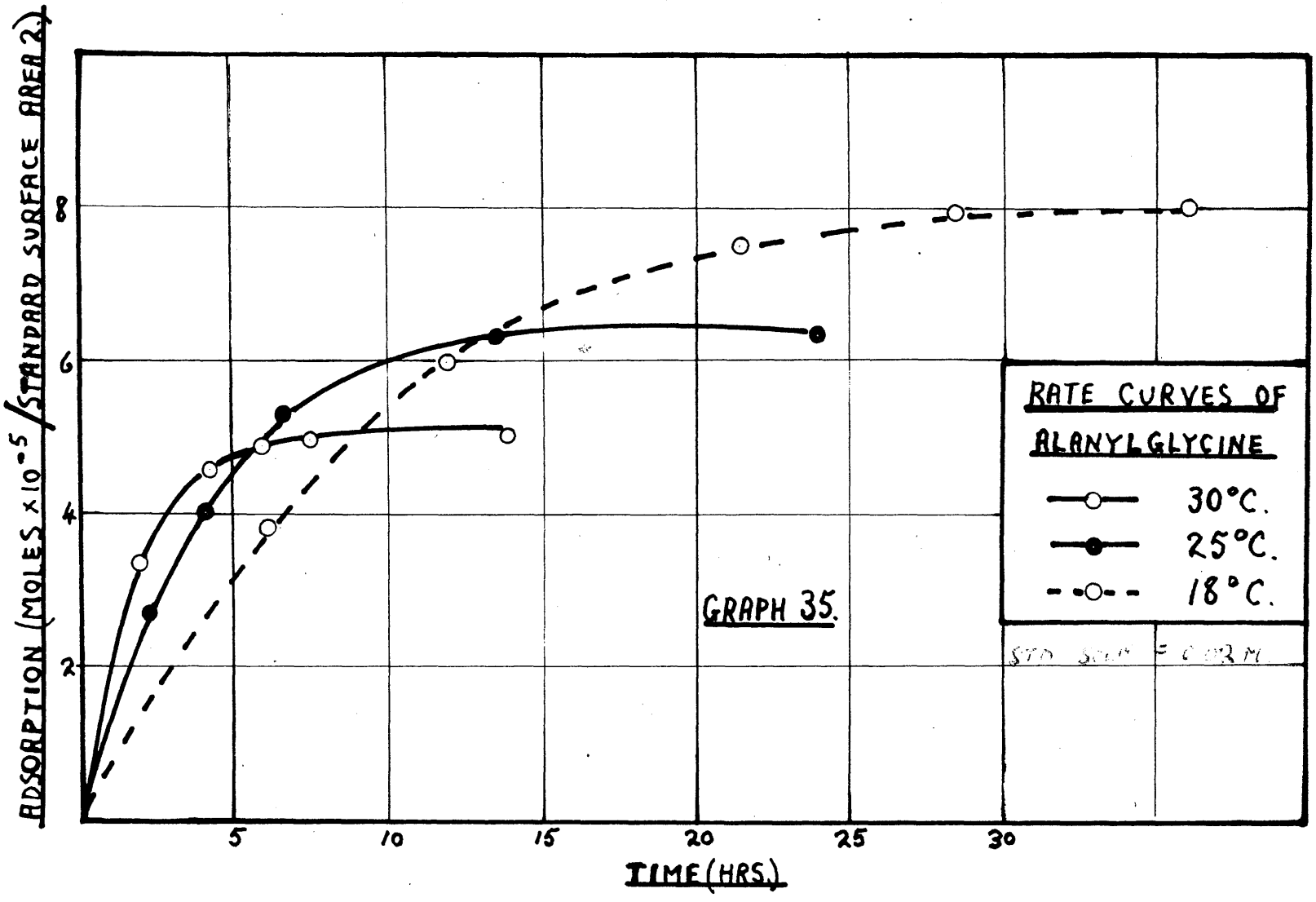
Table 17.

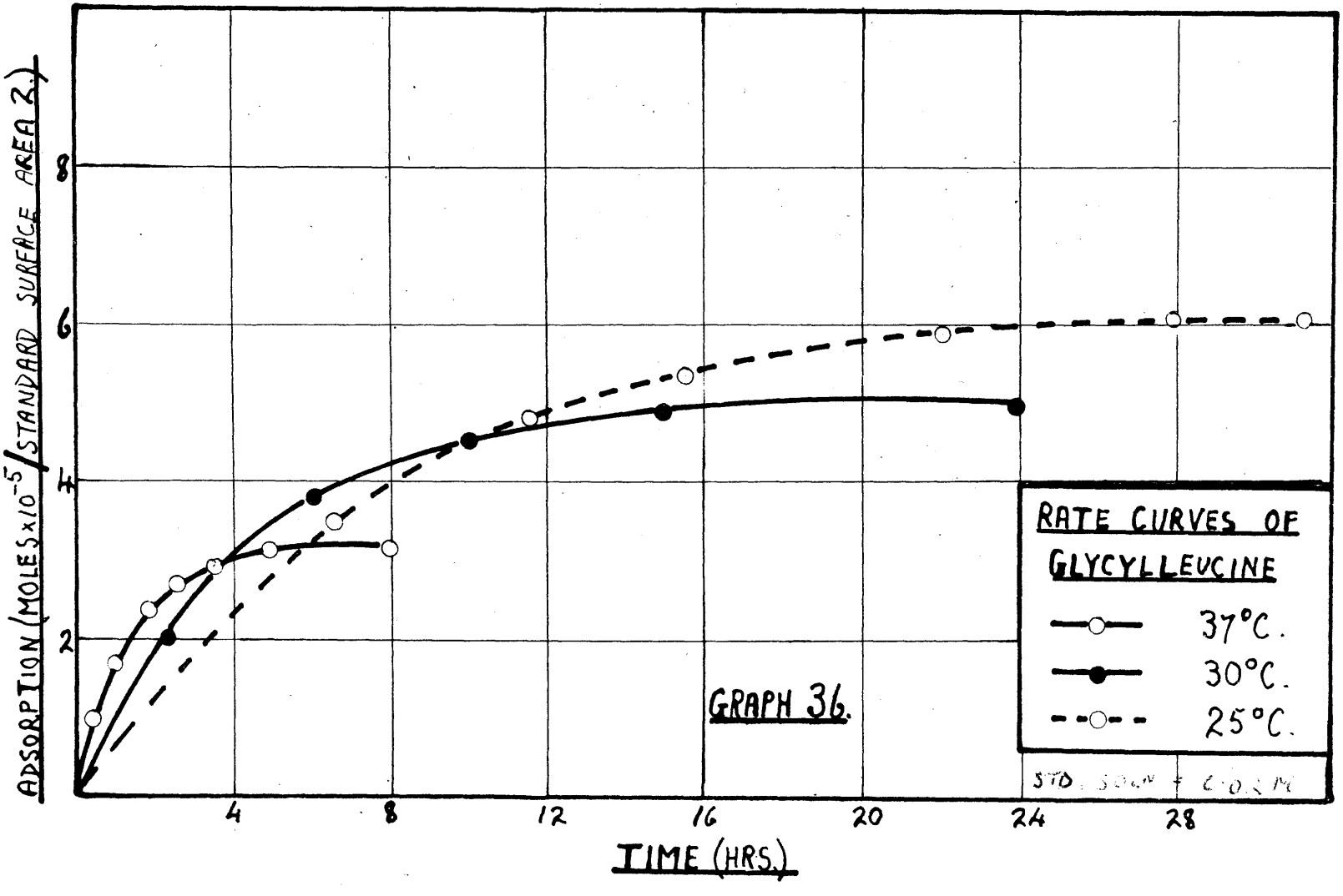
Peptide	Graph	(a_E)	$(k_1)_{25°C}$
Glycylglycine	34	8.64	8.90×10^{-3}
Alanylglycine	35	6.29	1.41×10^{-1}
Glycylleucine	36	6.34	1.25×10^{-1}
Leucylglycine	37	3.31	6.10×10^{-2}

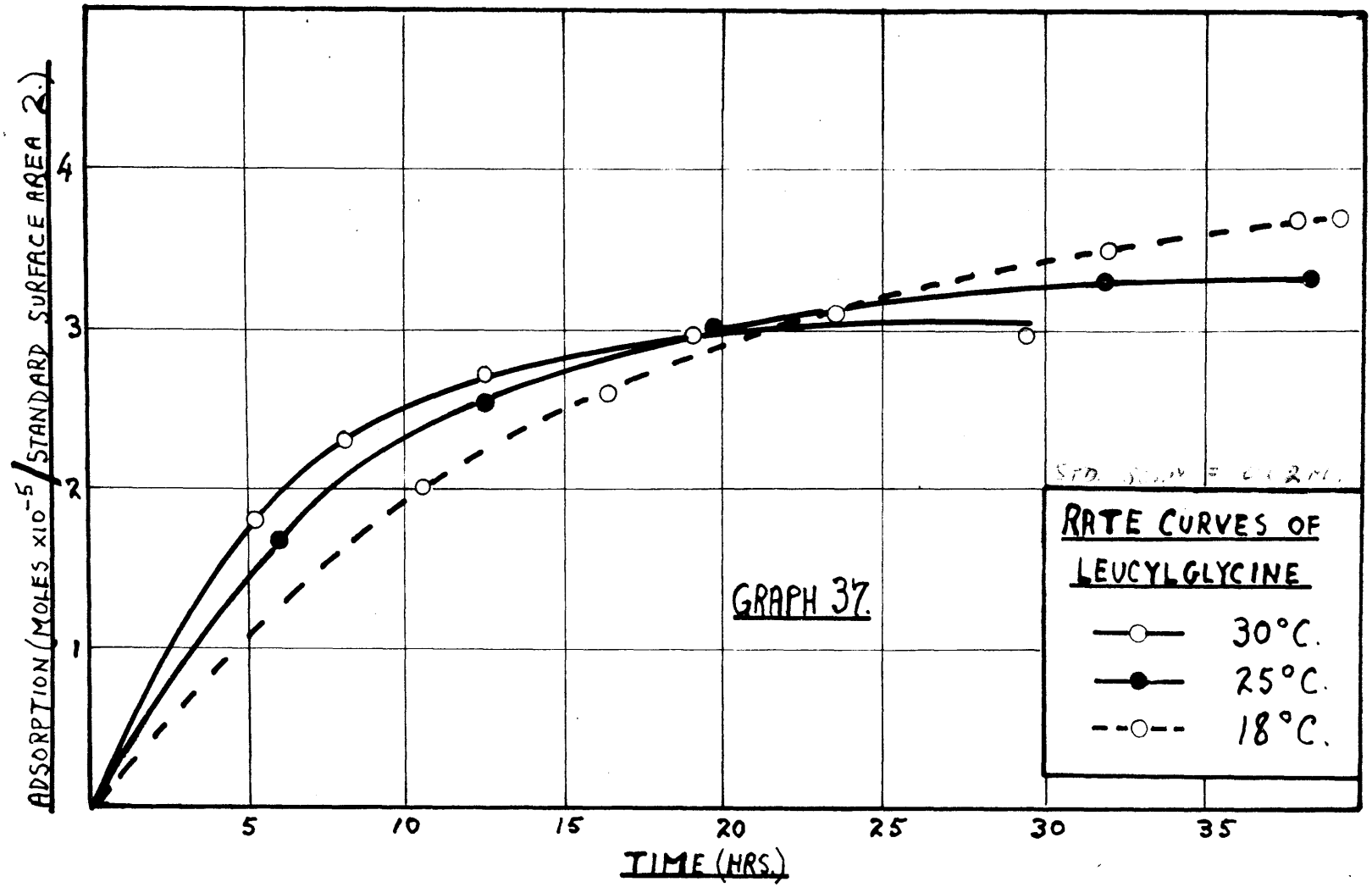
(a_E) = equilibrium adsorption in moles x 10^{-5}/standard surface area 2 from 0.02 M aqueous solutions at its isoelectric pH.

(k_1) 25°C = velocity constant of the adsorption process at 25°C.


ADSORPTION (MOLES x 10⁻⁵/STANDARD SURFACE AREA 2.)
8
6
4
2
20
40
60
80
100
120
140
TIME (HRS.)
RATE CURVES OF GLYCYLGLYCINE
37°C.
30°C.
25°C.
GRAPH 34
STD. SCAN = 0.02 M


ADSORPTION (MOLES x 10⁻⁵ / STANDARD SURFACE AREA 2)
8
6
4
2
5
10
15
20
25
30
TIME (HRS.)
RATE CURVES OF
ALANYL GLYCINE
30°C.
25°C.
18°C.
GRAPH 35.


