THE ADSORPTION OF AMINO-ACIDS AND PEPTIDES BY MONTMORILLONITE AND ILLITE

A thesis submitted

by

Ralph Henry Laby, M.Sc.

to the University of Adelaide

for the degree of

DOCTOR OF PHILOSOPHY

Department of Agricultural Chemistry

University of Adelaide

December, 1962.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis

Table of Contents

List of Figures

No. List of Tables Page No.

Summary

The adsorption of glycine and its di-, tri-, and tetra-peptides and also a number of naturally occurring amino-acids by montmorillonite and illite has been studied in considerable detail. Adsorption isotherms have been determined and their interpretation supported by X-ray diffraction investigations of the complexes formed.

The adsorption of neutral amino-acids and peptides by calcium montmorillonite or illite was found to give rise to linear isotherms. These could be interpreted by applying the mathematics of constant partition to the distribution of solute between the bulk phase and adsorbed phase water.

$$K = \frac{(\text{solute})_{\text{adsorbed}}}{(\text{solute})_{\text{bulk}}}$$

The volume of the adsorbed phase was taken as the Stern layer volume, in which the exchangeable calcium ions were assumed to occur. The change in free energy due to physical adsorption was calculated for each adsorbate. On the basis of these results a general theory for physical adsorption from aqueous solutions by clays has been proposed. The theory should apply to all adsorption of organic compounds where specific sites are not involved. When adsorbed molecules are not bonded specifically to the clay surface, it is possible to interpret physical adsorption as the formation of a solution of the organic compound in the water containing the

exchangeable cations which surrounds the clay particles.

For the amino-acids and simple peptides studied it has been shown that the following mechanisms contribute to the physical adsorption:

(1) Ion-dipole and surface dipole interactions, dependent upon the sign and magnitude of the dielectric increment of the adsorbate, and the density of charge of the substrate. The electrolyte concentration of the bulk phase should influence the extent of adsorption resulting from these interactions. Adsorption by illite is stronger than by montmorillonite due to the higher surface density of charge of illite.

(2) van der Waals interactions, dependent upon the molecular weight and the shape of the adsorbed molecules. Flat aromatic and straight chain aliphatic molecules give rise to efficient van der Waals contact.

(3) The salting-out effect of hydrocarbon side-chains. This effect can be significant for compounds of positive dielectric increment, when it opposes interactions (1) and (2).

Adsorption by sodium clays has been interpreted as a non-constant partition of adsorbate between the bulk and adsorbed phase solutions.

Proton transfer with hydrogen montmorillonite:

$$\text{clay } H_3O + NH_3^+ . \underset{\underset{R}{|}}{CH.COO^-} \rightleftharpoons \text{clay } NH_3^+ . \underset{\underset{R}{|}}{CH.COOH} + H_2O$$

and cation exchange involving the basic amino-acids

$$\text{clay Na} + NH_3^+.\,\underset{\displaystyle (CH_2)_4.NH_3^+}{\overset{\displaystyle CH.COO^-}{|}} \rightleftharpoons \text{clay } NH_3^+.\,\underset{\displaystyle (CH_2)_4.NH_3}{\overset{\displaystyle CH.COO^-}{|}} + Na^+$$

give rise to extensive adsorption, with isotherms of Langmuir type. Physical adsorption forces act in addition to the electrostatic bonding but are weaker.

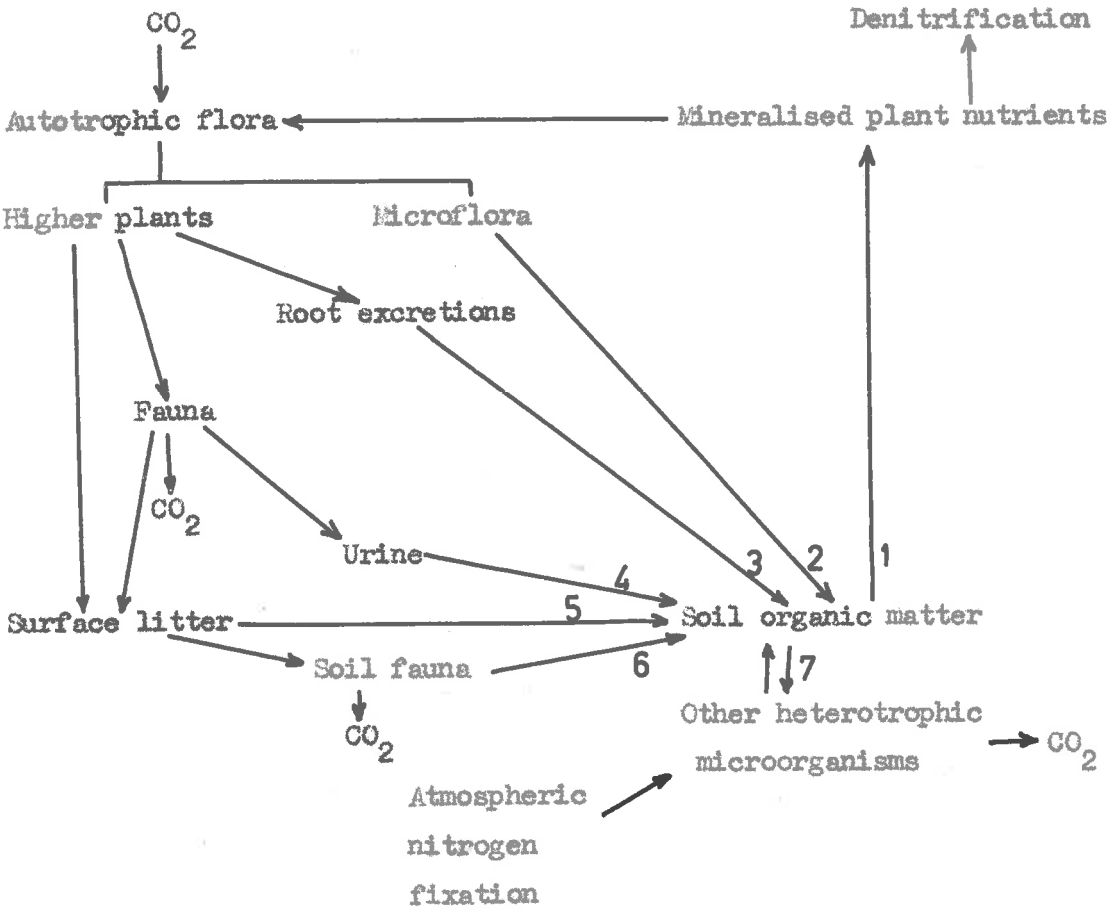
The X-ray investigations have shown that the intercalation of a solute of positive dielectric increment by moist calcium montmorillonite gives rise to an increase in the equilibrium basal spacing, from 19 Å to 21 or 22 Å. The increase is due to the increased dielectric constant of the interlamellar solution. The apparent contration in the van der Waals thickness of molecules adsorbed by montmorillonite can be adequately accounted for by keying into the hexagonal cavities of the internal surfaces of montmorillonite.

Attempts to examine complexes formed between amino-acids or peptides and montmorillonite by infra-red spectroscopy gave no useful results because the spectrum of the adsorbent largely masked those of the adsorbates. However, it was shown from the infra-red spectra of complexes formed between montmorillonite and several organic liquids that C-H • • • O interactions between methylene groups adjacent to electron withdrawing atoms in an organic compound and surface oxygens of montmorillonite do not occur. There is therefore no reason to believe that this previously postulated mechanism is involved in the adsorption of organic compounds by clays.

INTRODUCTION

The present work is a physico-chemical investigation of adsorption which, as a project in soil science, is primarily directed toward a fuller understanding of nitrogen transformations in the soil. Since these transformations are largely inseparable from those involving soil organic matter, the present work may have further implications in this latter and broader aspect of soil chemistry.

The organic matter of a soil is in equilibrium with the flora and fauna that the soil supports :-



The transformations 1 to 7 above, which largely determine the equilibrium level of soil organic matter, are almost exclusively performed by the microflora of the soil and will consequently involve water-soluble intermediates. Soil is a porous medium and the behaviour of these intermediates will be influenced by their interactions with the mineral surfaces of the pores. That is, the mobility of a water-soluble organic intermediate will depend on whether it is strongly adsorbed to give a mobile or a localised film of adsorbed molecules, weakly adsorbed or repelled from the mineral surfaces in the soil. The availability of an adsorbed organic compound for microbial or enzyme catalysed decomposition (Ensminger and Gieseking, 1939) will depend to some extent on its surface mobility and perhaps on its chemical properties in the adsorbed phase. However, the microbiological investigation of adsorbed intermediates was considered outside the scope of the present work.

The extent of interaction of an organic compound with mineral surfaces and the properties of the adsorbed molecules depend upon the bonding mechanisms involved in the adsorption complex and the adsorption affinity of the compound. The bonding mechanisms and adsorption affinity will be determined by the molecular properties of the organic compound. An evaluation of these surface interactions, which leads to an understanding of their significance in soil nitrogen and organic matter transformations, may be obtained from a physico-chemical investigation of adsorption from aqueous solution that has the following aims:-

(1) To elucidate the bonding mechanisms involved.

(2) To determine affinities or free energies of adsorption.

(3) To study the dependence of (1) and (2) upon the molecular properties of the adsorbates.

These are the three basic aims of the present investigation, the adsorption of amino-acids and peptides by montmorillonite and illite. Amino acids were chosen as adsorbates for the following reasons.

(i) Amino-acids are important nitrogen containing intermediates in soil organic matter transformations. Up to 40% of the nitrogen of soil organic matter occurs as amino-acids combined with other organic substances (Bremner, 1955) but in a chemical environment different to that of plant or animal protein. Therefore, proteolysis of the soil organic matter precursors must precede organic matter formation. The concentration of free amino-acids in the soil solution is extremely small (Bremner, 1950, 1952) since they are rapidly utilized by the soil bacteria. The relevent published work on the significance of amino-acids in the soil solution and their availability for microbial utilization is reviewed in Part I.

(ii) There is a growing awareness of the importance of the plant root-soil interface in the nutrition of the plant (Ohlrogge, 1962). The adsorption of amino-acids excreted by plant roots may be important in determining the extent to which the roots can modify their environment, particularly in relation to the microbial population existing in the rhizosphere.

(iii) The structures of the amino-acids and peptides and the physical properties of their aqueous solutions are known in considerable detail (Cohn and Edsall, 1943). It was envisaged, therefore, that in addition to the direct application of the present work to the understanding

of amino-acid transformations in the soil, the study of these compounds would probably fulfill the third basic aim of the present work, the establishment of relationships between adsorption affinities and molecular parameters. This investigation would also provide a basis for further adsorption studies using more complex but less thoroughly characterised organic adsorbates which were related to other intermediates of soil organic matter transformations.

Montmorillonite and illite were chosen as adsorbents because they occur frequently in soils and have surfaces available for adsorption that are predominantly internal and predominantly external respectively. The structures of these two minerals are discussed in detail in Part I and the structures of other clay minerals referred to in the text are given by Grim (1953).

The physico-chemical interpretation of the results of the present work (Part II) required an understanding of the adsorption mechanisms involved in clay-water systems and the properties of adsorbed water. The relevent literature is reviewed in Part I.

The manner in which clay mineral surfaces can influence microbial or enzyme catalysed decomposition of adsorbed organic compounds is illustrated in Part I by a brief review of the relevent literature on protein adsorption.

As part of the investigation of the bonding forces involved in the adsorption complex, C-H. . . O hydrogen bonding between methylene groups adjacent to electronegative atoms of an adsorbed organic compound and the surface oxygen atoms of montmorillonite is critically evaluated. This mechanism, initially proposed by Bradley (1945), is considered in

terms of the general requirements for hydrogen bonding in Part I. An interaction of the C-H ...O hydrogen bonding type which is too weak to describe as true bond formation, but which may contribute to the adsorption affinity of the compound is discussed in Part III.

In conclusion, the results are discussed in terms of their contribution to the understanding of soil organic matter transformations, and further experiments are suggested.

PART I LITERATURE REVIEW

1. The Structure of Montmorillonite and Illite

1. General

The elucidation of the main structural features of the micaceous minerals by Pauling (1930) and by Jackson and West (1930, 1933) began a new era in clay mineralogy when the behaviour and properties of the minerals could be discussed in terms of an alumino-silicate layer lattice structure.

Hofmann, Endell and Wilm (1933) recognised that montmorillonite occurred in an essentially pure, homogeneous condition amongst the bentonite deposits of the western United States. They showed that the X-ray diffraction results accorded with a structure based on that of pyrophyllite and hence resembling the micas, but characterised by a variation of the basal spacing with water content.

The structure of pyrophyllite is given in Fig. 1(a). The layer lattice can be considered as the fusion of an alumina layer (in which each Al^{3+} is octahedrally coordinated with four O^{2-} and two OH^{-}) between two silica layers (in which each Si^{4+} is tetrahedrally coordinated with four O^{2-}) giving an alumino-silicate layer four oxygen sheets thick. The two central sheets are composed of oxygen and hydroxyl ions in the ratio 2:1 and are close-packed arrays. The two outer sheets contain only oxygen ions in an open hexagonal array shown in Fig. 1(b).

The complete layer is electrically neutral when 2 out of every 3 octahedral spaces between the central oxygen sheets contains

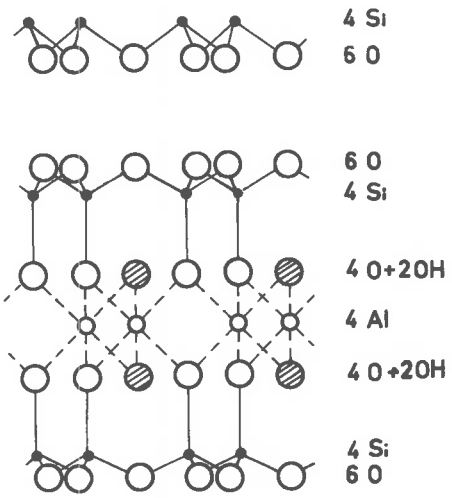
Fig. 1 (a). The structure of pyrophyllite.

Fig. 1 (b). The open hexagonal array of the outer sheets of oxygen ions of pyrophyllite.

Fig. 1 (c). The open hexagonal array of the outer sheets of oxygen ions of montmorillonite, incorporating the 7° rotation of the silica tetrahedra as proposed by Radoslovich.

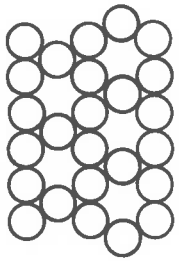
fig 1

(a)



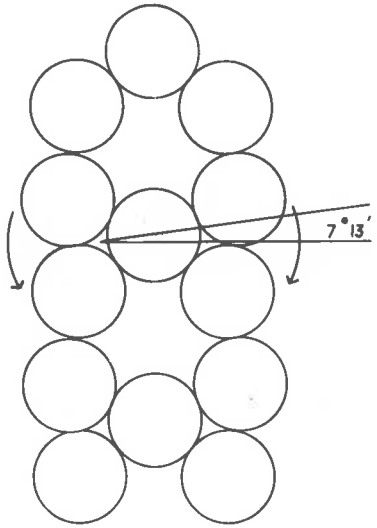
PYROPHYLLITE

(b)



OPEN PACKED
HEXAGONAL SURFACE

(c)



HEXAGONAL SURFACE
INCORPORATING
ROTATION OF $Si O_4$ TETRAHEDRA

an Al^{3+} (that is, pyrophyllite is a dioctahedral mineral) and every tetrahedral space in the silica layers contains a Si^{4+}.

ii. The structure of montmorillonite

The name montmorillonite was first used to denote a clay-like mineral found near Mont Morillon in France (Damour and Salvetat, 1847). According to the classification proposed by Brown (1955) montmorillonite is a dioctahedral member of the smectite group of minerals of the triphormic family. Other dioctahedral members of the smectite group are beidellite and nontronite.

Montmorillonite differs from pyrophyllite in that it exhibits intra-crystalline swelling by intercalation of water and other polar liquids, and has a cation exchange capacity of approximately 100 m equiv./ 100 g. The origin of this exchange capacity was attributed by Hofmann et al. (1933) to broken bonds at the edge of the clay crystals. They gave little attention to the possibility of isomorphous replacements occurring within the lattice, and chief credit for this idea must be given to Marshall (1935). About the same time, Holzmer (1935) and Gruner (1935) also suggested that isomorphous replacements could be of importance in smectites. The partial replacement of Si^{4+} by Al^{3+} in the tetrahedral layer and of Al^{3+} by Mg^{2+} or another bivalent cation of suitable size gives rise to a negative charge in the alumino-silicate layer which is balanced by the exchangeable cations. The modification of the pyrophyllite structure to give that of montmorillonite according to the isomorphous replacement theory is:

n H_2O	$(2x + y)M^+$	$(2x + y)$ +
6. O^{2-}		12 -
x Al^{3+} + $(4-x)Si^{4+}$		$(16-x)$ +
4. O^{2-} + 2. OH^-		10 -
$(4-y)Al^{3+}$ + y Mg^{2+}		$(12-y)$ +
4. O^{2-} + 2. OH^-		10 -
x Al^{3+} + $(4-x)Si^{4+}$		$(16-x)$ +
6. O^{2-}		12 -
		0

M^+ = exchangeable cation.

$(2x + y) \cong 0.66$

The success of the isomorphous replacement theory in explaining the widely divergent chemical compositions of the smectite group has led to its widespread acceptance.

However, in 1940, Edelman and Favejee postulated a modified montmorillonite structure in which they proposed that alternate silicon ions were placed above instead of below the open packed oxygen sheets. Hydroxyl ions were added above these silicons to complete their coordination. The negative charge on the clay lattice was thought to arise from the dissociation of the hydrogen ions from the projecting hydroxyl groups. These workers attempted to justify this model in terms of the difference between the adsorption properties of montmorillonite and halloysite and the other silicate minerals having surfaces composed of $Si_2O_5^{2-}$ units but with no considerable adsorptive properties. They later modified the model such that only sufficient

hydroxyl groups projected to explain the cation exchange capacity by complete dissociation. It is virtually impossible either to prove or disprove this theory by X-ray diffraction.

The model would not have received the attention that it did if it were not apparently supported by the work of Berger (1941), Deuel and Huber (1951), Slabaugh (1952) and Spencer and Gieseking (1952) who claimed to have prepared organic derivatives of montmorillonite by such reactions as :-

$$CH_3.COCl + Si.OH \rightarrow SiO.CO.CH_3 + HCl$$

The careful quantitative work of Greenland and Russell (1955) showed that although montmorillonite can take up chloride from thionyl chloride treatment and can retain acetyl tenaceously after acetyl chloride treatment, there is no positive evidence for the formation of covalent bonds between the clay mineral and the chloride and acetyl groups and further, it is highly probable that in previous reports of the preparation of organic derivatives of montmorillonite, physically adsorbed organic molecules have been considered covalently bound.

Further details of the montmorillonite structure of relevence to the present work are:-

(1) The negative charge of the alumino-silicate layer is largely due to the replacement of Al^{3+} by Mg^{2+} in the octahedral sheet. In addition there is a small replacement of Si^{4+} by Al^{3+} in the tetrahedral sheets and of Al^{3+} by Fe^{3+} in the octahedral sheet (Greene-Kelly, 1957).

(2) Radoslovich (1960) determined in detail the structure of muscovite and showed that there is a distortion of the open packed

hexagonal structure of the surface oxygen sheets similar to that described by Mathieson and Walker (1954). The distortion, illustrated in Fig. 1 (c), is related to a misfit between the tetrahedral and octahedral layers and is described as a rotation of the tetrahedral silica units about axes passing through each silicon ion and perpendicular to the a, b plane, together with a tilting of the tetrahedra in this plane. The rotation is approximately $\pm$ 5.5° for vermiculite (Mathieson and Walker, 1954), $\pm$ 13° for muscovite (Radoslovich, 1960) and $\pm$ 7° for montmorillonite (Radoslovich, private communication).