ADSORPTION (MOLES x 10^{-5} / STANDARD SURFACE AREA 2.)
2
4
6
8
4
8
12
16
20
24
28
TIME (HRS.)
RATE CURVES OF GLYCYLLEUCINE
37°C.
30°C.
25°C.
GRAPH 36.


ADSORPTION (MOLES x10⁻⁵ / STANDARD SURFACE AREA 2.)
1
2
3
4
5
10
15
20
25
30
35
TIME (HRS.)
GRAPH 37.
RATE CURVES OF LEUCYLGLYCINE
30°C.
25°C.
18°C.

very similar.

In Part 1, the influence of other functional substituents on the adsorption of an amino acid has been discussed, and it is probable that these groups will exert a similar influence on the adsorption of a dipeptide. However, two dissimilar α-amino acids, $NH_2.CHR.COOH$ and $NH_2.CHR^1.COOH$, may combine in two distinct ways to yield two isomeric but dissimilar dipeptides, differing in the positions of R and R^1 relative to the terminal ammonium and carboxylate groups. The following discussion on the experimental data for glycylleucine and leucylglycine illustrates that in addition to the nature of the secondary functional group, its location, with respect to the ammonium and carboxylate groups in the dipeptide, is of considerable importance in determining the adsorption behaviour of the molecule.

Under the same experimental conditions, the extent of adsorption of glycylleucine is approximately twice that of leucylglycine. The amount of glycylleucine adsorbed (6.34×10^{-5} moles) represents a limiting value of adsorption and corresponds to approximately a unimolecular layer. This may be seen more clearly in the isotherms of this dipeptide (graph 41) and will be discussed in greater detail in a later section, (section 4.2). The value for leucylglycine 3.31×10^{-5} moles does not represent a unimolecular layer, as may be seen from the isotherms of this dipeptide (graph 42). It is also observed that the velocity constant $(k_1)25^{\circ}C$ for the adsorption of glycylleucine is considerably greater than that for leucylglycine.

Since these two isomeric dipeptides contain the same constituent

groups, their molecular volumes will be approximately equal, and it is also probable that in their respective solutions, the molecules of each dipeptide interact with one another in a similar manner. It is clear, therefore, that the leucyl group when adjacent to the ammonium group, has a considerably greater influence on the rate and extent of adsorption of the molecule than when in the terminal position adjacent to the carboxylate group. The influence of the leucyl group in both positions is also reflected in the values of B_1, the frequency factors for the adsorption of each dipeptide; and these will be discussed in greater detail in a later section (4.4.). Interpreting the present data, it is probable that the leucyl group of leucylglycine is in contact with the silica surface, and possessing a limited freedom of movement will thus shield potential adsorption sites from other approaching molecules.

Comparison of the experimental data for glycylglycine, alanylglycine, and leucylglycine shows a marked decrease both in rate and extent of adsorption with increase in size of the carbon side chain adjacent to the ammonium group and to the silica surface. Since these values of adsorption do not represent complete covering of the available silica surface, as may be seen from the isotherms of the respective dipeptides (graphs 39, 40, 42), the decrease in extent of adsorption cannot be entirely due to the increase in molecular volume of the dipeptide. Further, under the same experimental conditions, both the rate and extent of adsorption for glycylleucine are almost identical with those for alanylglycine, thereby indicating that the influence of the methyl group adjacent to the ammonium group is virtually equivalent to that of

the larger isobutyl group in the terminal position adjacent to the carboxylate group. These facts again emphasise the greater influence of the alkyl group when adjacent to the ammonium group and to the silica surface.

Conclusions from Section 1.

(1) As for the simple amino acids, the introduction of an alkyl group into the dipeptide molecule increases its rate of adsorption. As the alkyl group increases in size both the rate and extent of adsorption of the dipeptide decrease. This phenomenon was also observed in the simple amino acids.

(2) It is probable that other secondary substituent groups will exert on the adsorption of the dipeptides influences similar to those observed for the simple amino acids.

(3) However, the position of the secondary substituent in the dipeptide molecule with respect to the ammonium and carboxylate groups is very important. It is observed that the isobutyl group decreases both the rate and extent of adsorption of the dipeptide in a more pronounced manner when adjacent to the ammonium group than when in the terminal position adjacent to the carboxylate group.

(4) When adjacent to the ammonium group, the isobutyl side chain may cover potential adsorption sites on the silica surface.

Section 2.

The effect of pH on the adsorption of the dipeptide.

As in Part 1, these experiments to determine the effect of pH on adsorption were carried out from aqueous unbuffered solutions of the dipeptides at 25°C. The dipeptides readily coagulated from solutions of pH greater than 7; consequently, adsorption values could not be determined in this pH range.

In table 18 are reported the maximum adsorption and the pH range over which it is attained for each dipeptide (see also graph 38). In general the adsorption behaviour of each dipeptide over the pH range studied in similar to that shown by the simple amino acids. In strongly acidic solutions, where the solvent is now dilute mineral acid, low adsorptions are again observed. Similarly, on approaching neutral or alkaline pH values, where there is dissociation of the positively charged ammonium group into the uncharged amino group, the extent of adsorption again decreases rapidly. This fact also confirms that adsorption of these dipeptides takes place through their positively charged ammonium groups. Of the four dipeptides, only glycylglycine and glycylleucine attain maximum adsorption at their isoelectric pH values, and it is noticeable that neither possesses a carbon side chain adjacent to the ammonium group, as do both alanylglycine and leucylglycine. Comparison of glycylglycine, alanylglycine, and leucylglycine shows that the pH range for maximum adsorption has decreased with increase in size of the carbon side chain adjacent to the ammonium group. These facts again emphasise the conclusions

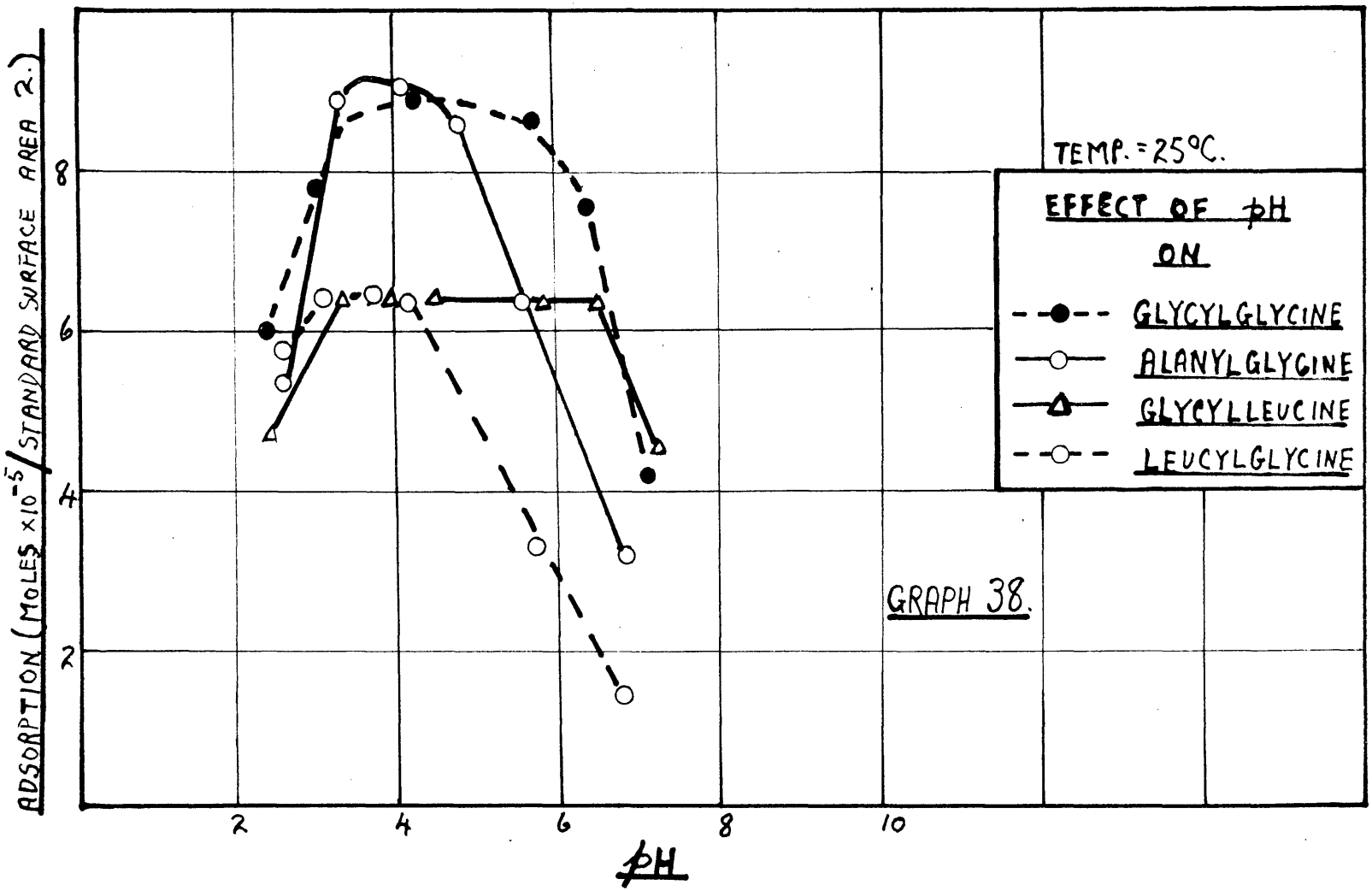
Table 18.

Temp. = 25°C.

Peptide	$a_{max.}$	$pH_{max.}$	Isoelectric[34] pH
Glycylglycine	8.86	3.4 - 6.0	5.60
Alanylglycine	9.0	3.3 - 4.7	5.64
Leucylglycine	6.40	3.2 - 4.2	5.70
Glycylleucine	6.40	3.2 - 6.5	5.73

$a_{max.}$ = maximum adsorption in moles x 10^{-5}/standard surface area 2.

$pH_{max.}$ = pH range over which $a_{max.}$ is attained.


ADSORPTION (MOLES $\times 10^{-5}$/STANDARD SURFACE AREA 2.)
8
6
4
2
2
4
6
8
10
pH
TEMP. = 25°C.
EFFECT OF pH
ON
GLYCYLGLYCINE
ALANYLGLYCINE
GLYCYLLEUCINE
LEUCYLGLYCINE
GRAPH 38.

deduced in the previous section concerning the greater influence of the secondary carbon chain when adjacent to the ammonium group.

The decrease in extent of maximum adsorption from 9.00×10^{-5} moles (for glycylglycine and alanylglycine) to 6.40×10^{-5} moles (for leucylglycine and glycylleucine) is due to the increase in molecular volume. The maximum adsorption shown here for glycylleucine and leucylglycine corresponds to a unimolecular layer as may be seen from the isotherms of these two dipeptides (graphs 41 and 42).

The pH range for maximum adsorption of glycylleucine is slightly greater than for glycylglycine, and it may consequently be deduced that the leucyl carbon chain adjacent to the carboxylate group has modified the adverse influence of this electronegative group. This effect strongly resembles that shown by the alkyl group in the simple amino acids.

Section 3.

Kinetics of adsorption.

The equations of Hill[44] and Washburn[45] were again tested with the rate data for the adsorption of the four dipeptides by a plot of adsorption vs time, and in each case a negative result was obtained. Consequently, the dipeptides are not adsorbed by a process involving either the diffusion of the molecules in solution to the silica surface or of capillary condensation. The Langmuir theory of adsorption was tested by applying the Langmuir rate equation to the experimental rate data for these dipeptides, plotting

$$\log_{10} \cdot a_e/(a_e - a_t) \quad \text{vs} \quad 6,$$

where a_e = adsorption at equilibrium

and a_t = adsorption at time t.

In each case the experimental data are in good agreement with the equation. From the values of the Langmuir rate constant (k) at 25°C, calculated from the above equation, values of the velocity constant $(k_1)25^{\circ}C$ for the adsorption of each dipeptide were calculated (see Part 1, section 3), and have been discussed in section 1.

It is thus clear that the kinetics of adsorption for the dipeptides are essentially similar to those for the simple amino acids. Consequently, the empirical assumptions of the Langmuir theory may also be applied to the adsorption of the dipeptides by silica. Thus, the dipeptide molecules are adsorbed to definite points of attachment on the silica surface and each such point can accommodate only one adsorbed

molecule. Further, as in the case of the amino acids, the active adsorptive centre of the dipeptide molecule, the ammonium group, is attached to the oxygen atom of the silica, and the primary force of adsorption is the mutual attraction between the positively charged ammonium group and the negatively charged oxygen atom of the silica.

Section 4.

Thermodynamic data.

4.1. Energy of Activation.

A study of the rates of adsorption of the four dipeptides at different temperatures, shown in graphs 34-37, indicates a distinct temperature coefficient; adsorption taking place with a characteristic velocity. For each dipeptide, and at each temperature, the velocity constant of the adsorption process (k_1) and of the desorption process (k_2) may be calculated in the manner described for the amino acids in Part 1, section 4.1. Applying the Arrhenius equation to these velocity constants, the energies of activation for the adsorption (E_1) and the desorption (E_2) of each dipeptide may be calculated and are shown in table 19. For each dipeptide, the frequency factors B_1 and B_2, for the adsorption and desorption process respectively, have also been calculated as described for the amino acids and are shown in table 19. Accurate values of E_2 and B_2 for glycylleucine could not be calculated since the values of $(1 - \theta_E)$ at 25°C and 30°C are very small and are, therefore, inaccurate.

Conclusions from Section 4.1.

(1) According to their values of E_1, B_1, E_2, and B_2, the four dipeptides may be divided into two groups as shown in table 19.

(2) The values of E_1, B_1, E_2, and B_2 for leucylglycine are comparable to those for the group 1 α-amino acids (Part 1, section 4.1, table 15).

(3) Similarly, the values for glycylglycine, alanylglycine, and

Table 19.

Peptide	E_1	B_1	E_2	B_2
Group 1.				
Leucylglycine	9.57	5.68×10^4	17.00	1.41×10^9
Group 2.				
Glycylglycine	16.3	6.66×10^7	30.00	1.94×10^{17}
Alanylglycine	17.0	7.03×10^8	29.5	4.34×10^{17}
~~Leucylglycine~~ Glycylleucine	15.0	3.1×10^8	-	-

E_1 and E_2 = kg. cals./mole.

B_1 and B_2 = moles/litre/sec.

glycylleucine are comparable to those for group 2 amino acids (β-alanine, lysine, etc.).

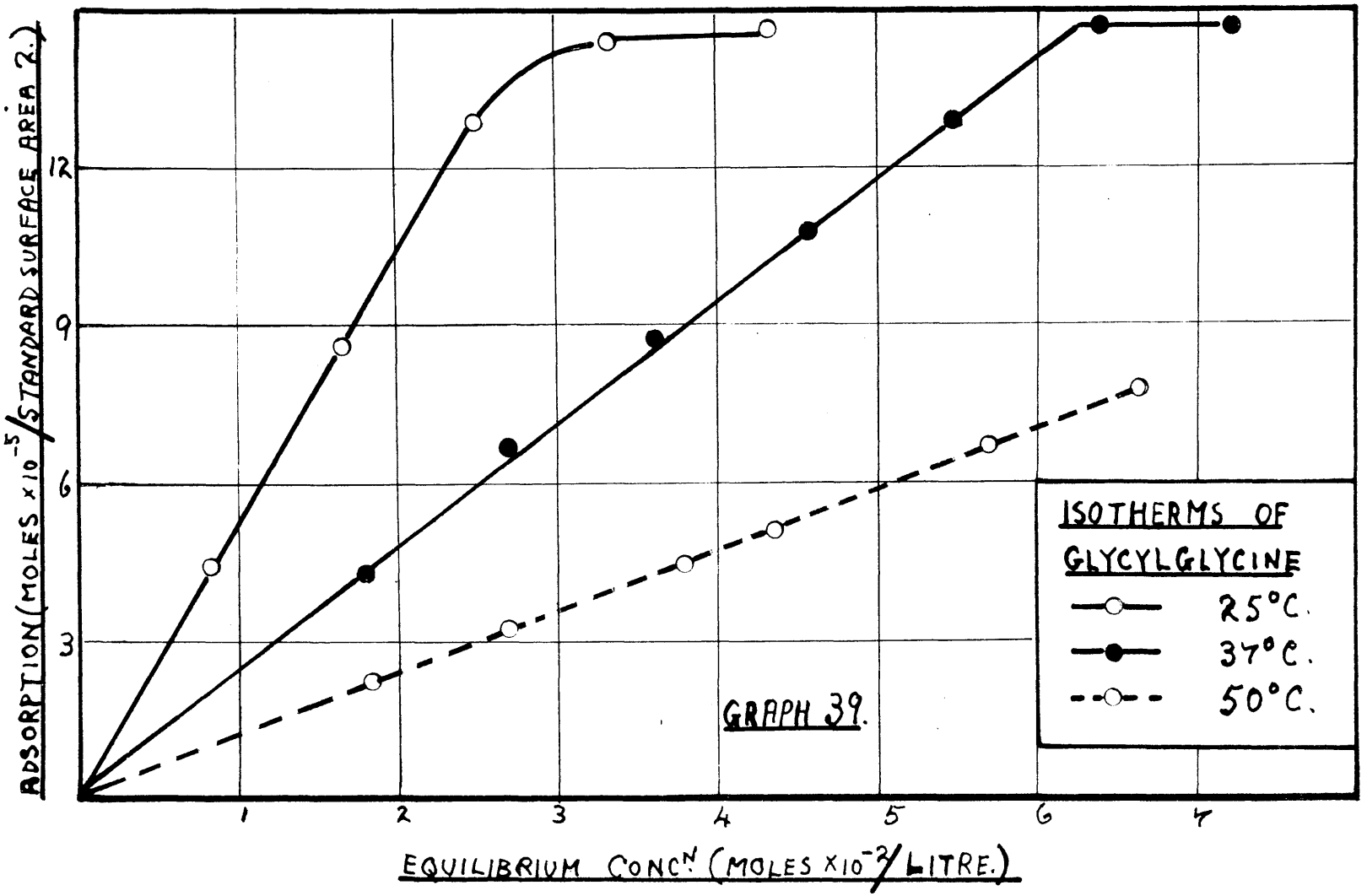
These data in table 19 will be discussed in greater detail in section 4.4 in conjunction with heat of adsorption data.

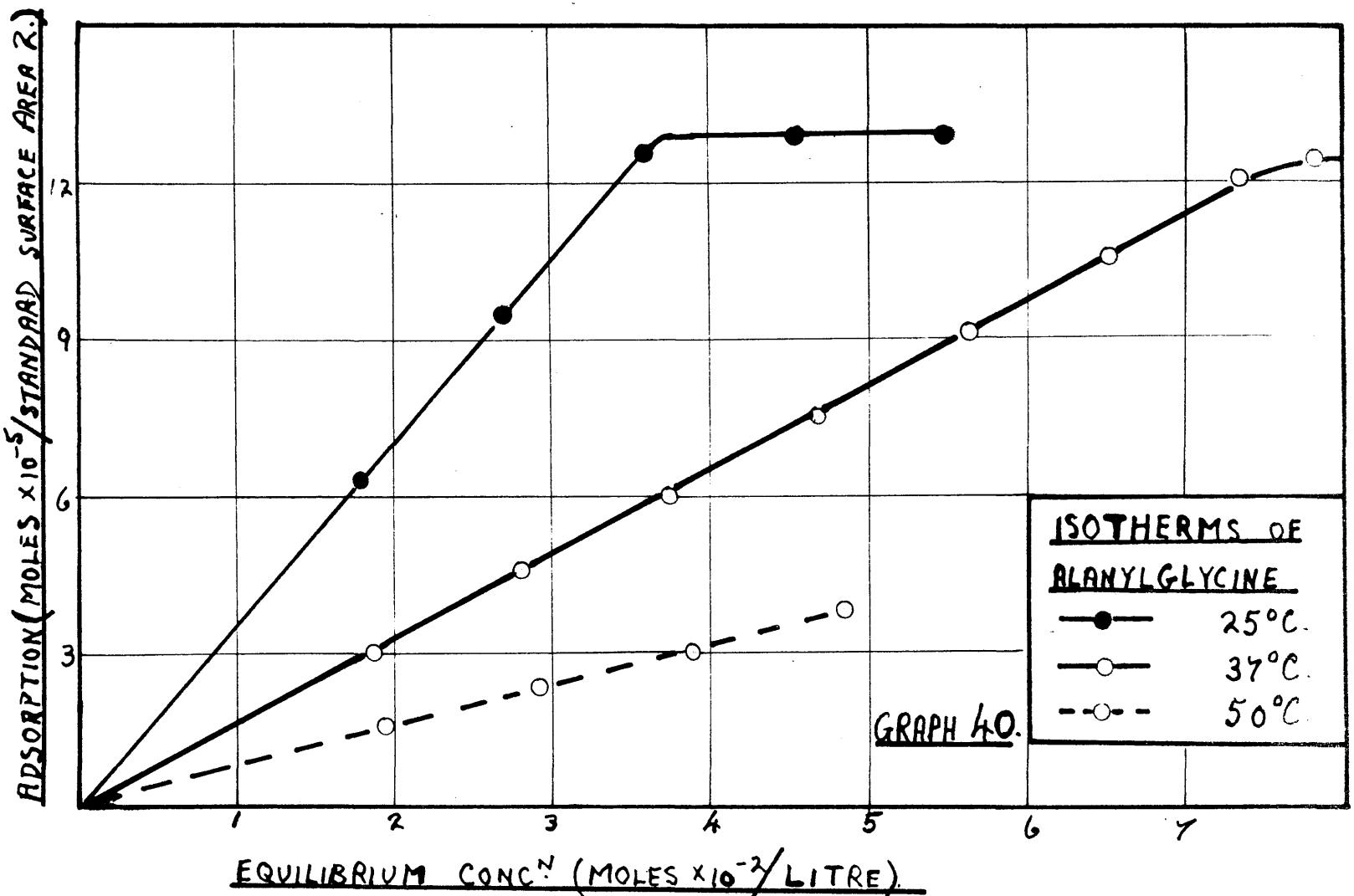
4.2. Isotherms.

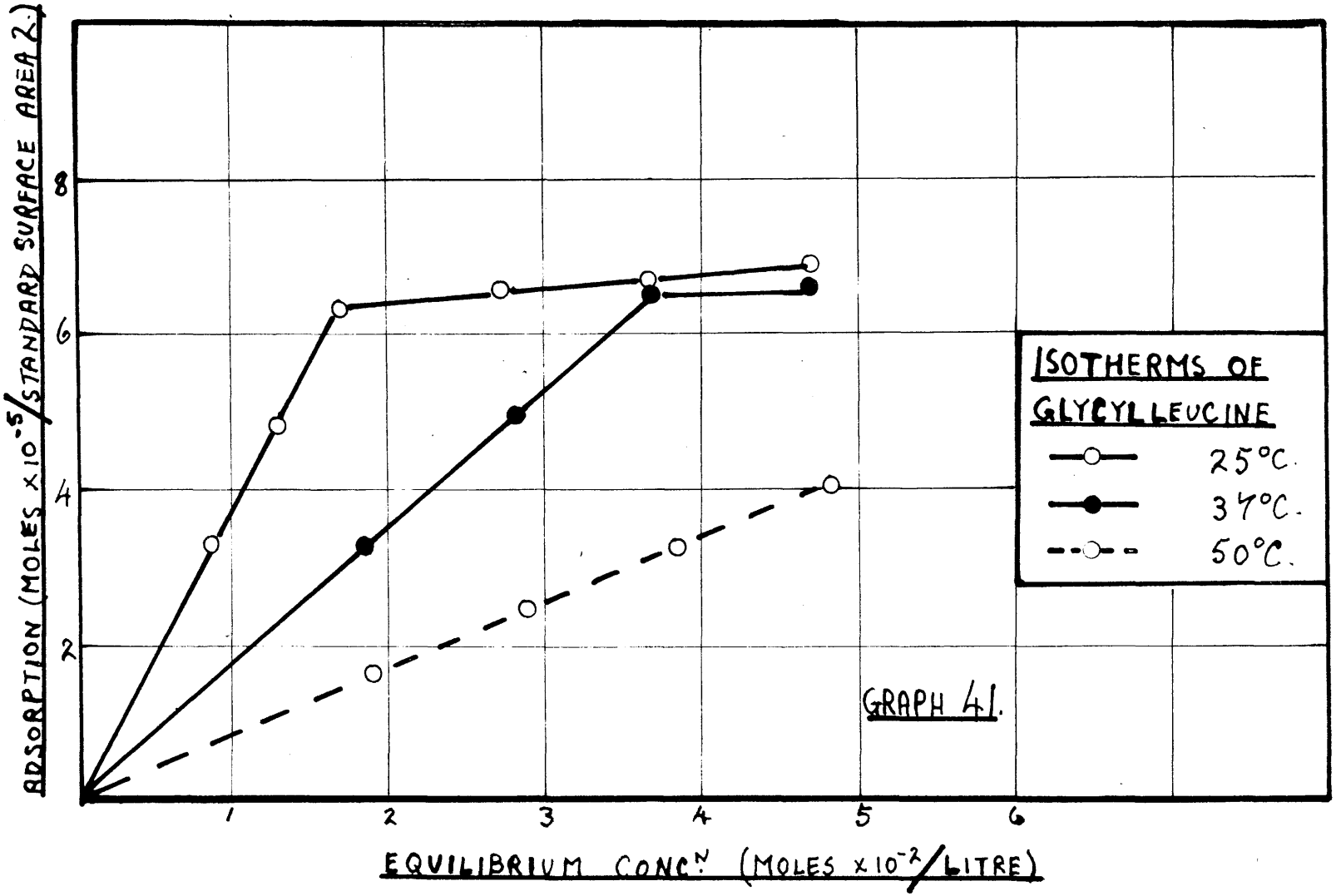
Isotherms at three temperatures have been determined for glycylglycine, alanylglycine, and glycylleucine, and are shown in graphs 39 - 41 respectively.