(3) In the study of complexes formed between montmorillonite and organic compounds by X-ray diffraction the results are frequently interpreted in terms of the thickness of the adsorbed layer of organic molecules, obtained by subtracting the alumino-silicate layer thickness from the observed basal spacing. The alumino-silicate layer thickness has been determined by adding twice the van der Waals radius for the oxygen ion (1.4 Å, Pauling, 1960) to the centre-to-centre thickness of the alumino-silicate layer, measured from one external sheet of oxygen ions to the other:

$$2 \times 1.4 + 6.6 = 9.4 \text{ Å}$$ (MacEwan, 1948; Greene-Kelly, 1955).

The O-O distance used in this calculation is that obtained from the data of Jackson and West (1930) for muscovite since it cannot be obtained with accuracy for the more poorly crystalline montmorillonite.

The recent work of Radoslovich (1960) indicates that the determination of the alumino-silicate layer thickness by this procedure may be in error. The rotation and tilting of the silica tetrahedra places one oxygen in every three approximately 0.15 Å closer to the

sheet of silicon ions. Also the mean O-O distance between adjacent oxygen ions in the open packed hexagonal sheets is 2.67 Å, suggesting an ionic radius of 1.34 Å. This is in close agreement with the ionic radius of oxygen chosen by Goldschmidt from the measurements of Wasastjerna (1.32 Å, see Pauling, 1960). From these considerations the above calculation of the alumino-silicate layer thickness becomes:

$$2 \times 1.3_5 + 6.5 \pm 0.1 = 9.2 \pm 0.1 \text{ Å}$$

The minimum basal spacing observed for collapsed montmorillonite is 9.5 Å (Greene-Kelly, 1955) and either this value or the calculated thickness of 9.4 Å has been used by those authors who discuss the thickness of the adsorbed layer of organic molecules (MacEwan's "Δ-value", 1948).

However, the minimum basal spacing of 9.02 Å observed by Walker (1956) for magnesium vermiculite heated between 250°C and 600°C is difficult to interpret in terms of an alumino-silicate layer thickness of 9.4 to 9.5 Å. In a private communication, Walker states that the low basal spacing is not completely realised if the vermiculite is finely powdered and suggests that the magnesium ion almost certainly migrates into the crystal lattice which may be modified in some way as a consequence. The Fourier analyses of Heller and Kalman (1961) lend support to this suggestion. The model of magnesium vermiculite made by Walker and which incorporates the distortion of the open packed sheets of oxygen ions, allows an interlocking or keying of the oxygen surfaces of adjacent alumino-silicate layers of approximately 0.2 Å. The contraction is not observed in magnesium saponite and the minimum basal spacing in calcium and sodium vermiculite is 9.31 and 9.55 Å

respectively.

These observations suggest that the difference (d(001)-9.5)Å is an under-estimate of the true interlamellar distence by approximately 0.3 Å and also that the basal spacing of collapsed montmorillonite is probably not a true measure of the alumino-silicate layer thickness.

Also both the observed tilting of the silica tetrahedra and the small uncertainty involved in considering the structure of montmorillonite in terms of X-ray data of muscovite or vermiculite prevent the accurate determination of the thickness of the interlamellar space. The significance of these observations will be made apparent later in the discussion.

iii. The structure of illite

The term illite is applied to a mica-like clay mineral with a non-expanding lattice. In the classification proposed by Brown (1955) it is structurally related to muscovite, a dioctahedral member of the mica group of 2:1 minerals of the triphormic family. An idealised general formula for the illite is:

$$(Si_{4-x}Al_x)Al_2(OH)_2O_{10}$$
$$\downarrow$$
$$(K,H_3O,\underline{Ca}_2,Na)_x$$

Apart from the differences in chemical composition the principal difference between illite and muscovite is the degree of crystallinity which reflects the greater compositional range permitted in the illite lattice. X-ray diffraction data for illites show a few broad, weak diffuse reflections in addition to the strong, broad basal reflection at 10Å.

This is in contrast to the numerous sharp strong peaks of muscovite. Correspondingly, electron micrographs reveal the platy, irregular aggregates of illite against the large clean-cut plates of muscovite (Molloy and Kerr, 1961). Illites are frequently interstratified with layers of an expanding lattice mineral, the amount of which varies with sample locality. The hydration of the expanding layers causes the 10Å peak to become asymmetrical or, for a relative large degree of interstratification, to shift to larger spacings (Grim and Bradley, 1937). The best indication of the extent of interstratification is obtained from a comparison of the external surface area, determined from the adsorption of non-polar gases, with the total surface area, measured by the adsorption of polar molecules or cations (Greenland and Quirk, in press). Polar compounds can be intercalated by the expanding lattice lamellae. The isomorphous substitution of illite is principally in the tetrahedral layer and is about double that of montmorillonite per unit cell. It is approximately one Al^{3+} in every six tetrahedral positions ($x \simeq 0.67$ in the above formula) compared with one Al^{3+} in every four tetrahedral positions of muscovite (Grim, 1953).

2. The Nature of Clay Adsorbed Water

During the present work, amino-acids and peptides were adsorbed from aqueous solution by clays, the final product being therefore an equilibrium mixture of clay, adsorbed amino-acid or peptide and adsorbed water. Consequently, it is of some importance to review the literature on the nature and properties of water in the adsorbed phase. In addition, many of the important developments in the physical chemistry of adsorption have resulted from the detailed

study of the adsorption of water. Recent review articles by Martin (1960) and Low (1961) cover the studies in this field.

1. The mechanism of clay-water interaction

The exchangeable cations play a major role in determining the manner in which water interacts with clays, both at high and low water contents.

As water is adsorbed by clay, the exchangeable cations become hydrated, especially if they are small and multiply charged. Consequently the water molecules may be oriented around the exchangeable cations in a definite manner, similar to that postulated by Mathieson and Walker (1954) and Walker (1955) for magnesium vermiculite.

At higher water contents when the exchangeable cations that are dissociated from the surface can be regarded as being in solution the activity of the water in the vicinity of the clay surface is lowered in the same manner as in ordinary aqueous solutions of salts. Consequently, water should move into the surface region or, expressed differently, clays may be expected to attract water by osmosis.

These two processes, hydration and osmosis, have been separately observed in swelling studies with expanding lattice minerals. Hendricks, Nelson and Alexander (1940) and others have shown that entry of water molecules into and removal from interlayer positions takes place in steps of about 3Å separation. As many as four layers of water molecules may enter the lattice in this stepwise fashion depending on the exchangeable cation.

Norrish and Quirk (1954) and Norrish (1954) have drawn attention to the second type of swelling in smectites at higher water contents.

This latter type of swelling is extensive and is due to the development of diffuse double layers at the alumino-silicate surfaces. The transition from stepwise to more extensive swelling occurs with small monovalent cations which have hydration energies sufficient to overcome the potential barrier due to electrostatic attractive forces acting between the alumino-silicate layers and the exchangeable cations. The hydration energies of the larger monovalent cations are insufficient to affect the transition so that their swelling is limited to two or three thicknesses of water molecules. With polyvalent cations the higher hydration energies are offset by the following factors which restrict the osmotic swelling of the clays.

(1) The osmotic pressure is lower than in a clay-water system containing monovalent exchangeable cations, since it is determined by the number of cations dissociated from the clay surface, and the ionic strength and activity of the resultant solution.

(2) The electrostatic attractive forces between the alumino-silicate layers and the exchangeable cations is higher and opposes the osmotic pressure. The attractive force increases with decreasing average dielectric constant of the interlamellar solution and since the polyvalent cations restrict the orientation polarization of a greater number of water molecules in their vicinity than do monovalent ions, the dielectric constant is very much reduced.

(1) and (2) are to some extent related, since the dissociation of the clay is restricted by the attractive force between the alumino-silicate layers and the exchangeable cations.

The swelling of smectites saturated with polyvalent cations is

limited to two or three molecular steps.

The possibility that hydrogen bonding between the water molecules and the surface oxygen ions contributes to clay water interactions has been investigated for many years. These hydrogen bonds appear to be quite feasible. For instance, Hendricks and Jefferson (1938), Macey (1942) and Forslind (1952) maintain that the hexagonal pattern of oxygen ions of the surface sheets of clay minerals can coincide at points with a similar pattern in a hydrogen bonded water structure (Bernal and Fowler, 1933). According to Low (1961) the perturbing effect of the oxygen ions of the surface (or more properly, the alumino-silicate layer as a whole) on the lone pair orbitals of the first layer of water molecules would tend to support further hydrogen bonding of a second and perhaps subsequent layers. However, the effect of hydration of the exchangeable cations, experimentally well established, will disrupt a hydrogen bonded water structure induced in such a manner on the surface and it is not known to what extent this disruption is important.

The infra-red data of Fripiat et al. (1960) supplies some important evidence of the nature of hydrogen bonding in the clay adsorbed water. They studied the I.R. spectra of hydrated samples of Li, Na, K and Sr montmorillonites and Li, K, and Sr vermiculites dehydrated at different temperatures. The adsorbed water in all samples dried at room temperature had O-H stretching frequencies approximately 500 cm^{-1} lower than the value for isolated water molecules of 3,700 cm^{-1}. This is approximately consistent with the depression observed for the O-H stretching frequehcy of ice

(500 cm^{-1}, Martin, 1947) which is undoubtedly due to hydrogen bonding. It is also consistent with the depression of the mean of the O-H stretching frequencies observed for crystalline hydrates (e.g. Li I.$3H_2O$ and $SrCl_2.6H_2O$) which is also approximately 500 cm^{-1} (Lucchesi and Glasson, 1956). Part of the observed depression in crystalline hydrates is due to electron withdrawal from water molecules of the hydration shell by the central cation.

Fripiat et al. (1960) also observed that the magnitude of the depression in the O-H stretching frequency of adsorbed water molecules decreased as the dehydration temperature increased, i.e. as the clay water content decreased. This suggests that the bonding is weakened as the water molecules are further separated at the surface by the dehydration process. These authors conclude that the depression in the O-H stretching frequency of adsorbed water molecules is due to hydrogen bonding between adsorbed water molecules and to the perturbation of the water by the electric fields of the cations and the surface.

The data of Mathieson and Walker (1954) and Walker (1955) support the conclusion of Fripiat et al. (1960). The single crystal X-ray data for the 14.4 Å phase of hydrated magnesium vermiculite (which is stable under normal atmospheric conditions) showed that the water molecules are arranged in a manner largely dictated by the Mg^{2+}, but with water-to-surface O-O distances of 2.90 Å. This separation is at the upper limit for significant hydrogen bonding as determined from the correlation between the depression in the O-H stretching frequency and the O-H...O distance (Lord and Merrifield, 1953). The empirical inverse linear relation given by Lord and Merrifield

is derived from infra-red and X-ray data for a number of hydrogen bonded systems including ice and hydrated carboxylic acids, and is in accord with Badger's generalization (1940) that the displacement in the O-H stretching frequency is a delicate measure of the force constant of the O-H...O bond and varies in an inverse linear ratio with the bond length.

The distance of closest approach of water molecules in the 14.4 Å phase of magnesium vermiculite is 2.78 Å (Mathieson and Walker, 1954, corrected value in Walker, 1955). This separation corresponds to a depression in O-H stretching frequency of approximately 500 cm^{-1} (Lord and Merrifield, 1953) which is in good agreement with the maximum depression given by Fripiat et al.(1960) for hydrated vermiculite and montmorillonite.

The water-to-surface O-O distance in the 14.8 Å phase of magnesium vermiculite (which is stable in contact with liquid water at room temperature) is 3.1 Å (Walker, 1955). This separation is too great for significant hydrogen bonding (Lord and Merrifield, 1953).

Finally, Young (1958) and Hockey and Pethica (1961) show that the physical adsorption of water vapour by silica is restricted to the silanol groups ($\equiv$Si - OH) and that heat treatment which irreversibly removes these groups, renders the silica hydrophobic to water vapour.

It may be concluded that, although the adsorbed water molecules are strongly hydrogen bonded to one another, there is no significant hydrogen bonding between the water molecules and the surface oxygen atoms of either smectites or silica. However, the available data on the nature of the Si-O bond suggests that the surface oxygens should

interact strongly with adsorbed water molecules and some attempt to reconcile these observations is necessary.

The structure of silicate minerals and oxides is commonly discussed in terms of the packing of constituent ions. This practice is largely based on the universal success of Goldschmidt's coordination numbers (which are derived solely from ionic radii) in the determination of silicate and oxide crystal structures. Verhoogen (1958) considered a wide range of physical properties of silicates and oxides and compared the experimentally observed values with those calculated for both ionic and covalent models. He concluded that these minerals behave mostly as purely ionic solids. However, the validity of these comparisons is questionable since it is probable that they cannot clearly distinguish between the models proposed for the following reasons:-

(1) There is little difference between the calculated values for the ionic and the covalent models for some of the compounds considered. This applies to lattice energies, Cauchy relations and atomic scattering factors.

(2) The experimental determination of diamagnetic susceptibility is very sensitive to the presence of paramagnetic impurities.

The electronic polarizabilities and ionic polarizations considered by Verhoogen are not sufficiently detailed to enable a clear determination of ionic character of the bonds in silicates.

Pauling (1960) assigns 50% or less ionic character to the Si-O bond of silica on the basis of electronegativities of the constituent atoms. Up to 80% covalent character has been determined by selecting different heats of formation (Young, 1958) while

Shaduri (1956) determined from approximate Slater's formulae that the amount of covalent character exceeds the ionic.

The adsorption of water vapour by silica (Young, 1958; Hockey and Pethica, 1961) and of a large range of organic and inorganic compounds by silica gels and porous glass (Terenin and Filimonov, 1959) is discussed in terms of a purely covalent Si-O bond.

It is difficult to interpret the fact that the surface oxygen atoms, with directed lone-pair orbitals as a result of covalent character of the Si-O bond, together with a high electron density due to partial ionic character, do not accept protons from water molecules to form strong hydrogen bonds, or by complete proton transfer to chemisorb water molecules. Proton transfer might be expected if the Si-O bond were largely ionic (Hambly, 1961).

The weak interaction between water molecules and the surface oxygen atoms may be a specific property of the water molecule, since Folman and Yates (1948, 1958) report that at low surface coverage of silica by water, the molecules are largely hydrogen bonded to each other rather than to the isolated hydroxyl group of the silica surface. In contrast to this, methyl alcohol apparently is hydrogen bonded to the surface hydroxyl groups.

It may also be a specific property of the silica or clay surfaces, since water molecules hydrogen bond strongly to zeolite (Frohnsdorff and Kington, 1958). The important difference is that the zeolites have non-planar and the clays and silica planar silicate surfaces.

It is possible that the electric fields of the clay surface and the exchangeable cations induce hydrogen bonding between water molecules at the expense of water-to-surface bond formation.

van der Waals or dispersion forces are probably responsible for a very small component of the total clay-water interaction. Low(1961) discusses this in some detail and concludes that the relative importance of these forces cannot at present be assessed.

Conclusion to Section 2, i, the mechanism of clay water interaction.

The most important contributions to the interactions between water molecules and clays are;

(1) The hydration of the exchangeable cations at low water content.

(2) The osmotic swelling, induced by the exchangeable cation solution at higher water contents.

Hydrogen bonding between adsorbed water molecules is influenced by the surface and the exchangeable cations and is stronger than in pure water. Other interactions including van der Waals forces are of smaller magnitude.

The net effect of these forces is to influence the properties of adsorbed water in several important ways. These properties are discussed in detail by Low (1961) and Martin (1960).

ii. The properties of water adsorbed by clays

a. The specific volume or density

Russell (1934) showed by pycnometer measurements that clays interacted differently with every liquid he investigated. From this he deduced that exchangeable ion-dipole interactions were important

in determining the density of the adsorbed liquid. His results also show that no inert liquid can be chosen from which a reliable value for the specific volume of the clay can be determined. This fact also has severely restricted the accurate determination of the specific volume of adsorbed water, and various attempts to overcome this difficulty have met with only indifferent success.

Low and Anderson (1958) derived an expression for the partial specific volume of adsorbed water which does not involve the determination of the specific volume of the clay. Their work is consequently the most successful of the experiments involving density determination and from it they concluded the following:

(1) The partial specific volume of adsorbed water is different from normal water to distances in excess of 60 Å from the clay surface.

(2) The partial specific volume increases as the clay surface is approached.

(3) Within 10 Å of the clay surface the specific volume is up to 3% greater than that of pure water (ice has a specific volume approximately 8% greater).

(4) As the temperature is lowered, the partial specific volume of the water increases.

(5) The exchangeable cations affect the partial specific volume of the water.

Low (1961) calculates the proportion of intact hydrogen bonds between water molecules in a 1:1 sodium montmorillonite:water paste at 25°C as being 55%. However, the calculation involves two

unacceptable assumptions:

(i) that the water has an ice-like structure and

(ii) that the effect of the exchangeable cations can be neglected.

Martin (1960) collected the data for density determinations of water adsorbed by sodium montmorillonite and showed that at water contents below 0.3g/g of clay the density exceeds that of bulk water, while at water contents above 1g/g of clay the adsorbed water density is within 2-3% of the value for pure water. At no water content does the density approach that required for ice ($0.90 g/cm^3$) or for a hexagonal network of water molecules ($0.92 g/cm^3$).

b. The viscosity of adsorbed water. Activation energy for flow.

Low (1961) clearly indicated that viscosity is a structure sensitive quantity. However, Martin (1960) emphasized that the absolute determination of the viscosity of adsorbed water from permeability measurements involves an assumption, either explicit or implied, concerning the flow path.

The presence of an indeterminate quantity in the calculation of both th density and the viscosity of adsorbed water reduces the validity of interpretation of the results in terms of a structure of the adsorbed water layer.

Low (1959) calculated the activation energy for flow from permeability data, thus avoiding the determination of fluid viscosity. The activation energy for flow in a paste containing 55% of montmorillonite increased with time to a value of 4350 cal./mole. of water after

three weeks aging. This may be compared with 3850 cal./mole. for bulk water in the same temperature range. From these results, he estimated that 48% of the hydrogen bonds remained unbroken in the adsorbed layer of water molecules.

Again Martin (1960) revealed an ambiguity in the interpretation of Low's results, claiming that they may equally be determined by a time-dependent change at the clay surface (e.g. by contamination with aluminium released from the crystal lattice during aging).

c. Freezing and super-cooling of adsorbed water

The fact that supercooling is very commonly observed in clay-water systems clearly indicates that the water differs from pure water. However, it is indeterminate whether the adsorbed water structure is more or less ordered than that of the bulk phase, because a change in either direction will account for this observation (Martin, 1960). However, if the adsorbed water had an ice-like structure, super-cooling would not be so pronounced.

d. The dielectric properties of adsorbed water

The dielectric constant ε_s in a static electric field of strength E is given by

$$\varepsilon_s = 1 + \frac{4\pi P}{E}$$

where P is the polarization induced by the field per unit volume of the dielectric and is made up of (1) the distortion polarization P_d largely due to the distortion of the electron clouds of an atom and (2) orientation polarization P_o due to orientation of the permanent dipoles in the electric field.

When the measurements are made in an alternating electric field, the permanent dipoles oscillate. On increasing the alternating frequency a point is reached when it is no longer possible for the permanent dipoles to orient themselves with the field. This is the region of anomalous dispersion, where the dielectric constant is given by:

$$\varepsilon = \varepsilon_o + \frac{\varepsilon_s - \varepsilon_o}{1 + i\omega\tau}$$

ε_o = the dielectric constant at very high frequencies and is due to distortion polarization only. This is the region of dielectric saturation.

$\omega = 2\pi\nu$ where ν is the frequency at which anomalous dispersion occurs.

τ = relaxation time

$i = \sqrt{-1}$

Resolving the complex quantity ε :

$$\varepsilon = \varepsilon' + i\varepsilon''$$

ε' = the effective dielectric constant

ε'' = the dielectric loss

In the region of normal dispersion, ε'' is very small.

In the region of anomalous dispersion it increases to a maximum and then falls off.

The relaxation time τ is related to the frequency at which this maximum (ν_m) occurs by the equation

$$\frac{1}{\tau} = \frac{\varepsilon_s + 2}{\varepsilon_o + 2} \cdot 2\pi\nu_m$$

Both τ and the related ν_m are structure sensitive quantities. For example, the dielectric relaxation time for an electrolyte solution decreases in an approximately linear manner with increasing concentration (Robinson and Stokes, 1959). This appears to be consistent with the views of Frank and Evans (1945) on the structure-breaking effects of ions, since a decrease in τ corresponds to a decrease in the restrictions placed on the water molecules by hydrogen bonding in an ice-like structure.