For each dipeptide, a sharply defined maximum adsorption is observed which is constant at each temperature and represents complete covering of the available silica surface. The shape of each isotherm suggests that these three dipeptides do not form multilayers on the silica surface. In each isotherm, adsorption increases linearly with equilibrium concentration, a behaviour identical to that shewn by the amino acids. Consequently, the discussion applied to the isotherms of the amino acids may also be applied to the isotherms of these dipeptides.

In addition to the above, isotherms at four temperatures for leucylglycine are shown in graph 42. The isotherms at 25°C and 37°C possess a sigmoid shape characteristic of multilayer formation[59]. However, before the first break-point, adsorption still varies linearly with equilibrium concentration of solution and this fact is observed more clearly in the isotherms at 50°C and 65°C. The extent of adsorption at the first break-point, both at 25°C and 37°C, is 6.2×10^{-5} moles approximately. Further adsorption then takes place and a constant maximum is finally attained at 12.00×10^{-5} moles at both temperatures. From the simple


ADSORPTION (MOLES x10⁻⁵ / STANDARD SURFACE AREA 2.)
12
9
6
3
1
2
3
4
5
6
7
EQUILIBRIUM CONCⁿ (MOLES x10⁻² / LITRE.)
ISOTHERMS OF GLYCYLGLYCINE
25°C.
37°C.
50°C.
GRAPH 39.


ADSORPTION (MOLES x10⁻⁵/STANDARD SURFACE AREA R.)
12
9
6
3
1
2
3
4
5
6
7
EQUILIBRIUM CONCⁿ (MOLES x10⁻²/LITRE).
ISOTHERMS OF
ALANYLGLYCINE
25°C.
37°C.
50°C.
GRAPH 40.

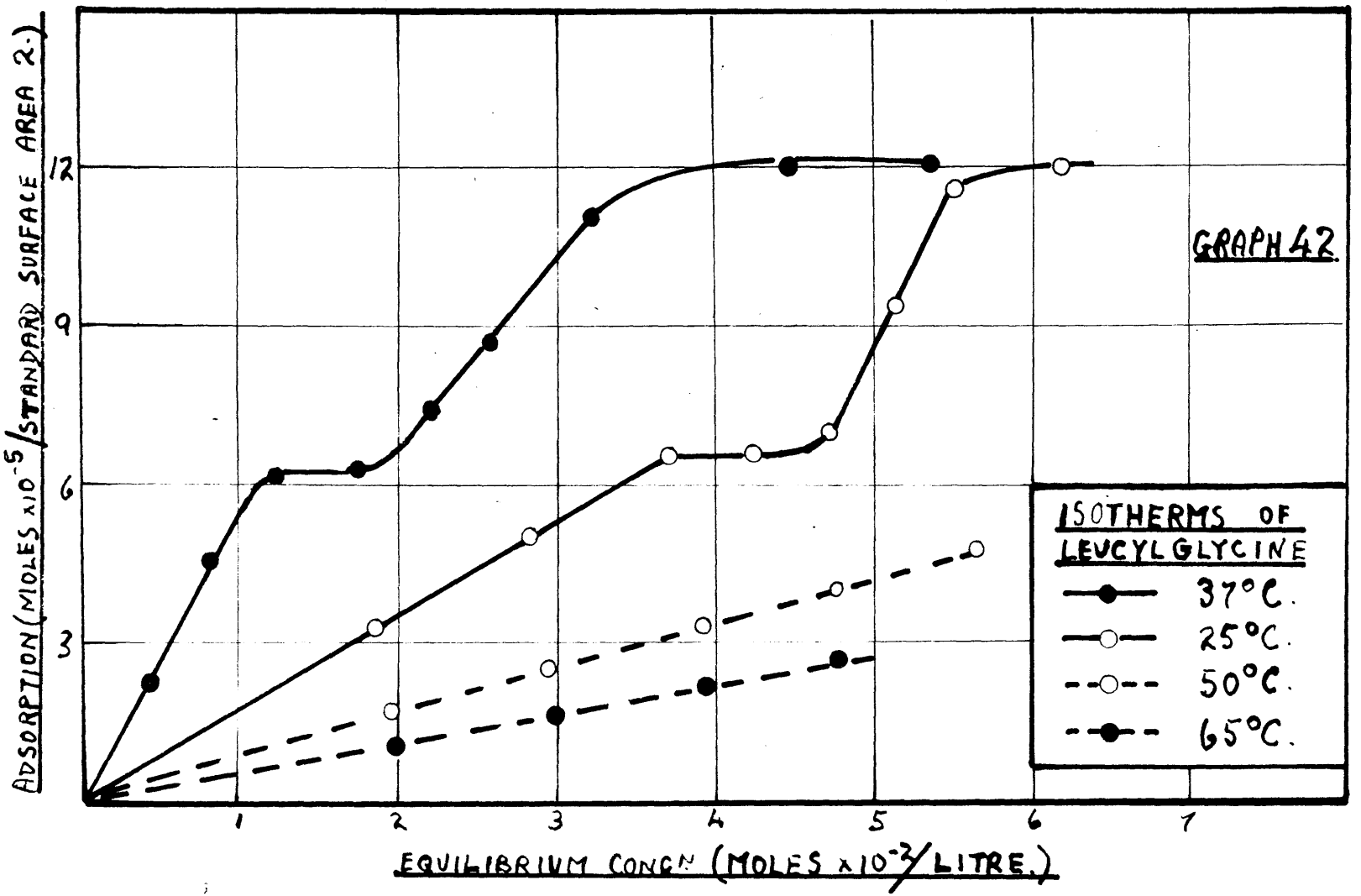

ADSORPTION (MOLES x 10⁻⁵/STANDARD SURFACE AREA 2.)
8
6
4
2
1
2
3
4
5
6
EQUILIBRIUM CONCⁿ (MOLES x 10⁻²/LITRE)
ISOTHERMS OF GLYCYL LEUCINE
25°C.
37°C.
50°C.
GRAPH 41.

numerical ratio of these two adsorption values it may be deduced that 6.2×10^{-5} moles represent a unimolecular layer and 12.0×10^{-5} moles a bimolecular layer. From graph 41 it is observed that a unimolecular layer of glycylleucine is also attained at 6.4×10^{-5} moles, which emphasises the preceding hypothesis for leucylglycine. The adsorption behaviour of leucylglycine could not be studied over a wider range than that shown in graph 42, since more concentrated solutions of the dipeptide could not be obtained.

An isotherm, similar in form to that observed for leucylglycine, has been obtained by Sheppard[49] for the adsorption of a dye cation on silver bromide. This isotherm also shows two clearly defined plateaus, corresponding to monomolecular and bimolecular layers respectively. Sheppard has found that the bimolecular layer is reversibly adsorbed although the monomolecular layer is irreversibly adsorbed.

For leucylglycine the extent of adsorption at 37°C is greater than at 25°C. This phenomenon is unprecedented and irregular since the adsorption at the higher temperatures, 50°C and 65°C, is considerably lower than that at 25°C. Although these isotherms have been duplicated, further work will be carried out on the adsorption of leucylglycine within the temperature range 25°C - 50°C before an interpretation of this phenomenon is attempted.

It is difficult to understand why leucylglycine should form multimolecular layers and not glycylglycine, although from the observed greater solubility of the latter it is possible that the greater interaction between these molecules in solution retards such multilayer


ADSORPTION (MOLES x10⁻⁵ / STANDARD SURFACE AREA 2.)
12
9
6
3
1
2
3
4
5
6
7
EQUILIBRIUM CONCⁿ (MOLES x10⁻³/LITRE.)
GRAPH 42
ISOTHERMS OF LEUCYL GLYCINE
37°C.
25°C.
50°C.
65°C.

formation. In the case of glycylleucine, the bulk of the large isobutyl side-chain adjacent to the carboxylate group will hinder multilayer formation through the carboxylate group.

4.3. Heat of adsorption.

As for the amino acids (Part 1, section 4.3.) it is possible to determine the heat of adsorption (ΔH^{o}) for each dipeptide from a knowledge of the concentrations of dipeptide in solution $(C)_S$ which are in equilibrium with the same amount of dipeptide adsorbed at two different temperatures by applying the equation

$$\Delta H^{o} = -R.T_1.T_2./(T_2-T_1) \left\{ \ln.(C)_{S1}/(C)_{S}. \right\}$$

In table 20 are shown the experimental data for each dipeptide from which mean values of ΔH^{o} (obs.), over the temperature range studied, were obtained by using the above equation.

From the relationship,

$$E_1 - E_2 = \Delta H^{o}$$

and using the data in table 19, values of ΔH^{o} were calculated and are shown in table 20 as H^{o} (calc.).

Conclusions from Section 4.3.

(1) The values of ΔH^{o} (calc.) are in good agreement with those of ΔH^{o} (obs.).

(2) The value of ΔH^{o} for leucylglycine is closely related to those for the group 1 α-amino acids. (Part 1, section 4.3, table 15).

(3) The values of ΔH^{o} for glycylglycine, alanylglycine and glycylleucine are closely related to those for the group 2 amino acids (β-alanine, lysine, etc.).

Table 20.

Peptide	$(C)_S$	$1/T \times 10^3$ (°K)	$-\Delta H^\circ$ (obs.)	$-\Delta H^\circ$ (calc.)
Group 1.				
Leucylglycine	1.50	3.36	5.78	7.5
	3.20	3.23		
	4.76	2.96		
Group 2.				
Glycylglycine	0.83	3.36	11.55	13.7
	1.80	3.23		
	3.77	3.10		
Alanylglycine	1.14	3.36	11.68	12.5
	2.46	3.23		
	5.26	3.10		
Glycylleucine	1.08	3.36	11.50	-
	2.30	3.23		
	4.85	3.10		

$(C)_S$ = concentration of dipeptide in solution in moles x 10^{-2}/litre which is in equilibrium with the same amount of dipeptide adsorbed at the three temperatures.

$\Delta H^\circ_{(obs.)}$ kg. cals./mole.

ΔH° (calc.) = $E_1 - E_2$ = kg. cals./mole.

(4) The value of ΔH^{o} for each dipeptide is negative in sign indicating that the adsorption process is exothermic.

4.4 Nature of the adsorption bond.

It has been shown that the centre of adsorption in each dipeptide molecule is the positively charged ammonium group and not the peptide link. From the conclusions of sections 4.1 and 4.3, it is evident that we may interpret the thermodynamic data for the dipeptides (tables 19 and 20) in the manner described for the amino acids. (Part 1, section 4.4.)

Consequently, the group 2 dipeptides (glycylglycine, alanylglycine, and glycylleucine) form in the adsorbed state three hydrogen bonds in addition to the fundamental polar bond between the positively charged ammonium group and the negatively charged silica. In solution, the group 2 dipeptides form one hydrogen bond. Similarly, leucylglycine (group 1) forms one hydrogen bond, in addition to the polar bond, in the adsorbed state.

The existence of the true polar bond between the ammonium group and the silica is again reflected in the high B_2 values for the group 2 dipeptides; the values of 10^{17} for the frequency factors indicate partial charge neutralisation in the reverse process from the adsorbed to the transition state. Conversely in the forward adsorption process the general trend of B_1 values for both groups 1 and 2 dipeptides is one of continuous separation of charges in the dipeptide molecule from the free to the adsorbed state.

The value of 9.57 kg. cals./mole, equivalent to the energy of

activation (E_1) for leucylglycine and which is part of E_1 for the group 2 dipeptides, arises owing to the following factors.