The water molecules in the hydration shell of a cation, on the other hand, are strongly bonded and orientation of the dipoles is greatly restricted. The relaxation time for water in hydrated salt crystals (e.g. $CaSO_4.2H_2O$) is approximately 200 times as great as for pure water (Lyast, 1958).

The dielectric properties of water adsorbed by talc, kaolinite, metahalloysite and halloysite at different relative humidities were studied by Muir (1954). He showed that the magnitude of the dielectric loss is proportional to the number of adsorbed molecules and made the following observations for the individual materials.

Talc. A single maximum in ε'' at 10 kc/sec. which increased in height through the humidity range 0-80%.

Kaolinite. Two maxima, one of which remained at 10 kc/sec. and increased in height through the humidity range 0-30% and the other of constant height which moved to higher frequencies with increasing relative humidity.

Metahalloysite and halloysite. A single maximum which came into the frequency range at 20% relative humidity and moved with increasing

height as the relative humidity was increased until at saturation, a ν_m of 10 Mc/sec. was reached.

Since talc has only oxygen surfaces and kaolinite has both oxygen and hydroxyl surfaces, Muir concluded that the stationary dielectric loss maximum, which occurred at the same ν_m for both minerals was due to water on the oxygen surfaces. Muir related the moving maxima for kaolinite and the halloysites to water on the hydroxyl surfaces. The constancy of the height of the moving maximum for kaolinite was attributed to dielectric loss occurring only with the initially adsorbed water layer, and its movement was attributed to the interaction of outer layers on the first.

Recently, Goldsmith and Muir (1960) extended the range of frequencies in the dielectric loss experiment to 0.09 kc/sec. In addition they measured the dielectric properties of the water adsorbed by pre-heated and non pre-heated clays which were saturated with different cations. On the basis of these experiments they concluded that (1) the dielectric loss is due to the conductivity arising from the solution of ions in the first few layers of adsorbed water, (2) the structure of the adsorbed layer is more ordered in the presence of smaller cations and (3) the structure is more ordered when the exchangeable cations are buried in the crystal lattice by heat treatment.

Kurosaki et al. (1957) recorded a peak in the dielectric loss curve for water adsorbed by glass at 100c/sec. The peak moved to higher frequencies with increasing surface coverage. This very low ν_m suggests that the hydrogen bonding of water to the glass surface

greatly restricts the orientation polarization.

No dielectric measurements have been reported for water adsorbed by montmorillonite or vermiculite, but it is reasonable to assume that the water molecules close to the exchangeable cations and perhaps also to the surface will have a low dielectric constant and a high relaxation time.

e. The thermodynamic properties of adsorbed water

The free energy of adsorbed water

The swelling of montmorillonite and other clays provides direct evidence that the partial molar free energy of the adsorbed water is less than that of pure water, until the adsorbed films become very thick, because even dilute clay suspensions exert a measurable swelling pressure. The relevent equations are as follows:

$\bar{F}_a - \bar{F}_1 = RT \ln {}^{P}/_{Po}$ for adsorption from the vapour phase.

$\bar{F}_a - \bar{F}_1 = \bar{v}_w \pi$ for adsorption from solution.

$\bar{F}_a$ = the partial molar energy of adsorbed water.

$\bar{F}_1$ = " " " " " pure water.

${}^{P}/_{Po}$ = the vapour pressure of water in equilibrium with the clay relative to that of pure water.

$\bar{v}_w$ = the partial specific volume of water

π = the swelling pressure at equilibrium

All effects which intensify the bonding of the water molecules at the clay surface will contribute to the lowering of the partial molar free energy of this water. Mooney, Keenan and Wood (1952a, b) studied the vapour phase desorption of water from montmorillonite saturated with a wide range of exchangeable cations. By applying the B.E.T. equation for monolayer adsorption to their desorption isotherms they detected little difference in the amounts of water adsorbed by K^+, Rb^+ and Cs^+ clays. As a result they assumed that these ions are not hydrated and assigned all the adsorbed water to the mineral surface. By subtracting the surface adsorbed water from the total water adsorbed by the other clays studied, the relative ionic hydrations were obtained. From this calculation the water held by the surface amounted to more than half of the total for H_3O^+, Li^+, Sr^{++} and Ba^{++} montmorillonites and about half of the total for Ca^{++} and Mg^{++}. This type of calculation is clearly over simplified for a number of reasons, the most important being that the presence of any cation at the surface lowers the activity of the adsorbed water. Thus a cation, hydrated or otherwise, contributes to the amount of water adsorbed by montmorillonite and the water can never be completely assigned to the mineral surface.

When discussing the effect of cation hydration on the adsorption of water by clays, the heat of adsorption is a more useful thermodynamic quantity.

The change in heat content of water adsorbed by clays

Orchiston (1955), working with homionic montmorillonites, demonstrated a linear relationship between the heats of hydration of

the cation and the C parameter in the B.E.T. equation (which is related to the heat of adsorption of a monolayer) thus emphasising the predominant role of the cations in the adsorption process.

The heat of adsorption of water on clays can also be determined from the Clausius-Clapeyron equation

$$\ln {}^{P_2}/_{P_1} = \frac{\overline{\Delta H}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

where $\overline{\Delta H}$ is the mean change in heat content on adsorption over the temperature range T_1 to T_2, and P_1 and P_2 are the equilibrium vapour pressures at constant water content at these temperatures. To determine these quantities, adsorption isotherms are required at each of the temperatures considered. The quantities determined in this manner (as opposed tocalorimetric procedures) are commonly termed isosteric heats of adsorption.

The isosteric heat of adsorption is greater than the heat of condensation of pure water (9,700 cal/mole) for both kaolinite (Goates and Bennett, 1957) and montmorillonite (Mooney et al. 1952a, Barshad, 1955) and varies with initial water content. For example, the isosteric heats of adsorption of water by a Japanese bentonite at different water contents are given in Table 1.

Table 1

The isosteric heats of adsorption of water by a Japanese bentonite at different water contents

Heat of adsorption k.cal./mole.	Water content mg/g clay
27.0	10
18.5	50
11.7	100
10.9	200

The figures in Table 1 were determined from B.E.T. plots for adsorption at 15°, 25°, 35° (Takizawa, 1960).

Differential heats of wetting of clays have been determined by Robins (1952), Rosenqvist (1955) and Zettlemoyer et al. (1955). The differential heat of wetting is the heat liberated per gram of liquid water added to a clay at constant temperature, pressure and water content. It differs from the heat of adsorption by the heat of condensation of pure water. All the heat of wetting studies referred to have two features in common, namely, that the heat of wetting becomes very close to zero when only a few layers of water have been formed, and the first layer or two are adsorbed with the release of very large amounts of energy (more than is released on the freezing of water). For example, Zettlemoyer et al.(1955) found differential heats of wetting for a natural bentonite clay ranging from about -380 cal./g. near zero water content to practically zero cal./g. at 20% water content. The data presented by Grim (1953), Slabaugh (1959) and Rosenqvist (1955)

show that the exchangeable cations influence the heats of wetting and adsorption. Generally speaking, the heats of wetting increase with decreasing ionic size.

The importance of the exchangeable cation in determining the heat of wetting is shown in Table 2 where the results for homionic vermiculite and a styrene-divinyl benzene co-polymer cation exchange resin, each with the same exchangeable cations (Keay and Wild, 1961) are compared.

Table 2

The comparison of the heats of wetting of a vermiculite and a cation exchange resin for a range of exchangeable cations.

Exchangeable cation	Heat of hydration (wetting) of vermiculite k.cal./mole exch. cation	Heat of wetting of polystyrene D.V.B. resin, k.cal./mole. exch. cation
Mg^{2+}	-41.0	-39.4
Ca^{2+}	-33.8	-24.6
Sr^{2+}	-36.3	-
Ba^{2+}	-25.2	-20.8
Na^{+}	-16.6	-9.1

Although the heats of hydration refer to a range of water contents which is rather arbitrary, they indicate that the nature of the exchangeable cation largely determines the heat of hydration of vermiculite.

The entropy of adsorbed water

This is a thermodynamic property which should enable the differentiation of those models proposed for clay adsorbed water, the molecules of which possess different degrees of order and/or freedom. Unfortunately, two important models discussed in conjunction with entropy results in the literature (a fixed adsorption structure with quasi-solid properties and a two dimensional liquid film) have approximately the same entropy. See Table 3 (Martin, 1959).

Table 3

Calculated entropy values for various models proposed for adsorbed water.

Model	Entropy (cal./mole.° at 20°C, 17.53 mm Hg)
Normal gas $S_g - S_l$	+ 35.9
Two dimensional gas $S'_g - S_l$	+ 29.3
Normal liquid $S_l - S_l$	0
Two dimensional liquid $S'_l - S_l$	- 6.6
Normal solid $S_s - S_l$	- 5.9

This fact has led to some confusion of interpretation of entropy data. Martin (1959) calculated the differential entropy ($\bar{S}_a - S_l$) and the integral entropy ($S_a - S_l$) of the adsorption of water by lithium and sodium kaolinite by using equations of the type:

$$(S_a - S_l) = \frac{(H_g - H_l) - (H_g - H_a)_s}{T} - R\ln\left(\frac{P_s}{P_o}\right)$$

When the equation is used to calculate the differential entropy of adsorption the subscript S becomes X, the increment added to achieve the change in entropy $\overline{S}_a$. When calculating the integral entropy of adsorption, the subscript S becomes ϕ, the relative spreading pressure calculated from the Gibbs equation:

$$\phi = T\int_0^P \frac{X}{p}\, dp$$

$H_g - H_l$ = the latent heat of vaporization of water at temperature T.
$H_g - H_a$ = the integral or differential heat of adsorption from the vapour phase at temperature T and vapour pressure p_s.

The molar integral entropy of adsorbed water S_a differs from the molar differential entropy $\overline{S}_a$ in that it is the entropy of the whole assembly of water molecules on the surface at the particular activity ϕ, while $\overline{S}_a$ is the change in entropy of the adsorbed water at a particular water content due to the addition of an increment of water.

Martin (1959) illustrated graphically the change in both differential and integral entropy with increasing relative pressure. The differential entropy varies in a complex manner with increasing relative pressure (as is observed in similar reports). However, the experimental curve for lithium kaolinite agrees very well with the ideal entropy curve calculated for a statistical model for physical

adsorption on an inert adsorbent, ultimately resulting in a mobile film at high surface coverage.

Martin discussed the nature of the mobile film in terms of the integral entropy of adsorption ($S_a - S_l$) which is positive over the the whole of the pressure range. He concluded from this observation that the adsorbed water is more random than pure water, the randomness being a result of the structure-breaking effect of the negative clay surface in an analogous manner to the structure-breaking effect of large anions such as Cl^-.

However, the integral entropies of adsorption of water by quartz up to a double layer of adsorbed water molecules (Whalen, 1961) and by a kaolinite with an unspecified exchangeable cation at monolayer coverage (Chessick and Zettlemoyer, 1961) are negative. At monolayer coverage of kaolinite by water molecules, ($S_a - S_l$) = -18.7 E.U. (Chessick and Zettlemoyer, 1961) or +2 to +8 E.U. (Martin, 1959). Both Whalen, and Chessick and Zettlemoyer used a form of the equation of Jura and Hill (1952) which is similar to that used by Martin. It is not clear why the results of these authors differ. The differences are probably too great to be explained in terms of the variability of kaolinite surfaces.

Whalen (1961) concluded that at low surface coverage of quartz, the adsorbed water vapour is immobile while at higher surface coverage a mobile film apparently exists.

Jurinak and Volman (1961) studied the water vapour adsorption by lithium, calcium and barium kaolinites and reported graphically the change in the differential entropy of adsorption with a surface

coverage parameter. For adsorption by heated lithium kaolinite (in which it is claimed that the lithium ions migrate into the crystal lattice) they conclude that an ice-like structure of water molecules exists on the surface. Also the surface water configuration in the presence of a hydrateable cation (Ba^{++} or Ca^{++}) is more ordered than the configuration based on water-surface interaction alone.

Barshad (1959) determined the partial molal entropy of adsorption of water by montmorillonite saturated with a wide range of cations. The reaction between clay and water was considered to be:

$$n_m \text{ montmorillonite (dry at } p = o) + n_w H_2O(\text{at } p_o) \rightleftharpoons n_m \text{ montmorillonite } n_w H_2O \text{ (at } p)$$

He concluded that the water molecules are in their lowest state of organization at low surface coverage, when the monolayer is incomplete and only a small proportion of the water molecules are associated with the exchangeable cations. Under these conditions, the water behaves almost as a two dimensional gas. At monolayer coverage, the water molecules are in their maximum state of orderliness.

Conclusion to Section e. The thermodynamic properties of adsorbed water.

The change in heat content on the adsorption of water by clay clearly demonstrates once again the importance of the exchangeable cations in clay-water interactions.

The interpretation of entropy data has led to proposals that the adsorbed layer of water molecules at different surface coverages is a two dimensional gas, a two-dimensional liquid (both less or more ordered than pure water) or an ice-like structure. Some of these interpretations

are contradictory and the interpretation of entropy data for clay-water and similar systems will be of doubtful validity until the thermodynamics of these systems is placed on a firmer basis. It is unknown to what extent the entropy changes in the region of adsorption and desorption hysteresis is expressed quantitatively by the relevant equations referred to in this discussion (Everett, 1957; Barrer and Kelsey, 1961). Also the extent to which statistical thermodynamics can be used to separate the contribution to the total entropy change by changes in the degrees of freedom and in the order of the molecules on adsorption (Barrer, 1958; Everett, 1958) is unknown. It may be necessary to derive a satisfactory model for the adsorbed layer of water molecules before these questions can be answered.

3. The Adsorption of Organic Compounds from Aqueous Solution

i. The adsorption of amino-acids and proteins by clay minerals

Attempts to explain the resistance of a large part of the soil organic matter toward microbial or enzyme catalysed decomposition include the suggestion that adsorption by soil clays might protect the organic matter, either by modifying its properties, or by preventing access of the microbes or extra-cellular enzymes to the adsorbed compounds (Ensminger and Gieseking, 1939).

Many of the reports on protein adsorption since 1939 have been concerned with this suggestion and it has been shown that intercalation by montmorillonite reduces the rate of protein hydrolysis by both bacteria and enzymes (Ensminger and Gieseking, 1939, 1942; Allison, et al., 1949; Pinck et al., 1951, 1954). Adsorption by non-expanding lattice clay minerals does not reduce the rate of protein hydrolysis (McLaren, 1954;

McLaren and Estermann, 1956; Pinck et al. 1954).

The adsorption of basic amino-acids lysine and arginine and the neutral amino-acid tryptophane by montmorillonite did not reduce the rate of utilization of these compounds by bacteria (Putnam and Schmidt, 1959). The rate of disappearance of amino-acids added to soils is extremely rapid (Quastel and Scholefield, 1949; Schmidt et al., 1960; Greenwood and Lees, 1960a, b) and the amount of amino-acids extracted from soil by 80% aqueous ethanol or cold water is of the order of μg/g (Bremner, 1950, 1952; Dadd et al. 1953; Simonart and Peeters, 1954; Simonart and Buysse, 1954; Dahr et al., 1955; Payne et al., 1956; Putnam and Schmidt, 1959).

However, Paul and Schmidt (1961) showed that electrolyte solutions extracted 5 to 25 times the amounts of the amino-acids extracted by 80% ethanol or water, and that the basic amino-acid lysine predominated in the extracted solutions.

A small number of physico-chemical investigations of the adsorption of amino-acids by clay minerals have been reported. These studies have been restricted to the interpretation of adsorption isotherms and the basal spacings of montmorillonite-amino-acid complexes (determined by X-ray diffraction). The aim of all these investigations has been the elucidation of the bonding mechanisms involved.

Talibudeen (1955) studied the complexes formed by the adsorption of glycine, alanine, phenyl-alanine, hydroxy-proline and arginine by montmorillonite at pH 2.5 and 4.0 by X-ray diffraction, and reported that both the basicity and size of the amino-acid molecules determine the extent of adsorption. The basicity is the more important property.

Mclaren et al. (1958) determined the isotherm at 25°C for the adsorption of alanine by montmorillonite at pH 2. They obtained X-ray data for the dried complexes in conjunction with this isotherm and showed that the basal spacings were not proportional to the amount of alanine adsorbed. They therefore concluded that any attempts to interpret X-ray data without relation to an adsorption isotherm are necessarily incomplete (e.g. the data of Talibudeen, 1955). This is an important conclusion, although the X-ray data of McLaren et al. for alanine are not accompanied by a sufficiently detailed adsorption isotherm.

Sieskind and Wey (1959) extended their earlier, systematic work on the adsorption of amines by montmorillonite (Sieskind and Wey, 1958) to the study of the adsorption of α-amino acetic acid (glycine), β-amino propionic acid, Y-amino butyric acid, δ-amino valeric acid and ε-amino propionic at a fixed concentration (0.025M) over the pH range from 1 to 11. The results, expressed as the amount of amino-acid retained (m equiv./100 g) against washing with water, plotted against pH, yielded a family of curves, similar in shape to acid-base titration curves, the amount held increasing with decreasing pH and reaching a maximum at pH 2. They found a linear relationship between the amount of amino-acid retained at pH 2 and the number of carbon atoms between the amine and acid groups. This last result can be interpreted as the increase in adsorption with increasing basic strength of the amino-acid, increasing molecular weight, or both. These authors conclude that the amino-acids are held as cations by the clay at pH values below their iso-electric points.

In a later paper, Sieskind (1960a) showed that amino-acid basicity

was more important than molecular weight in determining the amount retained at pH 2 against washing with water. She studied two series of compounds, the first being a number of isomeric amino-acids of different basic strengths and the second a range of α-amino-acids with similar strengths but different molecular weights. Her results show that the more weakly basic α-amino-acids are held to a very much smaller extent than the more strongly basic β- to ε-amino-acids, the maximum amounts retained being approximately 10 and 90 m mole./ 100 g respectively. The retention of glycine is greater than that of the other α-amino acids, with a maximum of approximately 50 m mole./100g. Sieskind (1960b) concluded from subsequent X-ray data for complexes obtained from the adsorption experiments that the greater amount of glycine remaining after washing was probably due to the more intimate contact between the planar glycine molecule and the clay surface.

Both Talibudeen (1955) and Sieskind (1960b) illustrated the importance of basicity in determining the amount of amino-acid adsorbed by montmorillonite at pH 2 to 2.5, but Sieskind supported this conclusion with more detailed and quantitative data. However, her data are dependent upon an arbitrary washing procedure used to determine the amount retained by the clay.

At pH 2, the amount of amino-acid adsorbed by clay is determined by an equilibrium of the type:

$$\text{clay } H_3O + RH^{\pm} \rightleftharpoons \text{clay } RH_2 + H_2O \qquad (1)$$

where clay H_3O = the exchangeable hydrogen ion,

$RH^{\pm}$ = the dipolar ionic form of the amino-acid, and

clay RH_2 = the adsorbed amino-acid cation RH_2^+

Desorption is the reverse of equilibrium (1) and it is theoretically possible to remove completely the adsorbed amino-acid by washing with water. The analogous desorption of amine cations adsorbed by montmorillonite is small but measurable (Cowan and White, 1958). The desorption of the more feebly basic amino-acids studied by Sieskind (1960a) is much more significant. This is clearly illustrated by comparison of her desorption data for alanine, where less than 5 m mole./100 g is held at 0.1 M with the equilibrium adsorption isotherm of McLaren et al. (1958) in which the corresponding value is approximately 30 m mole./100g.

Sieskind's (1960a) experimental procedure serves to distinguish the α-amino-acids clearly from the more strongly basic β- to ε- amino-acids, since the latter are not only more readily adsorbed, but are more difficult to desorb by washing. She justifies the experimental procedure adopted by maintaining that the determination of the amount of amino-acid adsorbed at equilibrium by analysis of the supernatant solution after separation of the clay is attended by errors that may be sufficiently large to obscure completely the adsorption phenomenon.