(1) Energy is absorbed by the dipeptide molecule, the effect being to increase the separation of its charges.

(2) The adsorption site on the silica surface will be solvated and energy is, therefore, required to desolvate the site before adsorption will take place.

(3) Similarly, the ammonium group of the dipeptide molecule must be desolvated before adsorption takes place.

Since leucylglycine forms only one hydrogen bond in the adsorbed state and the dipeptides of group 2 form three such bonds, it is evident that the isobutyl side-chain, when adjacent to the ammonium group, as in leucylglycine, exerts a steric "blocking" effect in the adsorbed state. The results of this steric effect have already been reported and discussed in section 1.

This steric effect is reflected in the very low B_1 value for leucylglycine. Since the geometrical requirements for the adsorption of leucylglycine are large, its values of E_1 and E_2 must be correspondingly smaller than those for group 2 dipeptides in order that their rates of adsorption and desorption shall be comparable.

Conclusions from Section 4.4.

(1) The fundamental mechanism of adsorption is the mutual attraction between the positively charged ammonium group of the dipeptide molecule and the negatively charged silica.

(2) The hydrogen atoms of the ammonium group may also form

hydrogen bonds with the electronegative oxygen atoms of the silica.

(3) If the dipeptide molecule contains a side-chain of large size adjacent to the ammonium group, the bulk of this side-chain produces a steric "blocking" effect and permits the formation of only one hydrogen bond in the adsorbed state.

Part 3.

Amino Acids: Adsorption on pure quartz.

The silica adsorbent used in the experiments described in Parts 1 and 2 was chosen because of its very large surface area, which is imperative in these adsorption experiments, and the fact that a large initial supply could be obtained. Further, a similar powder from the same source has been shown to be pathologially active[22]. However, the silica adsorbent contained a total impurity of 7.65%, composed of iron and aluminium, and it was, therefore, considered desirable that adsorption isotherms of two typical amino acids should also be obtained for pure quartz to see if these impurities influence the bond formation between the amino acids and the silica.

Section 1.

Experimental Methods.

1.1. Adsorbent.

The new adsorbent was prepared in the following manner.

Rock crystal, the purest obtainable form of quartz, was first crushed in a jaw-crusher into small lumps, and then ground in a roller-mill. The fine quartz below a 90 mesh B.S. sieve was separated off and then extracted with concentrated hydrochloric acid. After several acid treatments the presence of iron in the acid extracts could no longer be detected. The pure quartz was then ground in a mechanical agate mortar, in 5 gm. portions for 13 hours. Approximately 50 gms. of fine quartz of high purity was prepared in this manner and after drying in an air oven at 110°C, the material was stored until use in a vacuum desiccator.

The purity of this fine quartz was determined by evaporation of weighed samples with hydrofluoric acid and roasting the residues. On

this basis the quartz was found to contain 99.95% SiO_2. The surface area of the adsorbent, as measured by the Lea and Nurse air permeability method, was 3,600 sq.cms. per gram. When in suspension in distilled water, the fine quartz particles were observed to be negatively charged and under the influence of a direct electric current (250 v. D.C.) they flowed towards the positively charged electrode.

1.2. Amino Acids.

The amino acids chosen for these adsorption experiments on the pure quartz are α-alanine and β-alanine, so that two distinct values of ΔH° may be obtained which can be compared with those observed for the same amino acids on the impure silica. The purity of these amino acids has already been reported in the general Experimental section preceding Part 1.

1.3. Adsorption Procedure.

The procedure followed in the determination of the isotherms of these two amino acids on this pure quartz is exactly as described for the previous adsorbent in the general Experimental section; the adsorptions being determined from aqueous solutions of the amino acids at their isoelectric pH values.

Here, however, 4 gm. samples of the pure quartz were required for each adsorption test, and this quantity of adsorbent is equivalent to a surface area of 14,400 sq. cms., as measured by the Lea and Nurse method, which for reasons given in General Experimental Section is probably inaccurate. Consequently, in this Part 3 the extent of adsorption is

measured and reported in moles x 10^{-5}/standard surface area 3. (14,400 sq.cms.). The equilibrium concentration of solution for each adsorption is measured and reported, as in Parts 1 and 2, in moles x 10^{-2}/litre.

1.4. Analysis.

To determine the extent of adsorption of these amino acids, nitrogen analyses were carried out on the standard solutions before and after adsorption according to the semi-micro Kjeldahl method described in the general Experimental section.

Section 2.

Discussion of results.

2.1. Isotherms.

The isotherms for α-alanine and β-alanine at three temperatures are shown in graphs 43 and 44 respectively, and are observed to be similar in form to the isotherms of these same amino acids using the impure silica as adsorbent. (graphs 27 and 29.).

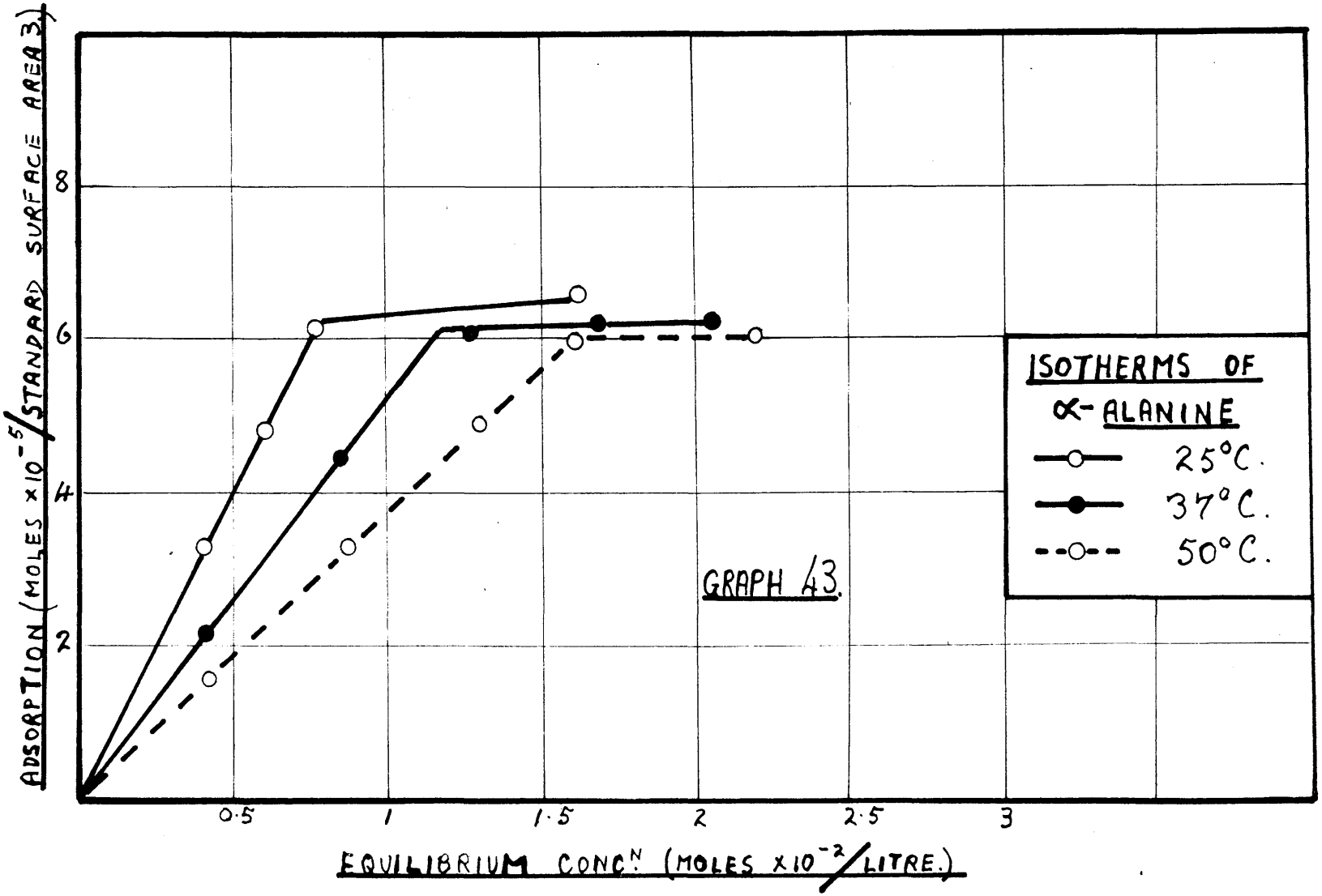
For both amino acids a sharp maximum adsorption is attained which is constant at each temperature and represents a uni-molecular layer.

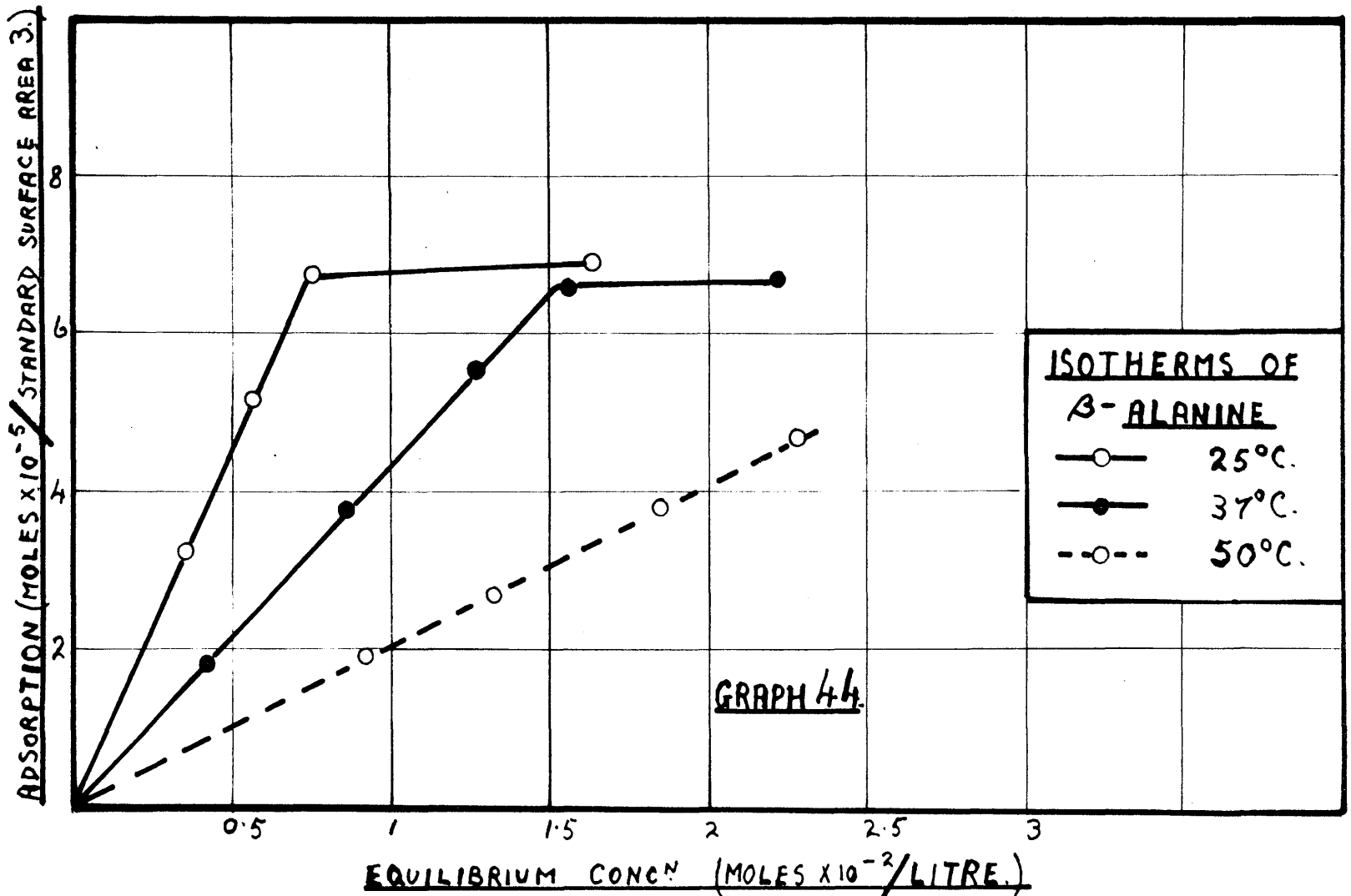
Since the quartz, like the silica, is also negatively charged, we may again conclude that the ammonium group, the active centre of the amino acid molecule, is attached to the oxygen atom on the surface of the quartz and the primary force of adsorption is the mutual attraction of the positively charged ammonium group and the negatively charged quartz. Consequently, it is observed that the primary mechanism of adsorption of the amino acids on the pure quartz is exactly the same as that on the impure silica.