The determination of adsorption isotherms by solution analysis was used by McLaren et al. (1958) to study the adsorption of alanine and has been adopted in the present work. It is shown from a consideration of the errors involved (see later in the discussion) that valid results can be obtained under carefully controlled experimental conditions.

The adsorption of amino-acids by cation exchange resins has been studied in great detail, chiefly with the aim of understanding the process of amino-acid separation. The proton transfer reaction with

the exchange resin in the acid form, has been most thoroughly studied, usually together with changing pH (e.g. Seno and Yamabe, 1960). The chemical equation describing the proton transfer reaction applies equally well when either an exchange resin or a clay is the substrate, but the extreme difference between the two types of surface prevents comparison of the contribution of physical forces to relative adsorption affinities.

The real value of equilibrium adsorption isotherms is that they allow the calculation of thermodynamic quantities for the system. These quantities can be interpreted in terms of relative adsorption affinities and the possible models for the adsorbed layer with greater certainty than by other procedures. This is well illustrated for vapour phase adsorption systems by the studies of Barrer and Kelsey (1961) and for adsorption from aqueous solution onto montmorillonite by Cowan and White (1958).

Cowan and White (1958) studied the adsorption of a homologous series of n-alkyl primary amine cations from aqueous solution (as chlorides) by sodium montmorillonite. The cation exchange reaction is:

$$\text{clay Na} + RNH_3^+ \rightleftharpoons \text{clay } RNH_3 + Na^+$$

From the adsorption isotherms, they determined the mass action quotient for the equilibrium, given by:

$$K_m = \frac{N_{RNH_3^+}}{N_{Na^+}} \cdot \frac{[Na^+]}{[RNH_3^+]}$$

where $N_{RNH_3^+}$ and N_{Na^+} are the amounts in m equiv./100g of amine

adsorbed and sodium ion remaining on the clay surface respectively and $\left[RNH_3^+\right]$ and $\left[Na^+\right]$ are the concentrations of the cations in the solution phase, at equilibrium.

The mass action quotient is related to the activity constant K_a by:

$$K_a = K_m K_\gamma$$

where K_γ is the corresponding quotient for the activity coefficients. Activities cannot be determined, but in so far as K_m is constant, K_γ is also constant.

The standard free energy for the exchange reaction is given by

$$\begin{aligned} -\Delta G^\circ &= RT \ln K_a \\ &= RT \ln K_m + RT \ln K_\gamma \\ &= -\Delta G^m + C \text{ for constant } K_\gamma \end{aligned}$$

Cowan and White (1958) expressed graphically the variation in $-\Delta G^m$ with the number of carbon atoms in the n-alkyl chains of the amine cations studied. The graph is linear for the cations between amylammonium and decylammonium, with an increment in $-\Delta G^m$ of 400 cal/ CH_2 group. Therefore the relative ease with which the alkylammonium ions replace the sodium ions between these limits is a linear function of molecular size.

From octylamine to tetradecylamine, adsorption exceeds the exchange capacity and is accompanied by a decrease in the pH. The amine adsorbed above the exchange capacity is present as uncharged molecules and adsorption is preceded by hydrolysis of the amine hydrochloride.

The analysis of adsorption data as described by Cowan and White

(1958) was used in the present work when an exchange reaction was involved.

ii. The interpretation of X-ray and infra-red data for complexes between organic compounds and montmorillonite or vermiculite.

a. C-H. . .O hydrogen bonding

Bradley (1945) proposed this form of bonding to account for the adsorption of glycols and glycol ethers. He showed that ethylene glycol and its dimethyl ether could at least partially displace water from the interlamellar region of montmorillonite. Since Hendricks (1941) had postulated earlier that the water molecules in a montmorillonite-water system are hydrogen bonded to the clay surface, Bradley concluded that the interaction between glycol or glycol ether molecules with the clay surface is comparable in magnitude to the O-H...O bonds that, according to Hendricks' postulate, would be broken on displacement of water. Bradley's Fourier analysis of the X-ray data for the ethylene glycol-montmorillonite complex shows that the C-H...O distance between the glycol molecules and the surface oxygen atoms is approximately 3.3 to 3.6Å. This distance is too great for O-H...O bonding to the surface (Lord and Merrifield, 1953) and Bradley concluded that glycol or glycol ether adsorption by montmorillonite led to the formation of C-H...O hydrogen bonds. The C-H...O distance of approximately 3.3 to 3.6 Å is the expected value for methylene-to-oxygen van der Waals contact (3.4 Å, Pauling, 1960).

It was shown earlier that the hydration of the exchangeable cations in a montmorillonite- or vermiculite-water system is of much greater significance than hydrogen bonding to the surface and therefore

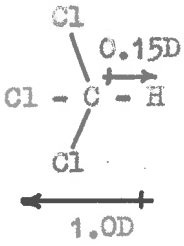
it cannot be concluded that the adsorption of glycol or glycol ether molecules is accompanied by the formation of C-H...O hydrogen bonds to the clay surface. Further, it is difficult to reconcile the conclusion that these bonds are formed, with the observation that the energetically more favourable O-H...O bonds to the clay surface do not occur on glycol or water adsorption. It is probable that the adsorption of glycols and glycol ethers by montmorillonite is accompanied by solvation of the exchangeable cations by these compounds.

MacEwan (1948) studied a wide range of glycol and glycol ether complexes with both montmorillonite and halloysite by means of X-ray diffraction and observed an apparent contraction in the van der Waals thickness of the adsorbed molecules. On the basis of Bradley's publication (1945) MacEwan attributed the apparent contraction to C-H...O hydrogen bonding between methylene groups and the surface sheet of oxygen atoms. The same conclusion was reached by Talibudeen (1955) from the apparent contraction in the van der Waals thickness of amino-acids and proteins on adsorption by montmorillonite. The last two authors suggest that the C-H...O bonds involve methylene groups adjacent to electronegative atoms which polarize the C-H bonds by the inductive effect.

Giles *et al.* (1956, 1957, 1959) have interpreted much of their own adsorption data in terms of C-H...O hydrogen bonding involving methylene groups.

Hambly (1961) showed that the formation of a strong hydrogen bond requires that the donor atom must have a sufficiently high electronegativity for its bond to hydrogen to become polarized and the exposed proton attract the elctrons in a lone pair orbital of the

acceptor atom. On this basis, C-H...O hydrogen bonds involving methylene groups are unlikely to occur since the central carbon atom is not electronegative with respect to the hydrogens. That is, the dipole of a C-H bond in sp^3 hybridization is directed with its positive end toward the central carbon atom (Partington, 1954). The presence of electronegative groups adjacent to the central carbon atom reduces the C-H bond dipole moment. For example, chloroform has a C-H bond moment of 0.15D compared with the more general value of 0.4 to 0.6D.



C-H bond dipole

molecular dipole

Chloroform forms a weak hydrogen bond to oxygen atoms with a bond energy of approximately 2.7 k cal. (Campbell and Kertzmark, 1960). This is because the molecular dipole, with its positive end toward the hydrogen atom orients the molecule toward the lone pair orbital of the acceptor atom. The lone pair electrons can then add to the polarization of the C-H bond induced by the 3 chlorine atoms.

This situation is not repeated with methylene groups adjacent to an electron withdrawing atom, because (1) the C-H bond dipole moment is greater than 0.15D (the electronegativity of one oxygen atom is 3.5 compared with the sum of the electronegativities of the three chlorine atoms of 9.0, Pritchard and Skinner, 1955) and (2) it is unlikely that the molecular dipole will assist hydrogen bond formation as effectively as described for chloroform.

Therefore, it is only in special cases when a methylene hydrogen is held close to a lone pair orbital of an acceptor oxygen atom by a favourable molecular conformation so that interaction is assisted that C-H...O hydrogen "bonding" has been recorded. For example, Prelog (1950), by a study of the first overtone of the CH_2 stretching frequencies of cyclohexanone, showed that there is a very weak intramolecular interaction between the α-methylene hydrogen atoms and the carbonyl oxygen atom.

Hoffmann and Brindley (1960) claim that an interaction of this type, too weak to be true hydrogen bonding, exists between methylene groups adjacent to electronegative atoms and the surface oxygen sheets of montmorillonite and enhances the adsorption of poly-functional ketones, esters and alcohols which contain a high proportion of α-methylene groups.

Tensmeyer, Hoffmann and Brindley (1960) studied the infra-red spectra of 2:5 hexanedione and 2:5:8-nonanetrione and the corresponding calcium montmorillonite complexes obtained from the earlier investigation of Hoffmann and Brindley (1960). They showed that the spectrum of nonanetrione supported in a KBr disc is closely similar to the spectra of the complexes containing one and two layers of adsorbed molecules. From the fact that the asymmetric deformation frequencies of nonanetrione in the solid phase or adsorbed by montmorillonite were higher than observed in the spectrum of the CCl_4 solution they deduced that weak C-H...O=C interactions of a hydrogen-bonding type occur in the crystal or adsorbed phase. However, this observation is not accompanied by lower C-H stretching frequencies in the solid or adsorbed phase than

in solution and the existance of C-H...C=C interactions is therefore doubtful. Splitting and slight elevation of C-H stretching and bending frequencies on crystallization of organic compounds due to interactions within the electric field of the crystal is commonly observed (Laby, 1959).

The above criticism of C-H...O hydrogen bonding does not apply to compounds containing C-H bonds in sp^2 or sp hybridization since the polarity of the bonds is opposite to that in methylene groups where sp^3 hybridization is involved. That is, the carbon atom is more electronegative in sp^2 or sp hybridized bonds than in a methylene group (Partington, 1954). C-H...O hydrogen bonds have been observed in formic acid esters and with substituted acetylenes (Brand et al. 1960).

Greene-Kelly (1955 a and b, 1956) studied the complexes between montmorillonite and a wide range of aliphatic and aromatic compounds by X-ray diffraction and concluded that C-H...O hydrogen bonding probably does not account for the observed contractions. He also claimed that at least part of the apparent contraction is due to "projection" effects but there is possibly also a true shortening of contact distances due to distortion of the molecules on adsorption. Greene-Kelly did not define projection effects, but they probably involve the interpretation of the perpendicular distance from an oxygen surface to the adsorbed layer of organic molecules in terms of van der Waals contact distances.

b. Other interpretations of X-ray data.

An alternative suggestion which has been offered to account for the apparent contraction in the thickness of organic molecules on adsorption by montmorillonite is that the molecules are keyed into the hexagonal depressions on the clay surface (Glaeser, 1951; Barrer and

Perry, 1961). Greene-Kelly (1956) maintained that keyed molecules (those which show an apparent contraction in molecular thickness) should diffuse out of the montmorillonite crystal at a slower rate than un-keyed molecules, which show no apparent contraction. In fact, very slow rates of diffusion have been observed for both types of complexes. However, projecting groups may be keyed into the surfaces thereby decreasing the diffusion rate without resulting in an apparent contraction.

Haxaire and Bloch (1956) studied the adsorption of a wide range of aromatic and aliphatic bases by montmorillonite and showed that in many cases adsorption was in excess of the exchange capacity. They also determined the basal spacings and MacEwan's Δ values for the resultant complexes. For the aromatic bases (including o-phenylene diamine, p-amino diphenyl, α-naphthylamine, p-phenylene diamine, methylene blue and benzidine) they showed that a good correlation existed between the amount adsorbed above the exchange capacity and the function π/Δ where π is the number of π electrons in the aromatic system and Δ is MacEwan's Δ value.

They concluded from this correlation that a strong bonding mechanism results from the interaction of the π electrons of the aromatic system with the clay surface and this produces a large contraction in the van der Waals thickness of the adsorbed molecule. This mechanism is said to be parallel to an oxidation-reduction reaction and is of significance only in those cases when the aromatic system can come into close proximity with the clay surface. That is, for

planar aromatic molecules.

The interpretation of the apparent contraction in the van der Waals thickness of an organic molecule on adsorption by montmorillonite or other expanding lattice minerals in terms of a bonding mechanism (e.g. C-H...O hydrogen bonding or the interaction of the π electron clouds of a planar aromatic molecule with the mineral surfaces) is invalid since the following uncertainties attend the interpretation.

(1) As discussed earlier, the assumed thickness of the alumino-silicate layer may be too great by a maximum of 0.3 Å and irregularities in the surface due to tilting of the silica tetrahedra may introduce an added uncertainty of $\pm$ 0.1 Å.

(2) The extent of distortion of a molecule on adsorption either by a change in bond angles or in the van der Waals radii under the influence of the electric field of the clay crystal is unknown.

(3) The amount of keying of adsorbed molecules into the cavities of the clay surface cannot be determined with any degree of certainty since it is dependent upon the distortion of the surface and the conformation of the adsorbed molecules.

The adsorption isotherms reported in Part II apply to the system where clay is in equilibrium with both water and adsorbate. Therefore, X-ray data for moist montmorillonite complexes prepared in conjunction with the isotherms is of considerable supplementary value. Of relevance to this aspect of the present work is the report by Walker and Garrett (1961) that magnesium vermiculite gives two types of swelling behaviour in the presence of amino-acid solutions: (1) Amino-acids adsorbed by cation exchange (ornithine, lysine and Y-amino butyric acid) confer on

the mineral swelling properties similar to lithium vermiculite. The degree of swelling decreases with increasing concentration of amino-acid. (2) When the adsorbed amino-acids are not held by cation exchange (glycine, β-alanine, γ-amino butyric acid and ε-amino caproic acid) the degree of swelling was observed to increase with concentration. The first type of swelling is due to development of diffuse double layers. The second type arises from an increase in the average dielectric constant of the interlamellar solution on intercalation of amino-acid dipolar ions, thus decreasing the electrostatic force of attraction between the exchangeable cations and the mineral surfaces. This effect was also observed during the present work (Greenland, Laby and Quirk, 1962). Barshad (1952) reported similar swelling data for calcium montmorillonite and magnesium vermiculite in the presence of amino-acid solutions and concluded that the high dielectric constant of these solutions is probably responsible for the expansion of the mineral lattices.

PART II EQUILIBRIUM EXPERIMENTS

1. Introduction

The amounts of amino-acids adsorbed by clays is a maximum at pH 2. As a consequence, adsorption isotherms have been studied at this pH alone, (Talibudeen, 1955; Sieskind, 1960a & b) where the proton transfer mechanism is involved and the amino-acids are held by cation exchange forces. It is not surprising therefore that emphasis has been placed on the basicity of amino-acids in determining the extent of adsorption. No attempt has been made to exploit the real potential of equilibrium adsorption studies.

However, the swelling studies of Barshad (1952), and Walker and Garrett (1961) suggest that other properties, in particular the exceptionally large dipole moments of amino-acids and the related high dielectric constant of their aqueous solutions determine their interaction with clays at pH values closer to 7. The investigation of amino-acid adsorption at neutral pH will assist in establishing the significance of the dipolar ionic structure in clay-amino-acid interactions. Also these studies may lead to a better understanding of the interactions between organic matter (containing amino-acids in combination) and the mineral fractions of soil and of the amino-acid transformations in the soil solution. The conflicting postulates on the nature of water adsorbed by clays which have been derived from thermodynamic considerations may be evaluated to some extent by these studies.

These and similar thoughts have determined the approach to the present work which is largely concerned with the interpretation of

adsorption isotherms and supplementary X-ray data for moist montmorillonite complexes. The work of Cowan and White (1958) and of Sieskind and Wey (1958, 1959) illustrates the value of a homologous series of compounds in studies on adsorption (and of course, many other types of investigation) and partly influenced the choice of glycine and its peptides glycyl glycine, diglycyl glycine and triglycyl glycine in the present work. These compounds have been studied in greatest detail and provide a basis for comparison for the remaining amino-acids investigated.

The interactions that occur between clay minerals and amino-acids or peptides have been investigated in three different systems.

(1) The adsorption of neutral and acid amino-acids and peptides by calcium montmorillonite and illite (section 4, i) and by the corresponding sodium clays (section 4, ii). Physical forces are involved and include ion-dipole, ion-induced dipole, surface dipole and van der Waals contributions.

(2) The adsorption of the same neutral and acid amino-acids and peptides by hydrogen montmorillonite (section 4, iii). Proton transfer is the most significant adsorption mechanism and operates in addition to physical forces.

(3) The adsorption of basic amino-acids and peptides by sodium and calcium montmorillonite and illite (section 4,iv) where cation exchange occurs, supported by physical adsorption.

The details of the isotherms studied are given in Table 4. Where possible, the relative affinities of the adsorbates and the significance of each type of adsorption mechanism are discussed in

terms of thermodynamic data.

2. Preliminary Experimental Details.

i. Description of the clay minerals and the preparation of homoionic suspensions.

The clay used for the preparation of montmorillonite suspensions was obtained from Ward's Natural Science Establishment Inc. It originated from the John C. Lane tract, Upton, Wyoming and was a sample of the standard clay mineral number 25b of the American Petroleum Institute Project 49. Its composition is given as:

$$(Si_{3.92}\ Al_{0.08})\ (Al_{1.55}Fe_{0.15}Mg_{0.33})\ (OH)_2\ O_{10}$$

$$\downarrow$$

$$Na_{0.27}\ (\tfrac{Ca}{2})_{0.03}$$

The unground clay, as received, dispersed readily in distilled water without evidence of flocculation. The fraction less than 2 microns equivalent spherical diameter was separated by mechanical dispersion in distilled water, followed by repeated sedimentation and decantation. X-ray powder photographs of the clay obtained from these suspensions showed that approximately 3% of quartz was present. No other extraneous lines were observed. The X-ray diffractometer trace is given in Fig. 2.

The illite sample came from County Grundy, Illinois and was obtained from the Illinois Clay Products Co., Joliet, Illinois. No chemical analysis has been obtained for this sample. After preliminary saturation of the clay with sodium ion, the less than 2μ e.s.d. fraction was separated by the same procedure used for montmorill-

Fig.2. An X-ray diffractometer trace for the montmorillonite used.

Fig.3. An X-ray diffractometer trace for the illite used.

fig.2

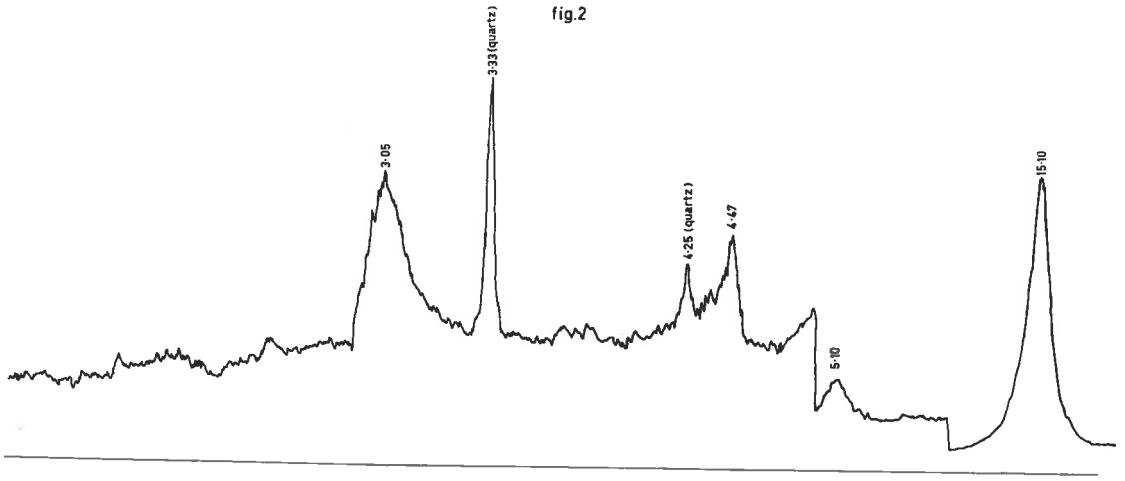
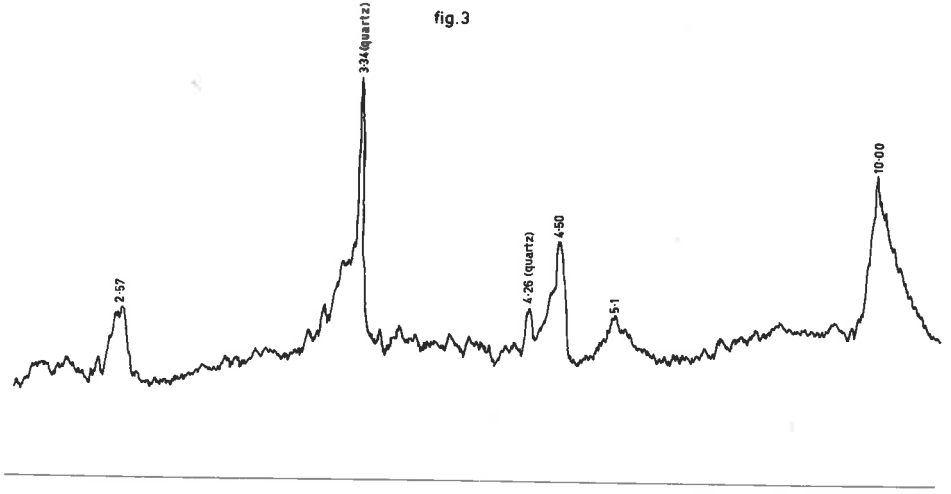

3·33(quartz)
3·05
4·25 (quartz)
4·47
5·10
15·10

fig.3


3·34(quartz)
2·57
4·26 (quartz)
4·50
5·1
10·00

onite. The X-ray diffractometer trace (Fig. 3) showed that less than 3% of quartz was present. No other extraneous lines were observed but the asymmetry of the 10 Å basal reflection suggests that the clay may be interlayered with an expanding lattice mineral (Molloy and Kerr, 1961). The extent of interstratification was shown to be small by comparison of the external surface area (106 m^2/g) determined by nitrogen adsorption with the total surface area (approximately 150 m^2/g.) determined from the adsorption of cetyl pyridinium bromide (Theng, 1961; Greenland and Quirk, in press). The internal surface area of approximately 44 m^2/g. indicates that the ratio of montmorillonite- to mica-like layers in the lattice is 1 to 15

The main difficulty encountered in saturating a clay with a given cation is the removal of aluminium present as an impurity on the surface (Martin, 1960). The complete removal of this impurity is impossible since the spontaneous alteration of the clay that occurs under the conditions of acidity at which surface-bound aluminium dissociates from the clay (Lin and Coleman, 1960) leads to translocation of the lattice bound aluminium which can further contaminate the surface (Bolt and Warkentin, 1956; Coleman and Craig, 1961). The aluminium impurity initially present probably consists of partly polymerised aluminium hydroxides and decomposition fragments of the clay (Bolt and Warkentin, 1956) and it contributes to the acidity of the sample (Low, 1955). Neither electrodialysis nor treatment with the hydrogen form of a cation exchange resin will remove surface bound aluminium (Thompson and Culbertson, 1959). It is therefore necessary to equilibrate the

clay with a salt solution of the saturating cation at a reduced pH. The optimum pH is approximately 3 (Lin and Coleman, 1960).

The details of the methods used to saturate montmorillonite with sodium, calcium and hydrogen ions and illite with sodium and calcium ions together with the experimentally determined exchange capacities, exchangeable cations, pH and electrolyte concentrations are given in Appendix I.

Calcium carbonate was present in the illite sample as received and a longer treatment with acidified salt solution was used to remove this impurity than was required for the saturation of montmorillonite suspensions.

ii. The amino-acids and peptides studied.

The structures and sources of supply and purity of these compounds are given in Appendix II.

iii. Separation of the clay from the supernatant solutions.

The amount of amino-acid or peptide adsorbed at equilibrium was determined by analysis of the supernatant solution which therefore had to be completely free of suspended clay. Since the electrolyte concentration was usually extremely low, it was found necessary to centrifuge the suspensions in a Spinco Model L ultracentrifuge at 23,000 r.p.m. (46,000 g.) for 25 min. The temperature change in the suspension during centrifugation was less than 1°C for all experiments. This was achieved by adjusting the refrigeration of the centrifuge.

iv. Estimation of the amount of amino-acid or peptide adsorbed

The supernatant solution obtained on centrifuging the suspension was analysed by a slightly modified Kjeldahl procedure (Appendix III)

All quantitative volumetric apparatus was of A grade quality. The calibrations were checked by weighing the volumes of water delivered or contained.

v. The adsorption experiment.

Adsorption isotherms were obtained by transferring accurately known portions of the clay suspensions to polystyrene tubes and adding to each, differing amounts of standard amino-acid or peptide solution. The solutions were standardised against the Kjeldahl procedure. The contents of the tubes were adjusted accurately to a common volume (usually 30 ml.) with distilled water and shaken overnight, at 20°C for the earlier experiments with glycine and its peptides, or 25°C for all other experiments. For the adsorption of the basic amino-acids, equilibration time was increased to 24 hours. The suspensions were then centrifuged and the supernatants analysed as described above. In the earlier experiments with glycine and its peptides and with the basic amino-acids the solutions were also analysed for the cation liable to be displaced by the clay. The pH of the supernatant solutions was determined in some experiments (see Table 4).

3. Results of the Equilibrium Experiments

The change in the amount of amino-acid or peptide adsorbed (m mole./100 g of clay dried at 110° or 350°) is plotted against the equilibrium concentrations (mole/l.) to give adsorption isotherms. The results are shown graphically in Figs.4 to 19 (for details of each Fig. see also Table 4). In some experiments reported (Table 4), the amount adsorbed was calculated on the basis of clay dried at 110°C for 24 hours, in conformity with standard practice for soil samples.

However, examination of differential thermal data for montmorillonite (MacKenzie, 1957) showed that drying at 350°C would give more reproducible results and further, this temperature corresponded to the loss of most of the adsorbed water without severely dehydrating the clay lattice. All subsequent experiments are referred to clay dried at 350°C.

Table 4

Details of adsorption isotherms illustrated in Figs. 4 to 19.

Figure number	Adsorbents	Adsorbates	Isotherm temp °C	Drying temp °C	pH determined
4	Na^+ montmorillonite	glycyl glycine, diglycyl glycine and triglycyl glycine	20	110	yes
5	Ca^{++} montmorillonite	glycine, glycyl glycine, diglycyl glycine and triglycyl glycine	"	"	"
6	H_3O^+ montmorillonite	as for 5	"	"	"
7	Ca^{++} montmorillonite	glycine and glycyl glycine	25 & 0	350	no
8	" "	diglycyl glycine and triglycyl glycine	"	"	"
9	Na^+ illite	as for 5	25	"	"
10	Ca^{++} illite	as for 5	"	"	"
11	Ca^{++} montmorillonite	α-alanine, β-alanine, leucine and serine	"	"	"

Table 4 contd.

Figure number	Adsorbents	Adsorbates	Isotherm temp °C	Drying temp °C	pH determined
12	H_3O^+ montmorillonite	as for 11	25	350	no
13	Na^+ illite	as for 11	"	"	"
14	Ca^{++} illite	as for 11	"	"	"
15	H_3O^+ montmorillonite	aspartic and glutamic acids phenyl alanine and p-amino benzoic acid	"	"	"
16	Ca^{++} montmorillonite	DL- and L-arginine hydrochlorides and glycine hydrochloride	20	110	yes
17	Na^+ montmorillonite	L-arginine, histidine and lysine hydrochlorides	20	110	yes
18	" "	carnosine and histidine hydrochlorides	25	350	no
19	Ca^{++} montmorillonite	histidine hydrochloride	"	"	"
	Ca^{++} illite	histidine hydrochloride	"	"	"
	Na^+ illite	carnosine and histidine hydrochlorides	"	"	"

The experimental results from which each of these isotherms was plotted are given in Appendix IV.

Fig. 4. Adsorption isotherms for glycyl glycine, +, diglycyl glycine, Δ, and triglycyl glycine, O, on Na^{+} montmorillonite.

Fig. 5. Adsorption isotherm for glycine, □, glycyl glycine, +, diglycyl glycine, Δ, and triglycyl glycine, O, on Ca^{++} montmorillonite.

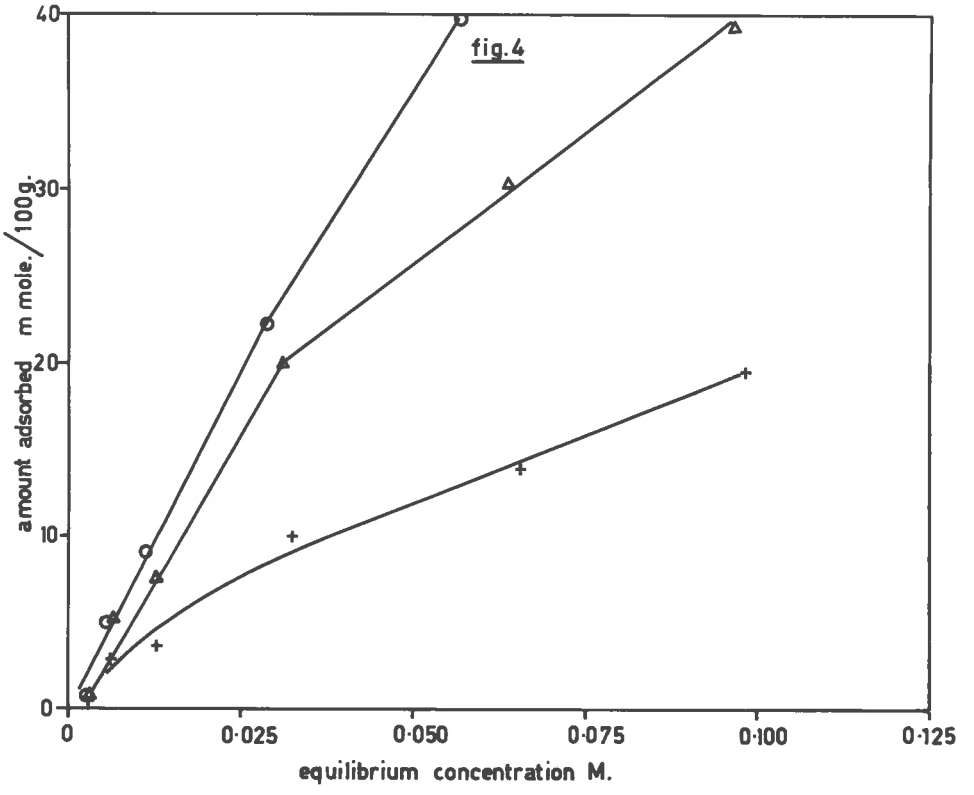


fig. 4
amount adsorbed m mole./100g.
40
30
20
10
0
0
0·025
0·050
0·075
0·100
0·125
equilibrium concentration M.

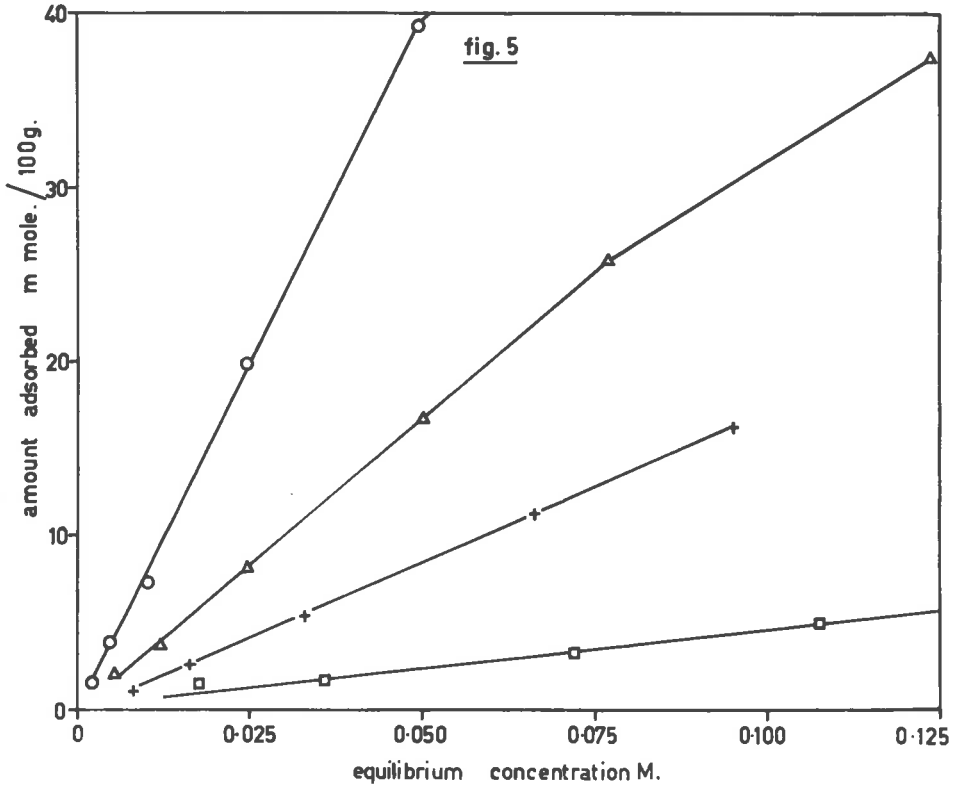


fig. 5
amount adsorbed m mole./100g.
40
30
20
10
0
0
0·025
0·050
0·075
0·100
0·125
equilibrium concentration M.

Fig. 6. Adsorption isotherms for glycine, □ , glycyl glycine, + , diglycyl glycine, Δ , and triglycyl glycine, ○ , on H_3O^+ montmorillonite.

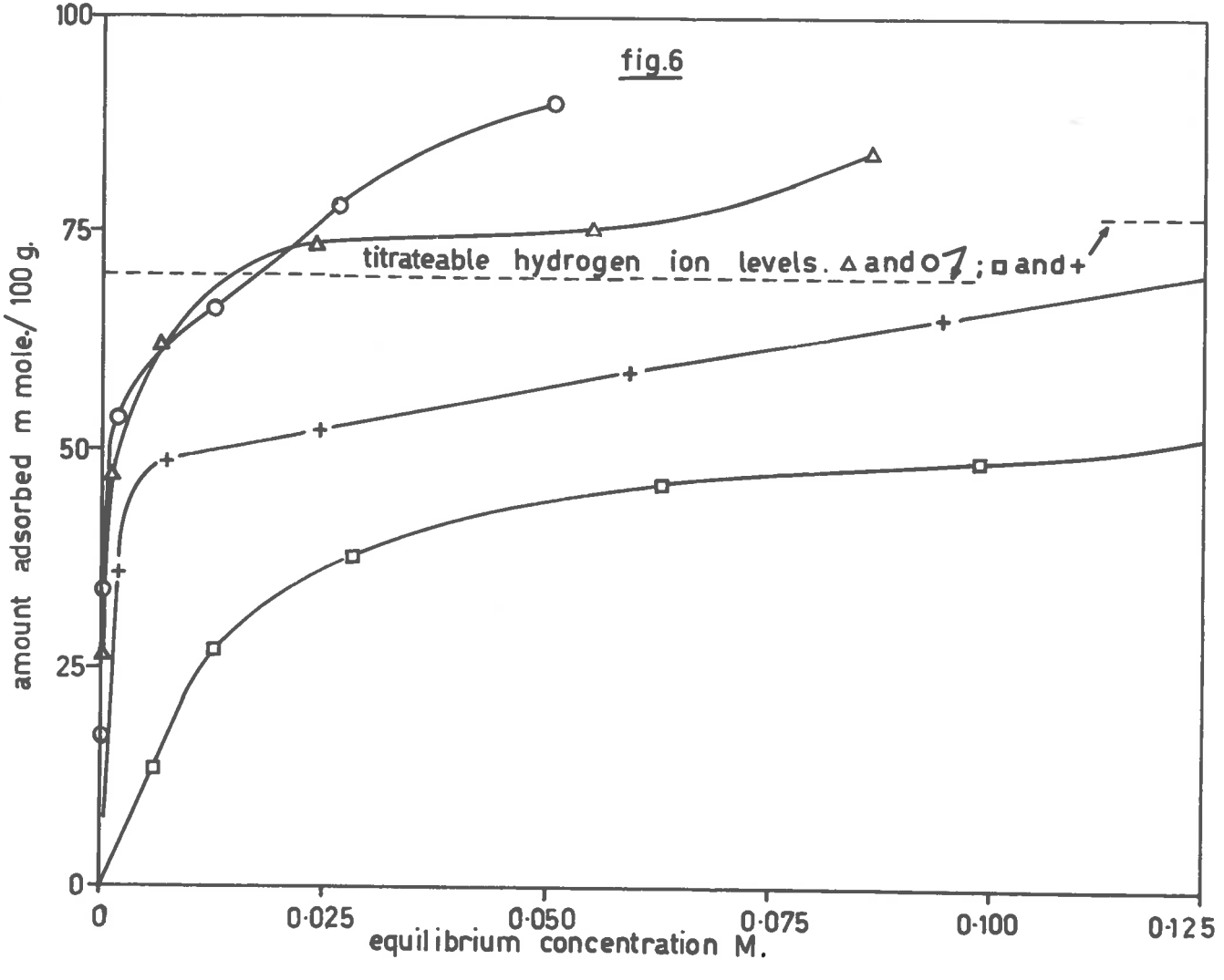
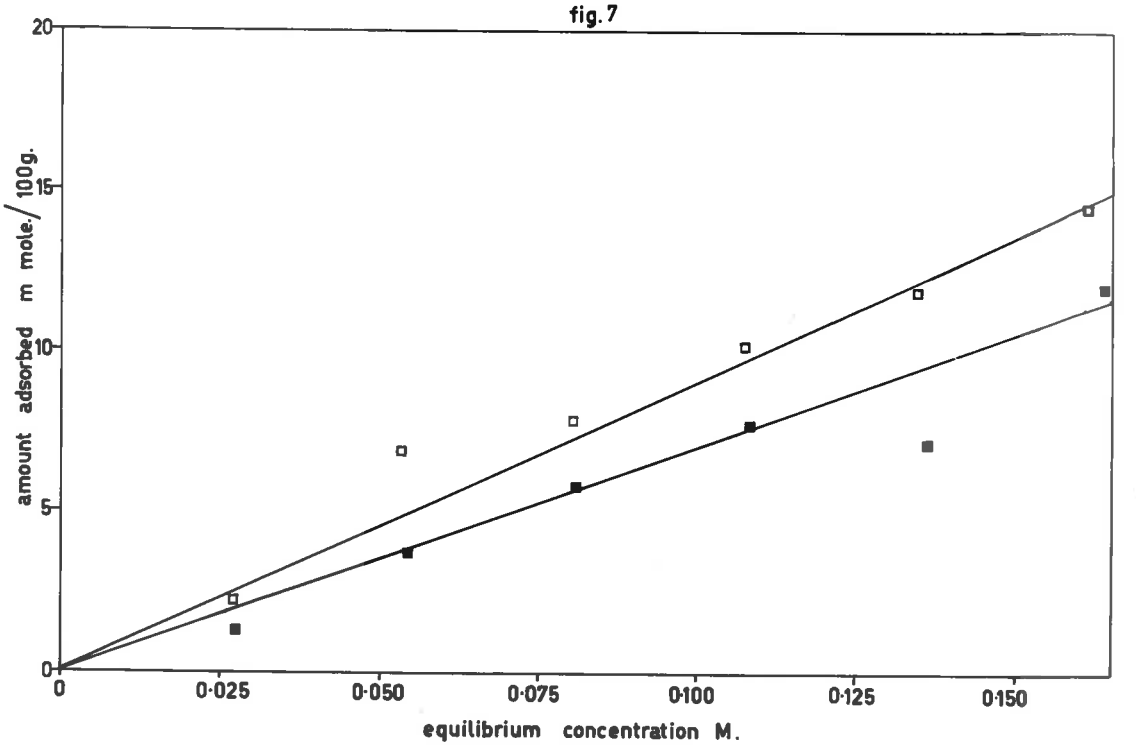


fig.6
100
75
50
25
0
amount adsorbed m mole./ 100 g.
titrateable hydrogen ion levels. △ and ○ ; □ and +
0
0·025
0·050
0·075
0·100
0·125
equilibrium concentration M.

Fig. 7. Adsorption isotherms for glycine on Ca^{++} montmorillonite at 0°C, □ and at 25°C, ■.