2.2. Heat of adsorption.

As in Parts 1 and 2, the heat of adsorption for each amino acid on the pure quartz may be calculated from a knowledge of the concentrations of amino acid in solution in equilibrium with the same amount adsorbed at two different temperatures.

Applying the experimental data for the isotherms at different temperatures to equation 14 (Part 1, section 4.3, p.70) mean values of


ADSORPTION (MOLES x 10⁻⁵/STANDARD SURFACE AREA)
8
6
4
2
0·5
1
1·5
2
2·5
3
EQUILIBRIUM CONCⁿ (MOLES x 10⁻²/LITRE.)
ISOTHERMS OF
α-ALANINE
25°C.
37°C.
50°C.
GRAPH 43.


ADSORPTION (MOLES X 10⁻⁵/STANDARD SURFACE AREA 3.)
8
6
4
2
0·5
1
1·5
2
2·5
3
EQUILIBRIUM CONCⁿ (MOLES X 10⁻²/LITRE.)
ISOTHERMS OF
β-ALANINE
25°C.
37°C.
50°C.
GRAPH 44.

ΔH° for each amino acid over the temperature range studied (25°C - 50°C) were obtained and are reported in table 21.

Conclusions from Section 2.2.

(1) The value of ΔH° for the adsorption of α-alanine on the pure quartz is 5.75 kg. cals./mole which is comparable with that of 5.73 kg. cals./mole on the silica. (Part 1, section 4.3).

(2) The value of ΔH° for β-alanine on the pure quartz is 11.5 kg. cals./mole which is also comparable with that of 11.45 kg. cals./mole on the silica.

(3) Both values of ΔH° on pure quartz are negative in sign, indicating that the adsorption process is exothermic as is the case for the silica.

2.3. Adsorption bond formation.

From these preceding conclusions it is evident that the relationship between the number of bonds broken in solution and those formed in the adsorbed state during the adsorption of each amino acid is virtually identical for both adsorbents, the pure quartz and the silica. Since the number of bonds broken in each amino acid solution is the same for experiments with both adsorbents, it may be concluded that similar bonds are formed by the amino acids on both adsorbents.

Thus we may conclude that the impurities contained in the silica adsorbent used in Parts 1 and 2 do not influence the mechanism of adsorption or the strength and nature of the adsorption bonds formed between the amino acid molecules and the silica.

Table 21.

Amino Acid	$(C)_S$	$\frac{1}{T} \times 10^3$ (^oK)	$-\Delta H^o$
α-Alanine	0.5	3.36	5.75
	0.73	3.23	
	1.06	3.31	
β-Alanine	0.5	3.36	11.5
	1.05	3.23	
	2.26	3.10	

$(C)_S$ = equilibrium concentration of amino acid in solution in moles x 10^{-5}/standard surface area 3, in equilibrium with the same amount of amino acid adsorbed at the three temperatures 25°C, 37°C and 50°C.

ΔH^o = kg. cals./mole.

Part 4.

Miscellaneous Adsorption Experiments.

Section 1.

Adsorption experiments with optically active D- and L-quartz.

Kögl[60, 61], has postulated a chemical theory of cancer based on his work on the hydrolysis of cancer tumours in which he detected the presence of amino acids of the D-series whereas in normal tissue the amino acids are all of the L-series. In a different field of research the adsorption of assymetric complex salts of cobalt on the optically active forms of quartz has been studied by Kuroya[62] who has found that there is a close relationship between adsorption and spatial configuration of the cobalt complex. After dipping a fine powder of D- or L-quartz into a racemic complex salt solution Kuroya found that an optical rotation had been developed in the solution indicating that one optical isomer of the complex salt had been preferentially adsorbed.

With these results in mind the following adsorption experiments were carried out to investigate the possibility that selective adsorption of one optical isomer of an amino acid could take place on each of the optically active forms of quartz. This investigation was approached in two distinct ways.

(a) Experiments were carried out in an endeavour to develop optical rotation in a racemic amino acid solution by treating it with each of the optically active forms of quartz.

(b) The extents of adsorption of both D(-)-valine and L(+)- valine on each of the two forms of quartz were determined to observe if either isomer of the amino acid was adsorbed to a greater extent on either

form of quartz.

1.1. Adsorption experiments with optically active quartz and DL-α-alanine.

Adsorbent.

Samples of optically active quartz, both the D- and L- forms, were ground in separate porcelain ball-mills with pebble balls, and the powder from each ball-mill which passed through a 300 mesh B.S. sieve was collected.

The purity of each quartz powder was determined by evaporation of weighed samples with hydrofluoric acid and roasting the residues. On this basis, the D-quartz powder was found to contain 99.79% SiO_2 and the L-quartz powder 99.71% SiO_2.

Standard DL-α-alanine solution.

A molar solution of DL-α-alanine in distilled water was prepared and the pH was adjusted with dilute hydrochloric acid to pH 4, since at this pH maximum adsorption of DL-α-alanine occurs. (See Part 1, section 2.1.). This solution was used in these adsorption experiments.

(The purity of the DL-α-alanine is reported in table A of the Appendix, section 1.)

Adsorption Procedure.

(a) A suspension of 15 gms. of the D-quartz powder and 100 ml. of the standard DL-α-alanine solution was made up and placed in the thermostat at 37°C.

(b) A similar suspension of 15 gms. of the D-quartz powder and 100 ml. of the standard DL-α-alanine solution was prepared and placed in the same thermostat.

Both suspensions were continually agitated by a mechanical stirrer.

After 70 hours in the thermostat, 25 ml. samples of both suspensions were filtered through Ford Sterimat filters and the filtrates tested for optical activity in a polarimeter using a sodium vapour lamp as a light source. The polarimetric readings were observed through a two decimeter tube.

Conclusion.

No difference was observed in the polarimetric readings of the standard DL-α-alanine solutions before and after contact with the D- and L-quartz powders. Consequently, we may conclude that no preferential adsorption of either optical isomer of α-alanine appears to take place on either D- or L-quartz powders.

It is unfortunate that the specific rotation of each optical isomer of α-alanine, and indeed of the α-amino acids in general, is very small; and if, as seems likely, any preferential adsorption is also small, the resulting optical activity may be below the experimentally observable limit. This conclusion is also supported by the fact that although the complex salts used by Kuroya[62] in his adsorption experiments on optically active quartz have a high specific rotation, the observed optical rotation developed in racemic solutions of the salts by preferential adsorption of one optical isomer was so small as to be just out with the experimental error.

Since the specific rotations of D- and L-cystine are fairly high, adsorption experiments on the D- and L-quartz powders were carried out

using a solution of DL-cystine.

1.2. Adsorption experiments with optically active quartz and DL-cystine.

Adsorbent.

The D- and L-quartz powders used in the following experiments have already been described in section 1.1.

Standard DL-cystine solution.

An approximately M/5 solution of DL-cystine in N.HCl was prepared and used in these adsorption experiments. The acid solvent was required because of the low solubility of the DL-cystine in water. (The purity of the amino acid is reported in table A of the Appendix, section 1.)

Adsorption procedure.

(a) A suspension of 5 gms. of the D-quartz powder and 15 ml. of the standard DL-cystine solution was prepared and placed in the thermostat at 25°C.

(b) A similar suspension using 5 gms. of the L-quartz powder was placed in the same thermostat.

Both suspensions were continually agitated; the temperature 25°C was selected to obtain high adsorption values.

After 10 days in the thermostat, the two suspensions were filtered through Ford Sterimat filters and the filtrates tested for optical activity in the polarimeter. The polarimetric readings were observed through a two decimeter tube.

Conclusion.

No difference was observed in the polarimetric readings of the standard DL-cystine solutions before and after contact with the D- and L-quartz powders. Consequently we may again conclude that no preferential

adsorption of either optical isomer of cystine takes place on either D- or L- quartz powders.

To the filtrates from the above suspensions (a) and (b) were added a further 10 gms. of D- and L-quartz powder respectively and the fresh suspensions were returned to the thermostat at 25°C. After a further period of 10 days, no optical activity was developed in either DL-cystine solution.

1.3. Adsorption experiments with optically active quartz and D(-)- and L(+)-valine.

Adsorbent.

The D- and L-quartz powders used in the following experiments have already been discussed in section 1.1.

Standard valine solutions.

An 0.02 M solution of both D(-)-valine and L(+)-valine in distilled water was prepared and used in these adsorption experiments. Both solutions had a pH value of 6.00 units.

The purity of these amino acids is reported in table A of the Appendix, section 1.

Adsorption Procedure.

(a) To each of two 10 gm. samples of the D-quartz powder were added 25 ml. of the standard D(-)-valine and L(+)-valine solutions respectively, and the suspensions were then allowed to revolve slowly in the thermostat at 25°C.

(b) Two similar suspensions, each containing 10 gms. of the L-quartz powder, were also made up and placed in the thermostat.

These experiments were carried out at 25°C since at this temperature the extent of adsorption is greater than at 37°C. After 48 hours in the thermostat, each suspension was centrifuged and the supernatant liquids analysed to determine the extent of adsorption.

Analysis.

The valine solutions before and after adsorption were analysed to determine their nitrogen content according to the semi-micro Kjeldahl procedure described in the general Experimental section preceding Part 1. From the nitrogen determinations the extent of adsorption in each suspension was calculated.

Results.

(a) adsorption of D(-)-valine on D-quartz = 3.59 x 10^{-5} moles.

adsorption of L(+)-valine on D-quartz = 3.59 x 10^{-5} moles.

(b) adsorption of D(-)-valine on L-quartz = 4.26 x 10^{-5} moles.

adsorption of L(+)-valine on L-quartz = 4.26 x 10^{-5} moles.

The different extent of adsorption in (a) and (b) is due to the different surface areas of the D- and L- quartz powders.

Conclusion.

From these results it is evident that both optical isomers of valine are adsorbed to the same extent on both the D- and the L-quartz powders.

Conclusions from Section 1.

The experiments described in this section indicate that neither D- nor L-quartz preferentially adsorb either optical isomer of the amino acids, α-alanine, cystine, and valine. Since both specific rotation and extent of adsorption are low for each of the above amino acids, then any small preferential adsorption may be impossible to detect.

Section 2.

Adsorption of cystine on silica.

During the experiments to determine the rates of adsorption of the series of amino acids discussed in Part 1, the rate of adsorption of cystine on the same silica adsorbent was also studied. The experimental procedure for cystine was exactly as described for the other amino acids in the General Experimental Section 3.1.1; the silica adsorbent was that used for the other amino acids in Parts 1 and 2, the surface area of the silica being that referred to as standard surface area 1. The cystine solution used was an 0.01 M solution in N HCl, since cystine is virtually insoluble in distilled water.

However, irregular results are obtained for cystine; the extent of equilibrium adsorption from the 0.01 M solution (equivalent to 12.2 x 10^{-5} moles of cystine/standard surface area 1.) is considerably higher than was expected in view of the values shown for the other amino acids (Part 1, section 1.2, tables 1 and 2a.), and the extents of adsorption of cystine at increasing time intervals are erratic and cannot be reproduced.

After considering possible explanations for these irregularities, it was finally concluded that the cystine solution had deteriorated and possibly hydrolysis of cystine had taken place, since the cystine solution was prepared some weeks previous to use in these adsorption experiments. To test this conclusion, adsorption experiments were carried out under the same conditions with a 'fresh' 0.01 M solution of cystine in N HCl and the

extent of equilibrium adsorption attained is 1.12×10^{-5} moles of cystine/ standard surface area 1. This result proves that some change has taken place in the 'old' cystine solution and that the compound which is strongly adsorbed is not cystine.

The stability of cystine in hydrochloric acid has been studied by Shinohara and Kilpatrick[63] who concluded that this solution is unstable and hydrolyses to produce a reducing substance which is mainly cysteine. These workers explain the hydrolysis of cystine by the following equations.

(1) R-S-S-R + H_2O = intermediate compounds = R-SH + R-S-OH

(like R-S-OH_2-S-R.)

(2) 2R-S-OH = R-SH + R-SO-OH

(3) R-S-OH + R-SO-OH = R-SH + R - SO_2-OH

It is probable, therefore, that in the adsorption experiments with the 'old' solution, the cystine had hydrolysed according to the above equations. In order to determine more exactly which of the above compounds was so strongly adsorbed, ultra-violet adsorption curves of the following solutions were determined using a Unicam ultra-violet spectrophotometer.