Adsorption isotherms for glycyl glycine on Ca^{++} montmorillonite at 0°C, — + — and at 25°C - - - + - - -

fig. 7


20
15
10
5
0
amount adsorbed m mole./100g.
0
0·025
0·050
0·075
0·100
0·125
0·150
equilibrium concentration M.

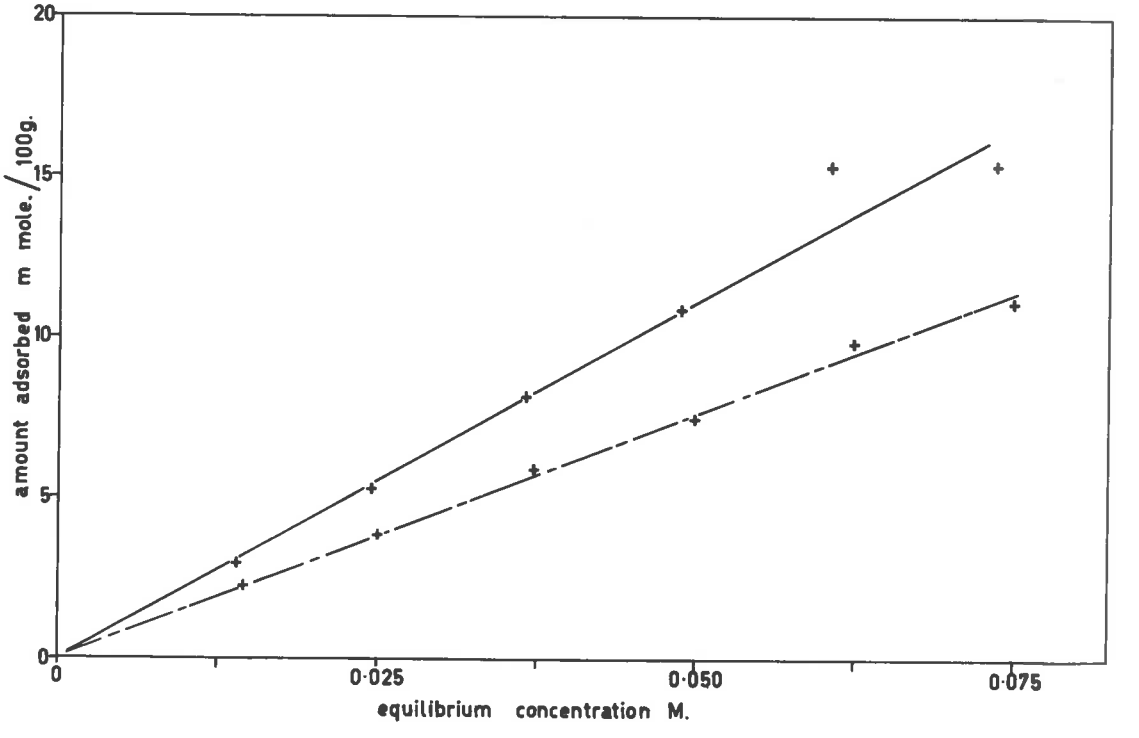
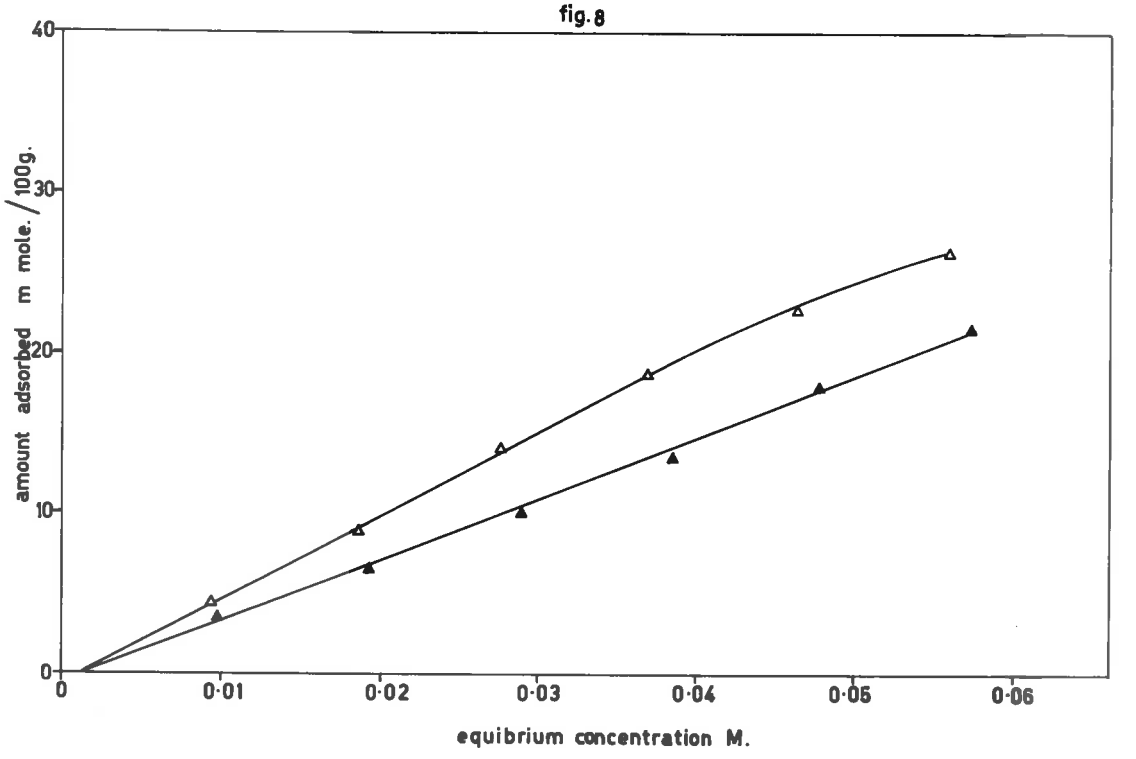

20
15
10
5
0
amount adsorbed m mole./100g.
0
0·025
0·050
0·075
equilibrium concentration M.

Fig. 8. Adsorption isotherms for diglycyl glycine on Ca^{++} montmorillonite at 0°C, △ and at 25°C, ▲.

Adsorption isotherms for triglycyl glycine on Ca^{++} montmorillonite at 0°C, ○ and 25°C, ●.

fig. 8


40
30
20
10
0
amount adsorbed m mole. / 100g.
0
0·01
0·02
0·03
0·04
0·05
0·06
equibrium concentration M.

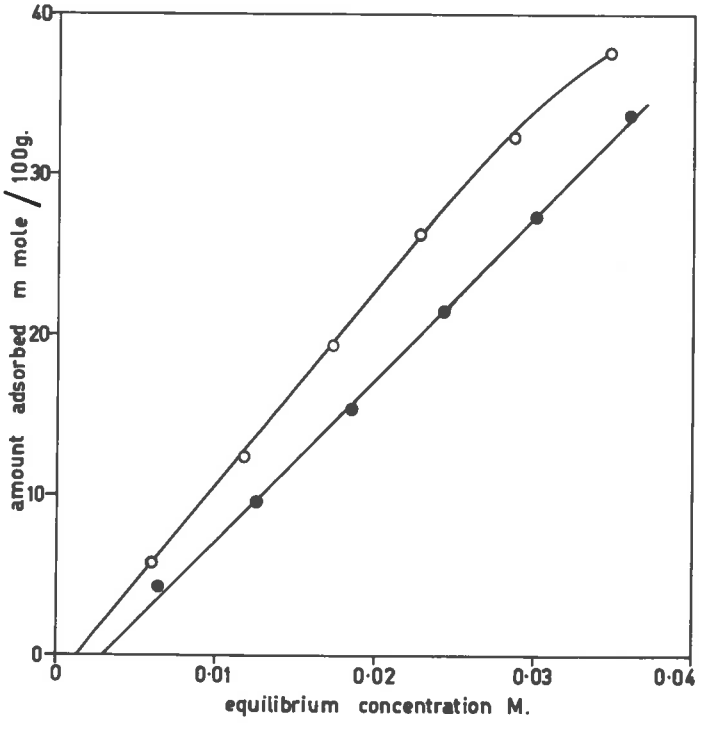

40
30
20
10
0
amount adsorbed m mole / 100g.
0
0·01
0·02
0·03
0·04
equilibrium concentration M.

Fig. 9. Adsorption isotherms for glycine, □, glycyl glycine, +, diglycyl glycine, △, and triglycyl glycine, ○, on Na^{+} illite.

Fig. 10. Adsorption isotherms for glycine, □, glycyl glycine, +, diglycyl glycine, △, and triglycyl glycine, ○, on Ca^{++} illite.

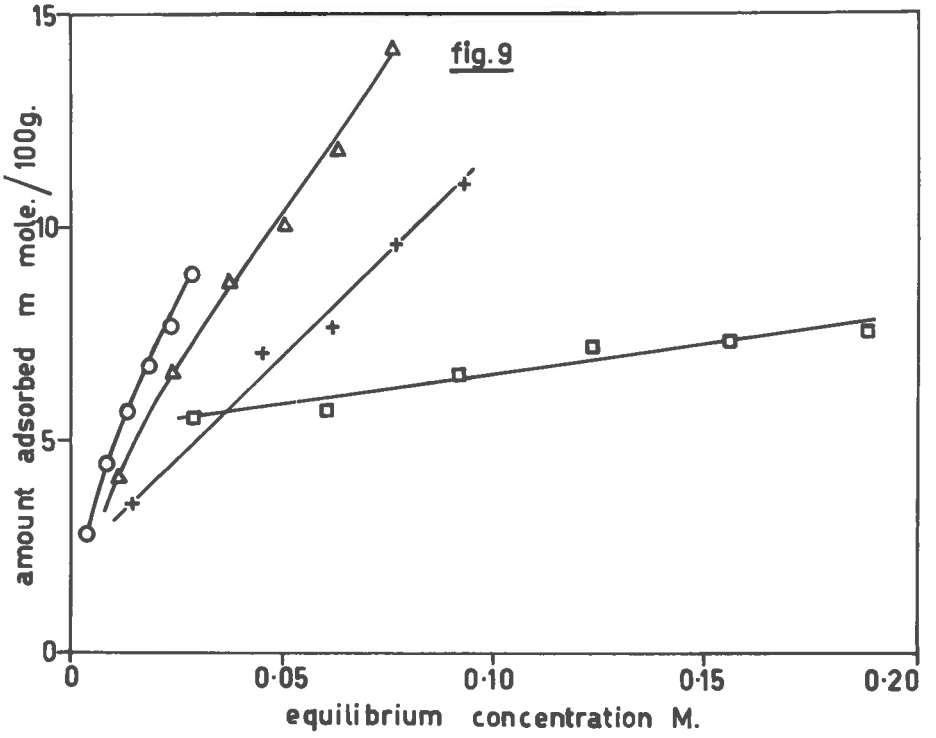


fig.9
amount adsorbed m mole./100g.
15
10
5
0
0
0·05
0·10
0·15
0·20
equilibrium concentration M.

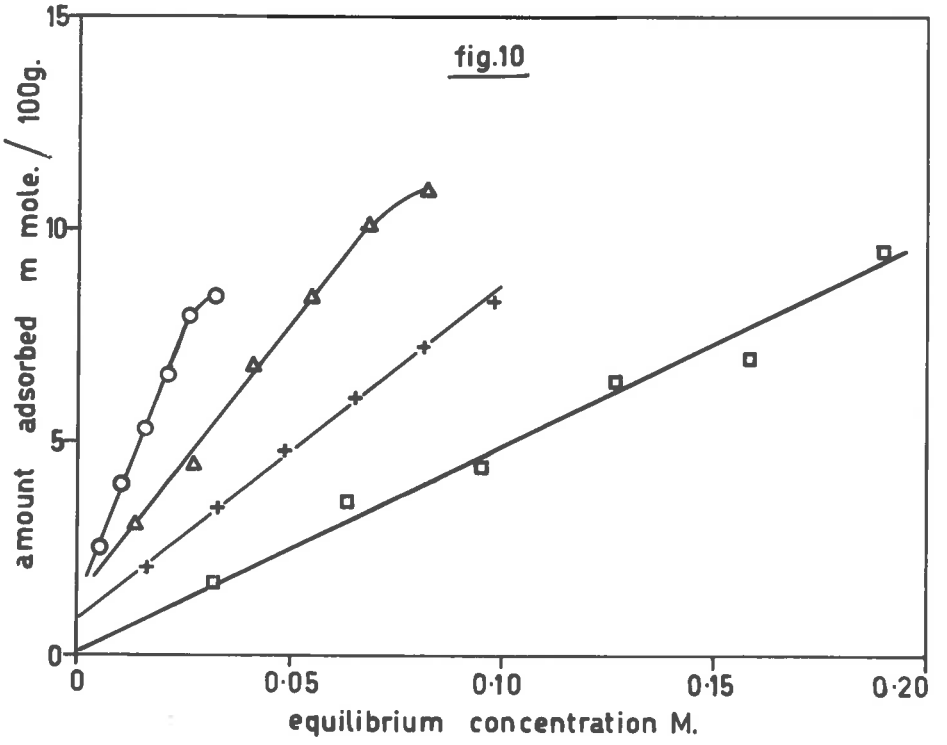


fig.10
amount adsorbed m mole./100g.
15
10
5
0
0
0·05
0·10
0·15
0·20
equilibrium concentration M.

Fig. 11. Adsorption isotherms for α-alanine, +, β-alanine, ×, leucine, ○, and serine, □, on Ca^{++} montmorillonite.

Fig. 12. Adsorption isotherms for α-alanine, +, β-alanine, ×, leucine, ○, and serine, □, on H_3O^+ montmorillonite.

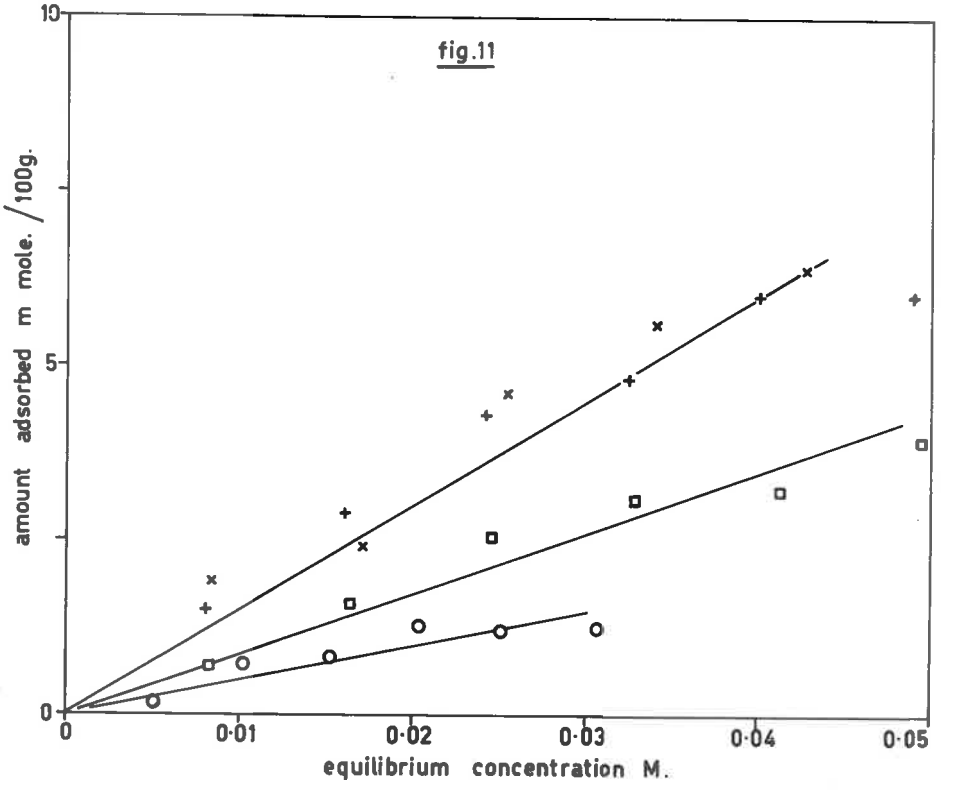


fig.11
10
5
0
amount adsorbed m mole. /100g.
0
0·01
0·02
0·03
0·04
0·05
equilibrium concentration M.

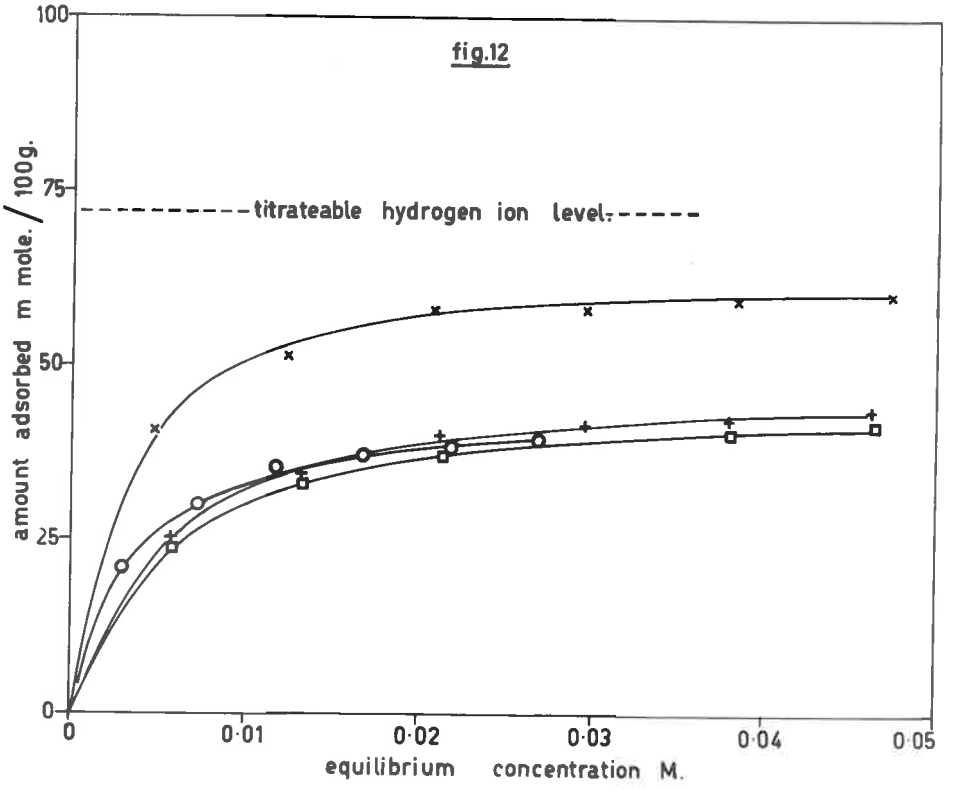


fig.12
100
75
50
25
0
amount adsorbed m mole. / 100g.
titrateable hydrogen ion level.
0
0·01
0·02
0·03
0·04
0·05
equilibrium concentration M.

Fig. 13. Adsorption isotherms for α-alanine, +, β-alanine, ×, leucine, ○, and serine, □, on Na^{+} illite.

Fig. 14. Adsorption isotherms for α-alanine, +, β-alanine, ×, leucine, ○, and serine, □, on Ca^{++} illite.

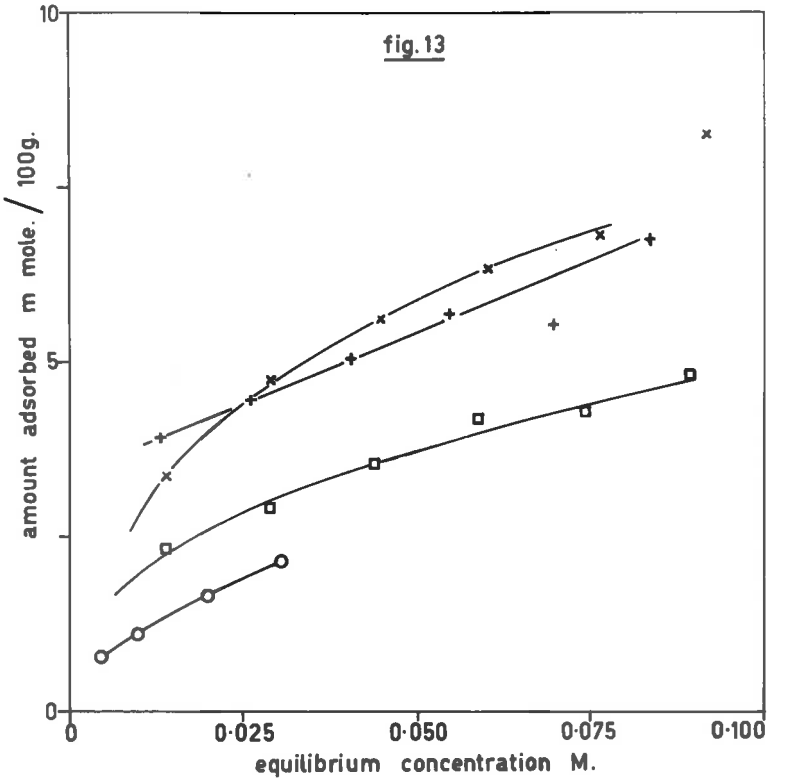


fig. 13
amount adsorbed m mole. / 100g.
10
5
0
0
0·025
0·050
0·075
0·100
equilibrium concentration M.

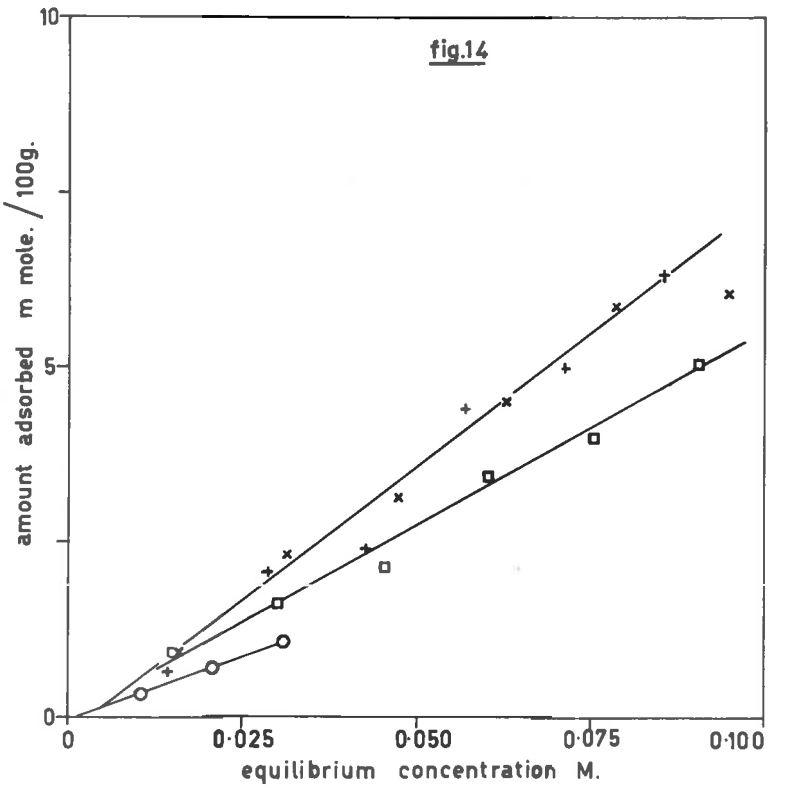


fig.14
amount adsorbed m mole. / 100g.
10
5
0
0
0·025
0·050
0·075
0·100
equilibrium concentration M.

Fig. 15. Adsorption isotherms for aspartic acid, ○, glutamic acid, □, phenylalanine, × and p-aminobenzoic acid, +, on H_3O^+ montmorillonite.

Fig. 16. Adsorption isotherms for DL-arginine, ◎, L-arginine, ○, and glycine, □, cations on Ca^{++} montmorillonite. The amounts of Ca^{++} released by DL- and L-arginine, Δ and by glycine, ■ on adsorption of these cations.

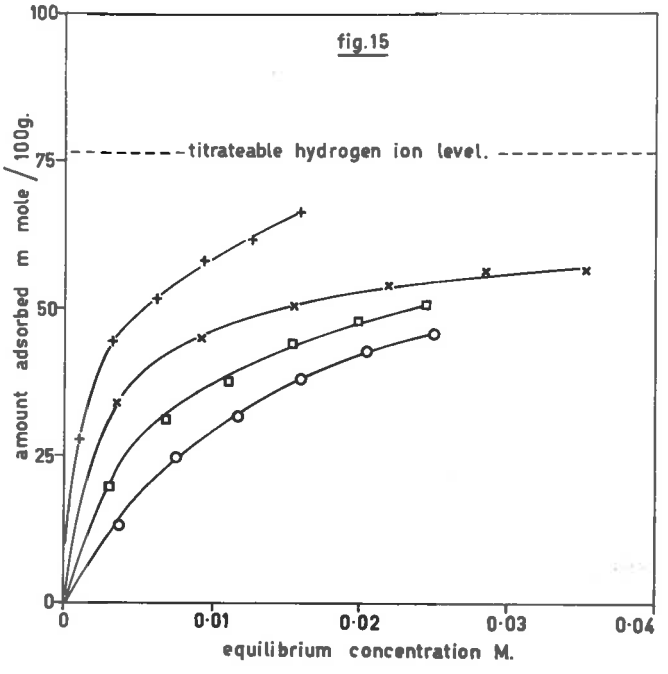


fig.15
titrateable hydrogen ion level.
amount adsorbed m mole / 100g.
100
75
50
25
0
0
0·01
0·02
0·03
0·04
equilibrium concentration M.

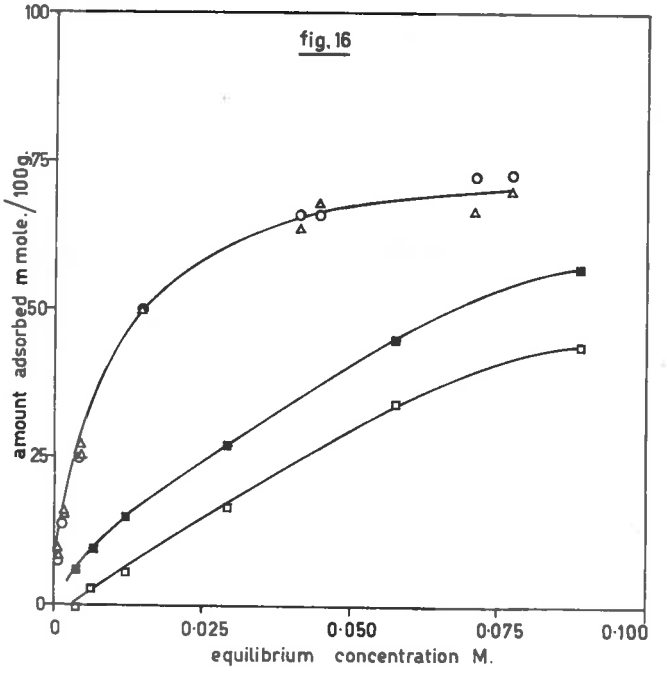


fig.16
amount adsorbed m mole./100g.
100
75
50
25
0
0
0·025
0·050
0·075
0·100
equilibrium concentration M.

Fig. 17. Adsorption isotherms for L-arginine, △, histidine, ○, and lysine, □, cations on Na^+ montmorillonite. The amounts of Na^+ released on adsorption of these cations are shown by the dashed curves.

Fig. 18. Adsorption isotherms for carnosine, Y, and histidine, ○, cations on Na^+ montmorillonite.

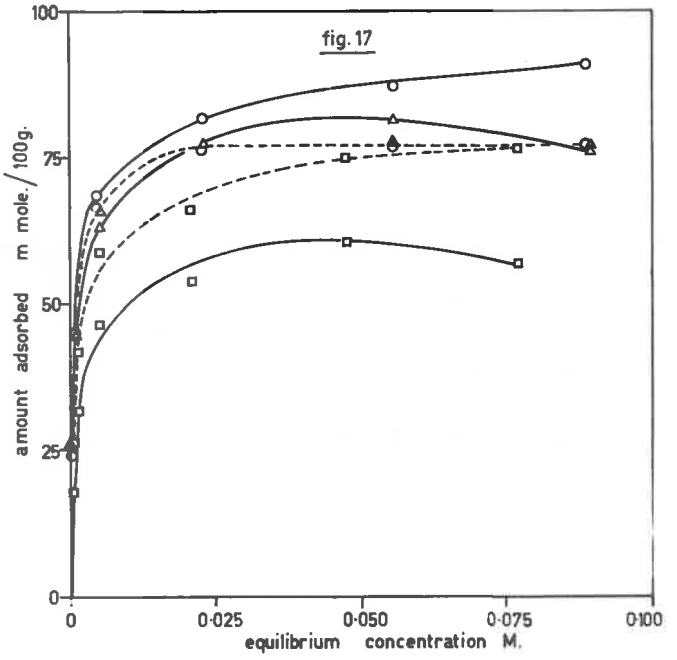


fig. 17
100
75
50
25
0
amount adsorbed m mole./100g.
0
0·025
0·050
0·075
0·100
equilibrium concentration M.

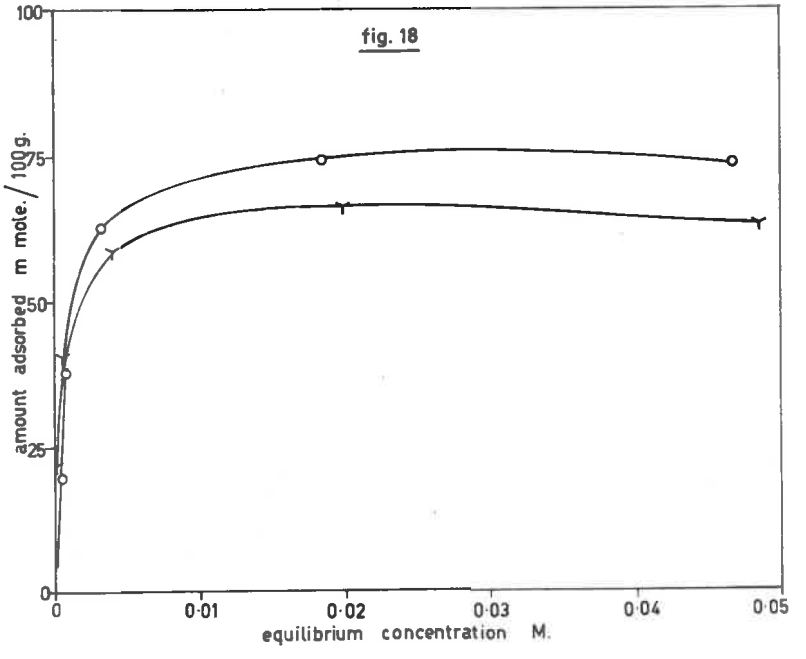


fig. 18
100
75
50
25
0
amount adsorbed m mole./100g.
0
0·01
0·02
0·03
0·04
0·05
equilibrium concentration M.

Fig. 19. Adsorption isotherms for histidine cations on Ca^{++} montmorillonite, □, Ca^{++} illite, ●, and Na^{+} illite, ○, and for carnosine cations on Na^{+} illite, Y.

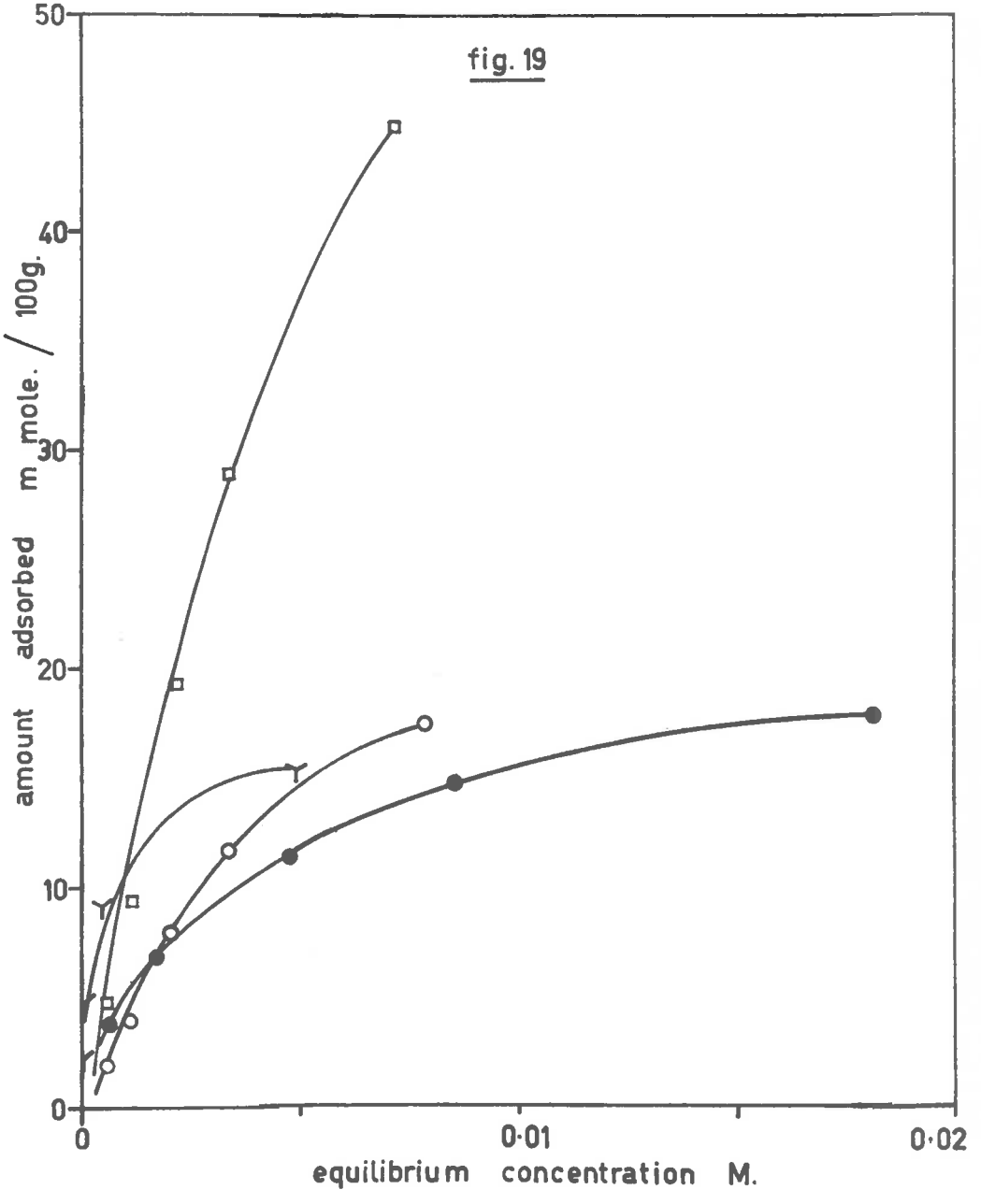


fig. 19
50
40
30
20
10
0
amount adsorbed m mole. / 100g.
0
0·01
0·02
equilibrium concentration M.

i. The method of calculation of experimental results.

This is illustrated by a random example, the adsorption of serine by sodium illite. The arithmetical processes involved in determining the amount adsorbed and the equilibrium concentration from the experimental data are given at the head of the columns in Table 5.

(1) Data used to determine volume of water and weight of clay per tube.

Temperature of suspension	22°C
Mean weight of clay suspension per tube	16.305 ± .002g.
Mean weight of clay per tube, based on drying at 350°C	2.2955 ± .0004g.
Mean volume of water delivered with clay suspension	14.05 ml.
Mean made up volume in each tube	29.05 ml.

(2) Standardisation of serine solution

10.00 ml. solution digested, distilled and titrated against 0.1187 N HCl.

Volume of HCl	15.23, 15.23 ml.
Normality of serine solution	0.1808 N

(3) Determination of equilibrium concentration and amount adsorbed

Six lots of standard serine solution were dispensed into tubes containing clay suspension (column a, Table 5). Volumes were made up to 29.05 ml., equilibrated and centrifuged.

10 ml. portions of the supernatant solution were taken for analysis, 0.02374 N HCl being used in the titration of ammonia distilled (column b). A 10 ml. and a 50 ml. burette were used for titrations below and above 10 ml. respectively.

Table 5

An example of the method of calculating the adsorption isotherms from experimental data. The adsorption of serine by sodium illite.

a	b	c	d	e	f	g
Volume of serine soln. added ml.	Titration volume ml.	Amount of serine added m mole. (a x .1808)	Amount of serine left m mole. (f x 29.05)	Amount of serine adsorbed m mole. (c-d)	equil. conc. $M \times 10^2$ $\left(\frac{b-\text{blank}}{10.00}\right) \times 0.02374$	Amount of serine adsorbed m mole./ 100g. $f \times \frac{100}{2.296}$
2.5	5.80, 5.80	0.4520	0.3983	0.0537	1.37	2.3
5	12.15,12.16	0.9039	0.8372	0.0667	2.88	2.9
7.5	18.45,18.54	1.3559	1.2745	0.0814	4.39	3.5
10	24.83,24.83	1.8078	1.7110	0.0968	5.89	4.2
12.5	31.30,31.42	2.2598	2.1614	0.0984	7.44	4.3
15	37.72,37.75	2.7117	2.6013	0.1104	8.95	4.8
blank	0.02,0.02					

ii. Errors.

The two important sources of error in the adsorption experiment are the dispensing of the standard amino-acid or peptide solution and

the estimation of the equilibrium concentration or the amounts of adsorbate left in the supernatant after adsorption. These together are responsible for the error in the amount of amino-acid, or peptide adsorbed (column e, Table 5):

(amount added - amount left) m mole./tube

(c - d)

The error in the amount adsorbed is reduced by

(1) Increasing the amount of clay per tube.

(2) Increasing the number of nitrogen atoms per solute molecule.

(3) Increased specific adsorption (m mole./g).

Therefore the error is a minimum for the high specific adsorption of arginine (4 N atoms) by calcium montmorillonite (0.9 g/tube) or triglycyl glycine (4 N atoms) by hydrogen montmorillonite (0.1 to 0.2 g/tube) and is a maximum for the low specific adsorption of neutral amino-acids containing 1 N atom per molecule by sodium montmorillonite (0.35 g/tube) or calcium illite (1.1 g/tube). The error proved too great to permit the estimation by this procedure of the adsorption of the less soluble amino acids (aspartic and glutamic acids, phenyl alanine and p-amino benzoic acid) by sodium montmorillonite or calcium illite.

An estimate of the error involved has been obtained statistically by regression analysis of two of the linear isotherms reported, the adsorption of triglycyl glycine by calcium montmorillonite (which, according to the above discussion should be the most accurate of the linear isotherms studied) and adsorption of glycine by calcium illite

(one of the least accurate isotherms studied). The details of the statistical analyses are given in Table 6.

Table 6

Details of the statistical treatment of errors for two linear isotherms the adsorption of triglycyl glycine and of glycine by calcium montmorillonite, and calcium illite respectively.

Adsorbate	Maximum amount adsorbed per tube, m mole.	r.m.s. error of lowest amount adsorbed, %	r.m.s. error of highest amount adsorbed, %	Slope	Standard error in slope	Error in K_m, %
triglycyl glycine	0.308	23	2.9	974.0	15.9	1.6
glycine	0.107	33	5.7	47.0	1.69	3.5

The constant K_m, the error in which is given in the last column of Table 6, is discussed in section 4,i.

Statistical analysis of non-linear isotherms was not attempted. However, a qualitative estimate of the errors involved in each isotherm can be obtained by a comparison of the maximum amount adsorbed for each experiment (given in Appendix IV) with those reported in Table 6 for glycine and triglycyl glycine. In qualitative terms, the errors are inversely related to the maximum amount adsorbed per tube.

Since the equilibrium experiment was performed in a system of three components, adsorption of solute may have resulted in desorption

of solvent which in turn would have diluted the bulk solution. The amount of solute adsorbed as determined by the above procedure is therefore an apparent adsorption. However, at the concentrations involved, the effect of this dilution (2% maximum) may be neglected. Transmission of solution between the surface and the bulk medium occurs during adsorption as a result of swelling (see X-ray data). This effectively prevents any attempt to correct the apparent adsorption by means of the apparent molal volumes of the solutes.