(a) the 'old' cystine solution, (graph 45)

(b) the 'fresh' cystine solution, (graph 45)

(c) the 'old' cystine solution after adsorption (graph 45)

(d) a 'fresh' cysteine solution, (graph 46)

(e) a 'fresh' cysteine/cystine mixture (graph 46)

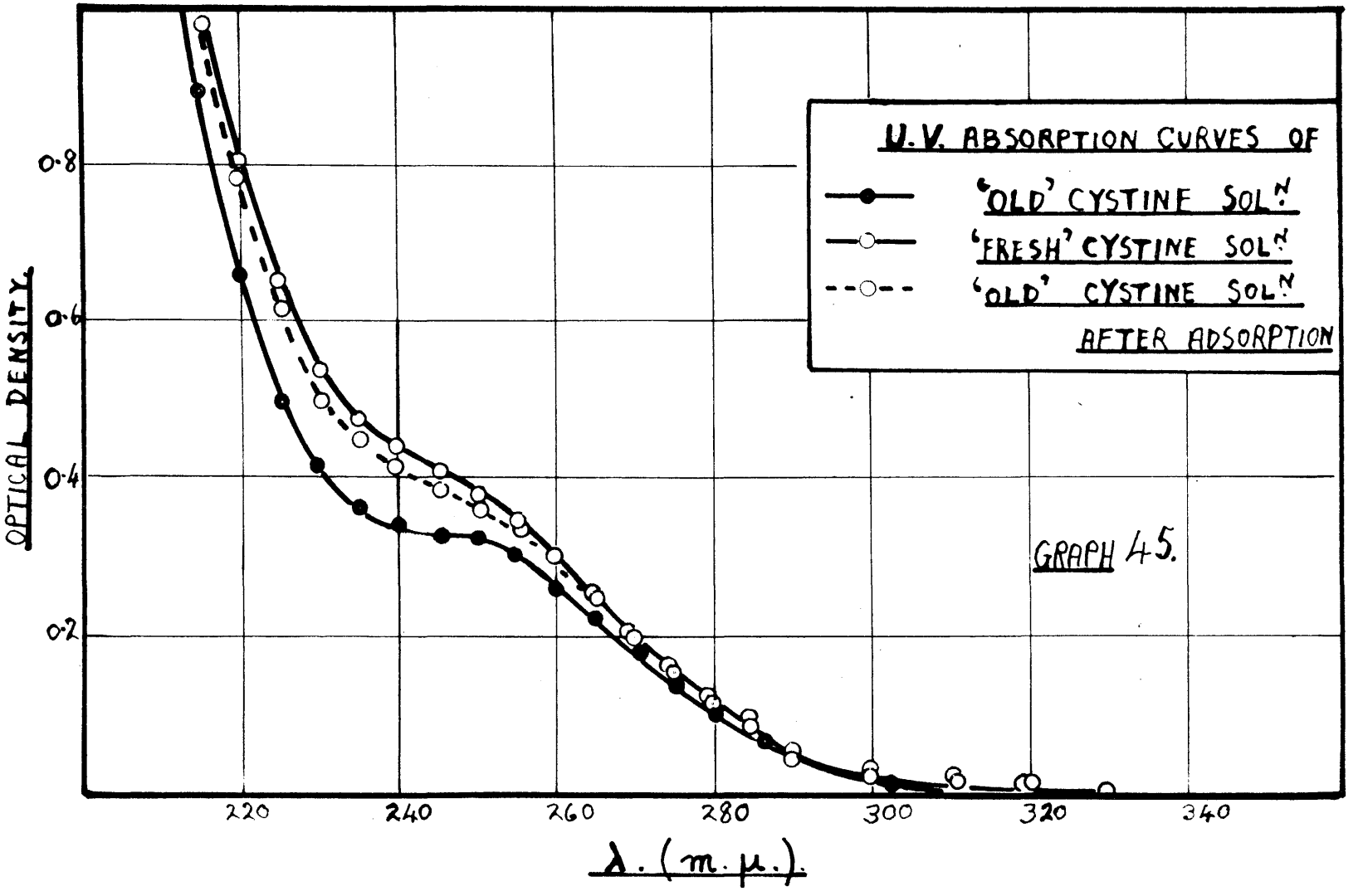
Solutions (a) - (d) were prepared from the respective 0.01 M standard solutions in N HCl by diluting to 0.001 M with N HCl.

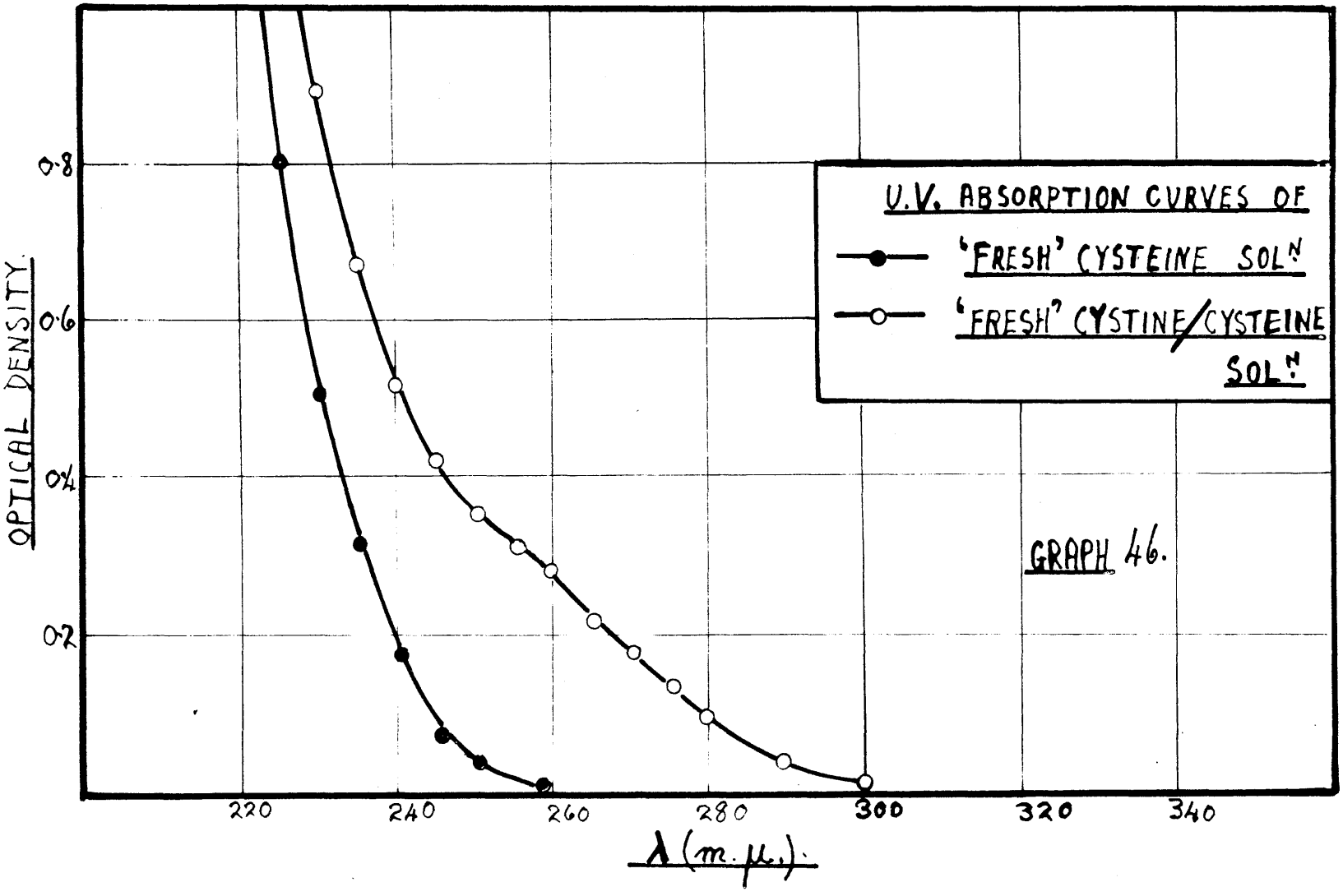
Solution (e) was prepared by diluting tenfold with N HCl a mixture of equal volumes of 0.01 M cystine in N HCl and 0.01 M cysteine in N HCl.

Graph 45 shows that the 'old' cystine solution contains some unknown compound which produces an inflection in the region 236-256 m.μ. This compound is strongly adsorbed on the silica, since the ultra-violet adsorption curve of the 'old' cystine solution after adsorption (graph 45) exhibits only a slight inflection and is virtually identical to the curve for the 'fresh' cystine solution. The adsorption curves for the 'fresh' cystine solution, the 'fresh' cysteine solution and the 'fresh' cystine/cysteine mixture (graph 46) are all smooth curves with no inflections and consequently the unknown compound which is strongly adsorbed on the silica is not cysteine, although adsorption of cysteine on the silica undoubtedly does take place, as has been shown in Part 1, section 1.2.2.

Examining equations (1) - (3) for possible compounds which might be the unknown, the following compounds R-S-OH, R-SC-OH, and R-SO_2-OH must be eliminated since these are all highly electronegative and according to the conclusions derived in Part 1, such compounds are not strongly adsorbed on the silica.

We must conclude, therefore, that the unknown compound, which is strongly adsorbed on the silica, is not shown in equations (1) - (3) and that its nature is probably much less polar or electronegative than those shown in equations (1) - (3) and further that it is probably a small molecule since cystine itself is adsorbed to a very small extent.


U.V. ABSORPTION CURVES OF
'OLD' CYSTINE SOLN
'FRESH' CYSTINE SOLN
'OLD' CYSTINE SOLN
AFTER ADSORPTION
GRAPH 45.
OPTICAL DENSITY.
0·8
0·6
0·4
0·2
220
240
260
280
300
320
340
λ. (m.μ.).


U.V. ABSORPTION CURVES OF
'FRESH' CYSTEINE SOLN
'FRESH' CYSTINE/CYSTEINE SOLN
GRAPH 46.
OPTICAL DENSITY.
0·8
0·6
0·4
0·2
220
240
260
280
300
320
340
λ (m.μ.).

Section 3.

Adsorption experiments with cholesterol on silica.

The presence of the silicic acid esters of cholesterol and analogous substances in silicotic lungs has been reported by Holzapfel[64], who has also discussed the importance of such 'organo-silicates' in silicosis[65]. Holzapfel has postulated that when silicic acid is dissolved from the surface of a quartz particle, free valencies are left on the quartz surface which may be neutralised by organic radicals. This worker also refers to Beger[66, 67], who has reported that albuminous coverings are formed round asbestos needles in silicotic lungs. However, these findings of Beger have been disputed by Koppenhofer[68].

Withthese views in mind, the following experiments were carried out to investigate the possibility of adsorption of cholesterol from aqueous media on silica.

Experimental.

Adsorbent.

In these cholesterol experiments, the silica adsorbent was that used for the amino acids in Part 1; the surface area of the silica being that referred to as standard surface area 1.

Adsorbate.

The cholesterol was obtained from Messrs. British Drug Houses Ltd.

(a) Since cholesterol has a very low solubility in water, an almost saturated solution of cholesterol in alcohol containing 10% of water was prepared and used in these adsorption experiments.

(b) A fine suspension of cholesterol in distilled water was also prepared in the manner described by Bergstrom and Wintersteiner[69]. This suspension was able to withstand, without loss of cholesterol, the centrifuging necessary to separate the silica from the cholesterol suspension. Adsorption experiments were also carried out using this cholesterol suspension.

Adsorption procedure.

The adsorption procedure followed in these cholesterol experiments was exactly as described for the amino acids of Part 1. The silica/cholesterol solution (or suspension) ratio was as for the amino acids and the temperature at which adsorption was studied was also 37°C.

Analysis.

The concentrations of cholesterol in both the cholesterol solution and suspension before and after contact with the silica were determined colorimetrically using the Leibermann-Burchardt reaction[70, 71]. The basis of this reaction is that a green colour is produced when acetic anhydride and concentrated sulphuric acid are added to an anhydrous solution of cholesterol in chloroform. The intensity of the colour was determined in a Spekker photoelectric absorptiometer. Since traces of water in the chloroform solution of cholesterol influence the rate of development and destruction of the colour, the samples of the cholesterol solution and suspension to be analysed were first carefully evaporated to dryness and then re-dissolved in anhydrous chloroform before adding the acetic anhydride and concentrated sulphuric acid.

Conclusion.

It has been found that cholesterol was not adsorbed on the silica from either the aqueous/EtOH solution or the aqueous suspension after 80 hours at 37°C. This result is to be expected since the cholesterol molecule does not contain a strong positively charged centre through which adsorption could take place.

GENERAL SUMMARY.

Summary of conclusions from Part 1 - Amino Acids.

Part 1, Section 1.

In this section the adsorption characteristics of a number of amino acids and related substances have been discussed in detail. In addition to the ammonium and carboxylate groups, the influence of other secondary substituents contained in the molecule on the adsorption behaviour of the amino acid have also been studied. The following general conclusions have been derived.

(1) In aqueous suspension, the silica adsorbent is negatively charged. Adsorption of the amino acids from aqueous solution occurs primarily by mutual attraction of the positively charged ammonium group of these molecules and the negatively charged silica.

(2) The rate and extent of adsorption of the amino acid are determined by the following considerations:-

a) the interaction of the molecules with one another in aqueous solution;

b) the nature and position of secondary substituent groups within the molecule;

c) the influence of these secondary substituents on the interaction between the amino acid molecules in solution;

d) the bulk of the secondary substituent in adsorbed molecules, which, for large substituents, will hinder further adsorption.

e) the distance between the ammonium and carboxylate groups;

f) the extent of substitution in the ammonium group.

Part 1, Section 2.

In this section the effect of pH on the adsorption of a number of amino acids has been studied and the following general conclusions were derived.

(a) The negative carboxylate group, especially when adjacent to the positive ammonium group, exerts an influence tending to retard or prevent adsorption.

(b) The alkyl group modifies this adverse effect of the carboxylate group. As the alkyl group increases in bulk the pH range for maximum adsorption increases, but there is a pronounced decrease in extent.

Part 1, Section 3.

The kinetics of adsorption have been studied in this section and it has been found that the adsorption of these amino acids on silica is not fundamentally a process of diffusion of the molecules in solution to the surface of the adsorbent, or of capillary condensation. The experimental data for each amino acid, irrespective of its molecular structure, have been found to show agreement with the Langmuir theory of the rate of adsorption on a free surface.

Part 1, Section 4.

In this section the energy of activation, heat of adsorption, and the frequency factor for the adsorption of each of several amino acids have been calculated from experimental data. From the values of the energy of activation etc., the nature of the adsorption bonds between the amino

acid molecule and the silica has been derived and may be summarised as follows.

(1) The primary mechanism of adsorption is the mutual attraction of the positively charged ammonium or imino group of the amino acid molecule and the negatively charged oxygen atom of the silica.

(2) In the adsorbed state, the ammonium group may also form hydrogen bonds with the electronegative oxygen atoms of the silica.

(3) The α-amino acids, in contrast to the β-, γ-, and higher acids, form only one hydrogen bond in the adsorbed state due to a steric "blocking" effect of the carboxylate group.

Summary of Conclusions from Part 2 - Peptides.

Part 2, Section 1.

In this section the adsorption characteristics of four dipeptides of related structure have been studied. The dipeptides are adsorbed on the silica through their positively charged ammonium group, and not through the peptide link, -CO.NH-. The rate and extent of adsorption of each dipeptide are determined by the same considerations as were described for the amino acids. However, in addition to the nature of a secondary substituent, its position in the dipeptide molecule with respect to the ammonium and carboxylate groups is of fundamental importance in determining the rate and extent of adsorption.

Part 2, Section 2.

The effect of pH on the adsorption of the dipeptides has been studied and conclusions have been derived which are similar to those described for the amino acids.

Part 2, Section 3.

The kinetics of adsorption of the dipeptides have been studied and the conclusions derived are as described for the amino acids.

Part 2, Section 4.

The energy of activation, heat of adsorption, and the frequency factor for the adsorption of each dipeptide were calculated from experimental data. The nature of the adsorption bonds between the dipeptide molecule and the silica has been derived and may be summarised as follows.

(1) The primary mechanism of adsorption is the mutual attraction between the positively charged ammonium group of the dipeptide molecule and the negatively charged silica.

(2) The hydrogen atoms of the ammonium group may also form hydrogen bonds with the electronegative oxygen atoms of the silica.

(3) If the dipeptide molecule contains a side-chain of large size adjacent to the ammonium group, the bulk of this side-chain produces a steric "blocking" effect and permits the formation of only one hydrogen bond in the adsorbed state.

Summary of Conclusions from Part 3.

In Part 3, isotherms of two amino acids on very pure quartz were discussed and were found to be fundamentally identical to the isotherms of the same amino acids obtained on the impure silica adsorbent used in Parts 1 and 2. Consequently, it has been concluded that the impurities in the silica adsorbent used in Parts 1 and 2, did not influence the mechanism of adsorption or the strength and nature of the adsorption bonds formed between

the amino acid molecules and the silica.

Summary of Conclusions from Part 4.

In Part 4 miscellaneous adsorption experiments were described. The possibility that preferential adsorption of one optical isomer of the amino acid could take place on one of the optically active forms of quartz was examined and it was concluded that such preferential adsorption either did not take place or was so small that it could not be detected experimentally.

It was found that cholesterol was not adsorbed on silica from either aqueous/alcohol solutions or from aqueous suspensions.

The adsorption on silica of the hydrolysis products of cystine in hydrochloric acid were also discussed.

APPENDIX.

Section 1.

Source of purity of the amino acids and related substances.

The amino acids and related substances were purchased from the following commercial firms:-

Messrs. British Drug Houses Ltd; Genatosan Ltd.; Light & Co.; Eastmann Kodak Ltd.; Hoffman - La Roche; Roche Products Ltd.:

The amino acid, its source, and calculated and observed % nitrogen are shown in Table A. The observed % nitrogen values were determined by the semi-micro Kjeldahl procedure described in the general Experimental Methods paragraph 4.

Table A.

Amino Acid	Source	% N (obs.)	% N (calc.)
α-Alanine	B.D.H.	15.67	15.73
β-Alanine	"	15.66	15.73
α-Aminobutyric Acid	"	13.58	13.59
Arginine (Monohydrochloride)	"	32.15	32.18
Asparagine	"	21.18	21.20
Betaine Hydrochloride	"	9.05	9.12
Citrulline	"	24.00	24.00
Cysteine	"	11.52	11.57
Cystine	"	11.60	11.66
Glutamic Acid	"	9.52	9.52
Glycine	"	18.58	18.66

Table A (cont'd).

Amino Acid	Source	% N (obs.)	% N (calc.)
Glycocyamine	B.D.H.	35.85	35.88
Glycylglycine	"	21.18	21.20
Guanidine Carbonate	"	46.64	46.66
Histidine	"	27.09	27.10
Hydantoic Acid	"	23.70	23.70
Leucine	"	10.65	10.69
Norleucine	"	10.62	10.69
Norvaline	"	11.90	11.96
β-Phenyl Alanine	"	8.44	8.48
Semicarbazide Hydrochloride	"	37.64	37.67
Serine	"	13.26	13.33
Taurine	"	11.17	11.20
Thiourea	"	36.82	36.85
Tryptophane	"	6.79	6.86
Urea	"	46.64	46.66
L(+)-Valine	"	11.89	11.96
D(-)-Valine	"	11.89	11.96
ε-Aminocaproic Acid	Eastman Kodak	10.63	10.69
δ-Aminovaleric Acid	"	9.10	9.12
Glycylleucine	Hoffmann-La Roche	14.88	14.89
Leucylglycine	"	14.88	14.89
Alanylglycine	Roche Products Ltd.	19.15	19.18

Table A (Cont'd)

Amino Acid	Source	% N (obs.)	% N (calc.)
Hydroxyproline	Light & Co.	10.65	10.69
Lysine Mono-Hydrochloride	"	19.16	19.18
Proline	"	12.10	12.17
Valine	"	11.90	11.96
Glutamine	Genatosan Ltd.	19.15	19.18
Sarcosine	"	15.69	15.73
γ-Aminobutyric Acid	Appendix	13.55	13.59
N-Acetylglycine	"	11.91	11.96

Section 2.

Preparation of N-Acetylglycine.

The method used to prepare N-acetylglycine is that described in 'Organic Syntheses' vol. 19, p.4.

The method may be briefly described as follows.

In a 100 ml. flask were placed a solution of 10 gms. of pure glycine in 30 ml. of distilled water. To this solution 25 gms. of 95% acetic anhydride were added in one portion and the mixture vigorously stirred for fifteen to twenty minutes. The solution which had become hot was then left overnight in a refrigerator, to allow the N-acetylglycine to crystallise out. The product was then collected on a Buchner funnel, washed with ice-cold water, and dried at 100 - 110°C.

Yield = 8 gms. = 66% approx. of the theoretical amount.

Melting point (reported) = 207-208°C.

Melting point (observed) = 207°C.

For % nitrogen see Appendix section 1, table A.

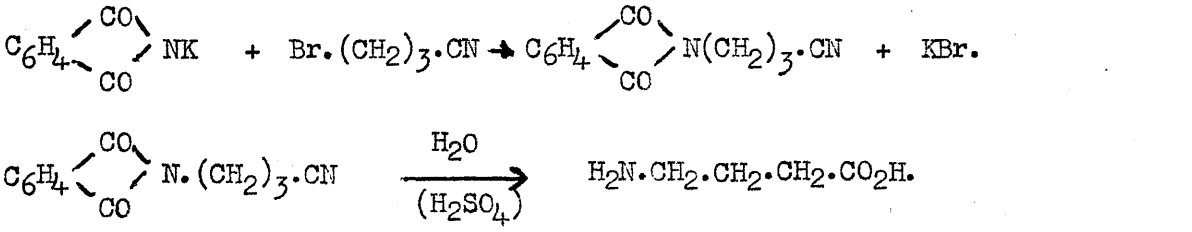
Section 3.

Preparation of γ-aminobutyric Acid.

The method used to prepare γ-aminobutyric acid is that described in 'Organic Syntheses', vol. 17, p.4.

The potassium phthalimide was purchased from Messrs. British Drug Houses, Ltd., and the γ-bromobutyronitrile from Messrs. Eastman Kodak Ltd.

The method may be briefly described as follows.



In a 500 ml. round-bottomed flask fitted with an air condenser were placed 34 gms. of finely powdered potassium phthalimide and 25 gms. of γ-bromobutyronitrile. The flask was heated in an oil bath maintained at 150 - 180°C. for 1.5 hours and then allowed to cool. The excess potassium phthalimide and the potassium bromide formed were removed by extraction with several portions of boiling distilled water until the wash water gave no test for the bromide ion. The flask was then cooled and the product caused to solidify and the remaining water decanted as completely as possible. The solid was treated with 65 ml. of concentrated sulphuric acid, and the mixture warmed gently in an oil bath under a reflux condenser until all the γ-phthalimidobutyronitrile was brought into solution. Through the reflux condenser 100 ml. of distilled water was

carefully added and the solution refluxed vigorously for three hours. The mixture was cooled, allowed to stand overnight and the phthalic acid filtered off. The filtrate was transferred to a large evaporating dish, 700 ml. of distilled water was added, and then an excess of barium carbonate in small portions. The mixture was evaporated nearly to dryness on the steam bath and the residue stirred thoroughly with 700 ml. of distilled water and again evaporated. These treatments removed completely all the ammonia evolved. Finally, 700 ml. of distilled water was stirred with the solid and the mixture filtered on a large buchner funnel. The precipitate was washed with three portions of 200 ml. of hot distilled water and the filtrate and washings concentrated to a volume of 100 ml. on the steam bath. After adding 2 gms. of activated charcoal, the solution was filtered through a No. 42 Whatman paper, and the charcoal washed with several small portions of hot distilled water. The filtrate was concentrated on the steam bath to the point of crystallisation and 300 ml. of absolute alcohol added to precipitate the amino acid. The mixture was stirred well so that the yellow impurities were retained in the solvent and, after cooling, the colourless, crystalline product was collected and washed with alcohol.

The alcoholic filtrate was treated with barium hydroxide to convert the pyrrolidone contained in the filtrate to the barium salt of the amino acid. The excess barium hydroxide was precipitated with carbon dioxide and the barium carbonate removed by filtration. A slight excess of sulphuric acid was added to liberate the amino acid from its barium salt and an excess of barium carbonate added to remove the sulphate ions.

The mixture was digested on the steam bath until effervescence ceased, filtered, and the precipitate washed with hot water. The filtrate and washings were decolourized with activated charcoal and concentrated on the steam bath to the point of crystallisation. The amino acid was precipitated with absolute alcohol, filtered and washed with absolute alcohol. The amino acid was recrystallised by dissolving in distilled water and reprecipitated with absolute alcohol.

Yield = 6 gms. = 40% approx. on the basis of γ-bromobutyronitrile used.

Melting point (observed and reported) = 193°C (decomp.)

For % N see Appendix section 1, table A.

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