4. Discussion of the Equilibrium Experiments

In the following discussion, the amount of amino-acid or peptide adsorbed by the clays is considered at times in terms of θ, the proportion of the total surface occupied by adsorbate molecules. θ_r is this ratio assuming a rectangular packing of the adsorbed molecules on the clay surface and θ_m is the ratio based on the projected molecular areas of the adsorbed molecules. The method of determining θ values is discussed in detail in Appendix V.

i. The adsorption of neutral amino-acids and peptides by calcium montmorillonite and calcium illite.

a. The significance of the cation exchange reaction

The supernatant solutions for the adsorption of glycine and its peptides by calcium montmorillonite were analysed by E.D.T.A. titration. The method of estimation (Appendix I) is sufficiently sensitive to detect less than 0.01 m equiv./100g released from the exchange sites and the adsorbates did not interfere at the concentrations involved. The maximum amounts of calcium liberated on adsorption of

glycine, glycyl glycine, diglycyl glycine and triglycyl glycine were 0.2, 0.5, 2.0 and 2.0 m equiv./100g respectively and the pH was reduced from 6.6 to a minimum of 5.6. The minimum pH observed on adsorption of each of these compounds is sufficiently close to the iso-electric pH for the concentration of the cationic form to be very low. For example, it can be shown that at pH 5.6, less than 0.5% of the total amount of diglycyl glycine in solution is present in the cationic form, corresponding to a maximum equilibrium concentration of 6×10^{-4}M. The hydrogen ion concentration at pH 5.6 is 2.5×10^{-6}M. Therefore the calcium is probably displaced from the surface by the cationic form of the amino-acid or peptide, and to a lesser extent by hydrogen ions. The maximum amount of glycine and its peptides adsorbed by cation exchange is between 2 and 5% of the total adsorption. This amount of cation exchange is probably responsible, at least in part, for the observation that most of the linear isotherms obtained for adsorption of the glycine peptides by calcium clays in the present work do not quite pass through the origin.

b. <u>The significance of isotherm linearity for adsorption by calcium clays</u>.

Inspection of the relevant isotherms reveals that their most obvious feature is their linearity. The adsorption isotherms for diglycyl glycine and triglycyl glycine (Figs. 5,8,10) are linear up to 25 and 40 m mole./100 g (θ_r = 0.18 and 0.34) respectively for calcium montmorillonite and up to 10 and 8 m mole./100g (θ_r = 0.48 and 0.47) respectively for calcium illite. The magnitude of these values of θ_r definitely excludes the possibility that Figs. 5, 8 and 10 are the linear

initial slope portions of more extensive isotherms of, for example, a Langmuir type, represented by:

$$p(\text{ or } c) = K_4 \frac{\theta}{1-\theta}$$

Langmuir isotherms are approximately linear at very low surface coverages when $(1-\theta) \simeq 1$ (Olivier and Ross, 1957). The isotherms for the remainder of the solutes studied, glycine and glycyl glycine (Figs. 5,7,10) and α-alanine, β-alanine, leucine and serine (Figs. 11, 14) are linear over the whole of the range of concentrations studied. However, since the maximum values of θ_r are lower and the errors are higher for these adsorptions, the possibility that the isotherms approximate to a Langmuir type cannot be excluded on the basis of θ_r values alone. Further and better reasons are given later for describing these isotherms as truly linear and therefore all the adsorptions of neutral amino-acids are considered together in the following discussion.

When a linear isotherm is observed in practice, one of two situations apply;

(1) The adsorption is very weak and $(1-\theta) \simeq 1$

(2) Experimental conditions (rather rarely observed) exist which effectively allow the total number of sites to remain constant as adsorption progresses. Provided that molecular interactions are unimportant, both mobile (Volmer equation) and immobile (Langmuir equation) adsorption models yield linear isotherms under these conditions (Olivier and Ross, 1957).

The discussion above applies to adsorption from solution if a

concentration term is substituted for the relative pressure, provided that adsorption of solute can result in desorption of solvent.

In their extensive survey of adsorption from solution, Giles et al., (1960) classify linear isotherms as C type isotherms (which, strictly speaking, are linear right up to their maximum possible adsorption). The basic requirement of a C type isotherm is that the number of equal energy sites does not diminish as sorption progresses (2 above). To satisfy this, either (i) the solute, but not the solvent, must act as a swelling agent for the clay (that is the solute molecules "create" the space that they occupy) or (ii) successive layers of adsorbed solute molecules must be adsorbed with equal energy (Giles, 1954; Giles et al., 1960). This second alternative is less likely, particularly with a clay substrate where it has already been shown that the properties of adsorbed water vary to a marked extent with increasing water content.

On the other hand, the first alternative (i) is supported by the swelling data of Barshad (1952) and Walker and Garrett (1961). Since their X-ray data is not accompanied by adsorption isotherms, it is not known whether or not the observed swelling is sufficient to accomodate the adsorbed solute.

In the present work, the basal spacing (determined by X-ray diffraction) of moist montmorillonite complexes obtained directly from the separation stages of equilibrium experiments involving the adsorption of glycine and its peptides have been used to determine the increase in interlamellar volume (cm^3/g clay). These have been compared with the increase in solute volume obtained from the apparent molal volume of the

solutes and the amounts adsorbed. The results of these calculations for the upper limits of isotherm linearity are given in Table 7.

Table 7

The calculated solute volumes and increase in water volumes of the interlamellar space of calcium montmorillonite on adsorption of glycine and its peptides at the upper limits of isotherm linearity.

a	b	c	d	e	f
solute	Apparent molal volume of solute+	Amount of solute adsorbed (from isotherms)	Interlamellar volume increase, $\frac{d(001)-19}{2} \times$ surface area $\times 10^{-4}$	Interlamellar solute volume, $\frac{b \times c}{1000}$	Increase in interlamellar water vol. on solute adsorption, (d-e)
	cm^3/mole.	m mole/g	cm^3/g	cm^3/g	cm^3/g
glycine	44	0.15	0.016	0.007	0.009
glycyl glycine	77	0.11	0.035	0.009	0.026
diglycyl glycine	114	0.22	0.109	0.028	0.081
triglycyl glycine	170	0.33	0.035	0.068	-0.033

+ apparent molal volumes were obtained from the literature (Cohn and Edsall, 1943) except for triglycyl glycine when it was claculated from the density of the saturated solution (Appendix VI).

The last three columns of Table 7 show that the observed swelling is more than adequate to accomodate the solute molecules up to the limit of isotherm linearity for all adsorbates except triglycyl glycine

when only about one half of the adsorbed molecules can be accomodated by the increase in interlamellar volume.

Thus, mechanism (i) above, proposed to satisfy the basic requirement of a linear isotherm, applies to the present system in rather a unique manner, since the solute induces additional swelling of the substrate by the solvent.

An explanation of isotherm linearity has been proposed for the adsorption of neutral amino-acids and peptides by calcium montmorillonite. However, it is of no value at this stage in determining the nature of the bonding forces involved on adsorption of solute and in addition it does not apply directly to the linear isotherms observed in the case of adsorption by calcium illite where interlamellar adsorption is probably small (30% or less of the total).

Therefore it is necessary to investigate the adsorption data in a more quantitative manner in an endeavour to solve these problems.

c. Linear isotherms in terms of constant partition

Giles et al., (1960) discussed C isotherms in terms of a constant partition of solute between solution and substrate right up to the maximum of adsorption. This is a useful concept, particularly in a three component system where there is an adsorbed layer of solvent. The solute can then be considered as being partitioned between the bulk and adsorbed phase solvents. The partition constant, which is the equilibrium constant for the reaction:

$$\text{solute}_{(\text{bulk})} \rightleftharpoons \text{solute}_{(\text{adsorbed})}$$

is given by:

$$K = \frac{[\text{solute}]_{\text{adsorbed}}}{[\text{solute}]_{\text{bulk}}}$$

An arbitrary depth must be assigned to the adsorbed layer in order to obtain a value for the concentration of solute in this layer ($[\text{solute}]_{\text{adsorbed}}$).

This concept is useful for the additional reason that it eliminates the necessity of discussing adsorption in terms of specific sites. In a three component system, the sites are all occupied by solvent molecules in the first instance and adsorption of solute is usually accompanied by desorption of solvent. However, in the present system, the adsorption by montmorillonite is further complicated by movement of water into the adsorbed phase on the intercalation of glycine and its di- and tri-peptides as a result of swelling, or away from the adsorbed phase when the tetrapeptide is intercalated, since swelling is to some extent restricted. The mathematical consideration of the adsorption process is therefore greatly simplified if sites can be ignored in the early stages of the discussion.

In gas-liquid chromatography, a partition as described above clearly applies to the distribution of the volatile species between the gas phase and the stationary liquid phase (which corresponds to the adsorbed layer of solvent above) and in equilibrium studies of gas-liquid chromatography systems, the isotherms that are produced are almost always linear and are termed partition isotherms, (Freiguard and Stock, 1961). Partition constants are readily determined because

there is no doubt about the distinction between the two phases and also, the volume of the adsorbed phase solvent is exactly known, being equal to the volume of liquid added to the inert solid supporting medium to produce the stationary liquid phase.

A description of the adsorption of amino-acids and peptides by calcium clays in terms of a constant partition of solute is not as clearly defined as in gas-liquid chromatography. Nevertheless the application of partition mathematics is equally valuable.

Amino-acids and peptides, being generally more soluble in aqueous salt solutions than in pure water, would also be more soluble in the water that contains the cations associated with the clay and which may be regarded as an electrolyte solution. The volume of water in which the interlamellar ions occur in a calcium montmorillonite suspension is readily obtained from the basal spacing of the fully expanded clay (19 $\mathring{A}$) and the specific surface area (725 m^2/g, see appendix V). The interlamellar thickness of water is 19-9.5 = 9.5 $\mathring{A}$ and there is $9.5/2 = 4.75$ $\mathring{A}$ of this water thickness associated with each surface. This value is close to the Stern layer thickness of 5 $\mathring{A}$ proposed by Verwey and Overbeek (1948) and for convenience of discussion the amino-acids and peptides are considered to form a solution in the Stern layer water, the volume of which is taken as $5 \times 725 \times 10^{-4} = 0.36$ cm^3/g of calcium montmorillonite. The calcium ions of the external surfaces of montmorillonite and illite in suspension are not confined to the Stern layer, but form a diffuse layer of greater thickness in which the Ca^{++} will decrease with distance from the surface. However, up to 80% of the external exchangeable calcium

is probably within 5 Å of the surface (Quirk, private discussion) and the adsorbed amino-acids and peptides are therefore assumed to be in solution in the Stern layer of calcium illite the volume of which is taken as $5 \times 106 \times 10^{-4} = 0.053$ cm^3/g. This volume may be toosmall by up to 30%, since the nitrogen surface area of 106 m^2/g has been used rather than the total surface area (150 m^2/g) determined by cetyl pyridinium bromide adsorption.

The adsorption of amino-acids and peptides by calcium montmorillonite and calcium illite in terms of partition of solute between the bulk and Stern layer solvents is therefore expressed by the equilibrium:

$$RH^{\pm}_{bulk} \rightleftharpoons RH^{\pm}_{Stern}$$

and the equilibrium constant is:

$$K_a = \frac{a_{Stern}}{a_{bulk}} = \frac{\left[RH^{\pm}\right]_{Stern}}{\left[RH^{\pm}\right]_{bulk}} \cdot \frac{\gamma_{Stern}}{\gamma_{bulk}}$$

$$= K_m K_\gamma$$

where $RH^{\pm}$ is the abbreviated symbol for the dipolar ionic structure of the amino-acids or peptides and K_m and K_γ are the mass action and the activity coefficient quotients respectively.

The standard free energy for this equilibrium is :

$$-\Delta G^\circ = RT \ln K_a$$

the standard being unit activity of the solute in both bulk and Stern layer solutions.

In so far as K_m is constant, K_γ is also constant and

$$-\Delta G^\circ = -\Delta G^m + C$$

The standard free energy of physical adsorption by calcium clay is discussed in terms of $-\Delta G^m$.

The applicability of thermodynamic calculations to adsorption systems has been discussed by Barrer and Kelsey (1961). They concluded that at least qualitatively correct interpretations of prominent physical aspects should be obtained. For their particular two component system of hydrocarbon adsorption by alkyl-ammonium montmorillonite, no other procedure could be used with comparable qualitative success. The work of Cowan and White (1958) is another example of the successful application of thermodynamic calculations to adsorption (of n-alkylammonium ions) by montmorillonite from aqueous solution.

The calculated values of K_m and $-\Delta G^m$ for the adsorption of the amino-acids and peptides by both calcium montmorillonite and calcium illite ~~are~~, presented in Table 8 and Figs. 20 and 21, show the variation in $-\Delta G^m$ with molecular weight for the adsorption by each of these substrates respectively.

Fig. 20. The variation in $-\Delta G^m$ with molecular weight for the adsorption of amino-acids and peptides by Ca^{++} montmorillonite.

Fig. 21. The variation in $-\Delta G^m$ with molecular weight for the adsorption of amino-acids and peptides by Ca^{++} illite. The fine line below the points for adsorption by illite is the reference line from Fig. 20 (adsorption by montmorillonite) and is included for comparison.

Fig. 21a. The variation in $-\Delta G^m$ with δ for the adsorption of glycine and its peptides by Ca^{++} montmorillonite.

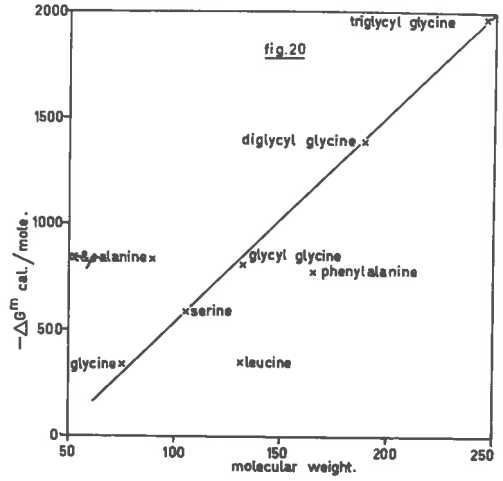


fig.20
triglycyl glycine
diglycyl glycine
glycyl glycine
phenylalanine
serine
glycine
leucine
2000
1500
1000
500
0
50
100
150
200
250
$-\Delta G^m$ cal./mole.
molecular weight.

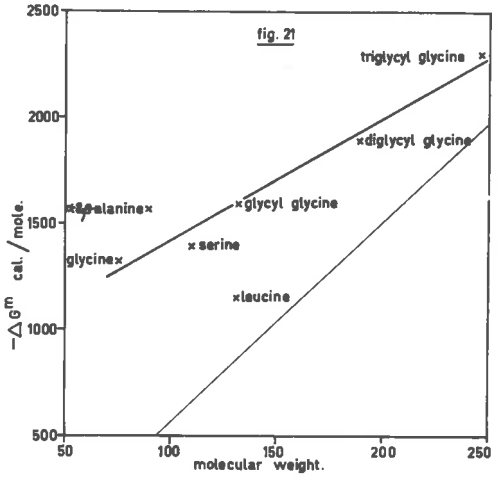


fig. 21
triglycyl glycine
diglycyl glycine
glycyl glycine
serine
glycine
leucine
2500
2000
1500
1000
500
50
100
150
200
250
$-\Delta G^m$ cal./mole.
molecular weight.

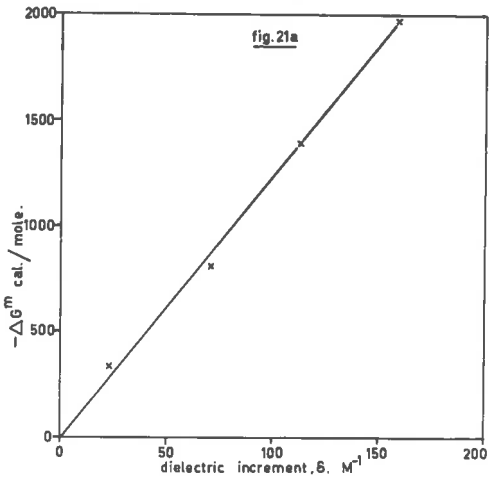


fig.21a
2000
1500
1000
500
0
50
100
150
200
$-\Delta G^m$ cal./mole.
dielectric increment, δ. M^{-1}

Table 8

Dielectric increments (δ) and the mass action quotients (K_m) and free energies ($-\Delta G^m$) for physical adsorption of amino-acids and peptides by calcium montmorillonite and calcium illite.

Solute	Molecular weight	δ M^{-1}	Calcium montmorillonite		Calcium illite	
			K_m	$-\Delta G^m$ cal./mole.	K_m	$-\Delta G^m$ cal./mole.
glycine	75	23	1.4	175	9.3	1320
glycyl glycine	132	71	5.0	930	14.5	1590
diglycyl glycine	189	113	9.6	1320	24.3	1890
triglycyl glycine	246	159	23.3	1830	48.5	2300
α-alanine	89	23	4.1	837	14.3	1570
β-alanine	89	35	4.1	837	14.3	1570
leucine	131	25	1.8	342	6.9	1150
serine	105	ca 23	2.4	509	10.4	1390

To determine the average enthalpy change and the entropy change the adsorption of glycine and its peptides by calcium montmorillonite was repeated at 0°C and 25°C (Appendix IV nos. 4 and 5). ΔH is given by the van't Hoff equation:

$$\log_{10} \frac{K_m^0}{K_m^{25}} = \frac{-\Delta H}{4.576} \left(\frac{1}{273.2} - \frac{1}{298.2} \right)$$

Table 8

Dielectric increments (δ) and the mass action quotients (K_m) and free energies ($-\Delta G^m$) for physical adsorption of amino-acids and peptides by calcium montmorillonite and calcium illite.

Solute	Molecular weight	δ M^{-1}	Calcium montmorillonite		Calcium illite	
			K_m	$-\Delta G^m$ cal./mole.	K_m	$-\Delta G^m$ cal./mole.
glycine	75	23	1.4	175	9.3	1320
glycyl glycine	132	71	5.0	930	14.5	1590
diglycyl glycine	189	113	9.6	1320	24.3	1890
triglycyl glycine	246	159	23.3	1830	48.5	2300
α-alanine	89	23	4.1	837	14.3	1570
β-alanine	89	35	4.1	837	14.3	1570
leucine	131	25	1.8	342	6.9	1150
serine	105	ca 23	2.4	509	10.4	1390

To determine the average enthalpy change and the entropy change the adsorption of glycine and its peptides by calcium montmorillonite was repeated at 0°C and 25°C (Appendix IV nos. 4 and 5). ΔH is given by the van't Hoff equation:

$$\log_{10} \frac{K_m^0}{K_m^{25}} = \frac{-\Delta H}{4.576} \left(\frac{1}{273.2} - \frac{1}{298.2} \right)$$

and ΔS is obtained from:

$$\Delta G^m = \Delta H - T\,\Delta S$$

The results are given in Table 9 and the variation in ΔH and ΔS with molecular weight is given in Figs 22 and 23 respectively.

Table 9

The mass action quotients, free energies, enthalpies and entropies of physical adsorption of glycine and its peptides by calcium montmorillonite.

Solute	K_m(0°C)	K_m(25°C)	$-\Delta G^m$(0°C) cal./mole.	$-\Delta G^m$(25°C) cal./mole.	$-\Delta H$ cal./mole	$-\Delta S$ E.U.
glycine	2.28	1.77	447	339	1660	4.4
glycyl glycine	5.42	3.61	917	760	2640	6.3
diglycyl glycine	14.5	10.5	1450	1390	2050	2.2
triglycyl glycine	34.3	27.9	1920	1970	1340	-2.1

The experimental errors in the values of K_m probably fall between 1.6 and 3.5% (see Table 6 and section 3, ii)

d. The validity of partition mathematics as applied to the present system.

Giles et al. (1960) concluded that the application of partition

Fig. 22. The variation in $-\Delta H$ with molecular weight for the adsorption of glycine and its peptides by Ca^{++} montmorillonite.

Fig. 23. The variation in $-\Delta S$ with molecular weight for the adsorption of glycine and its peptides by Ca^{++} montmorillonite.

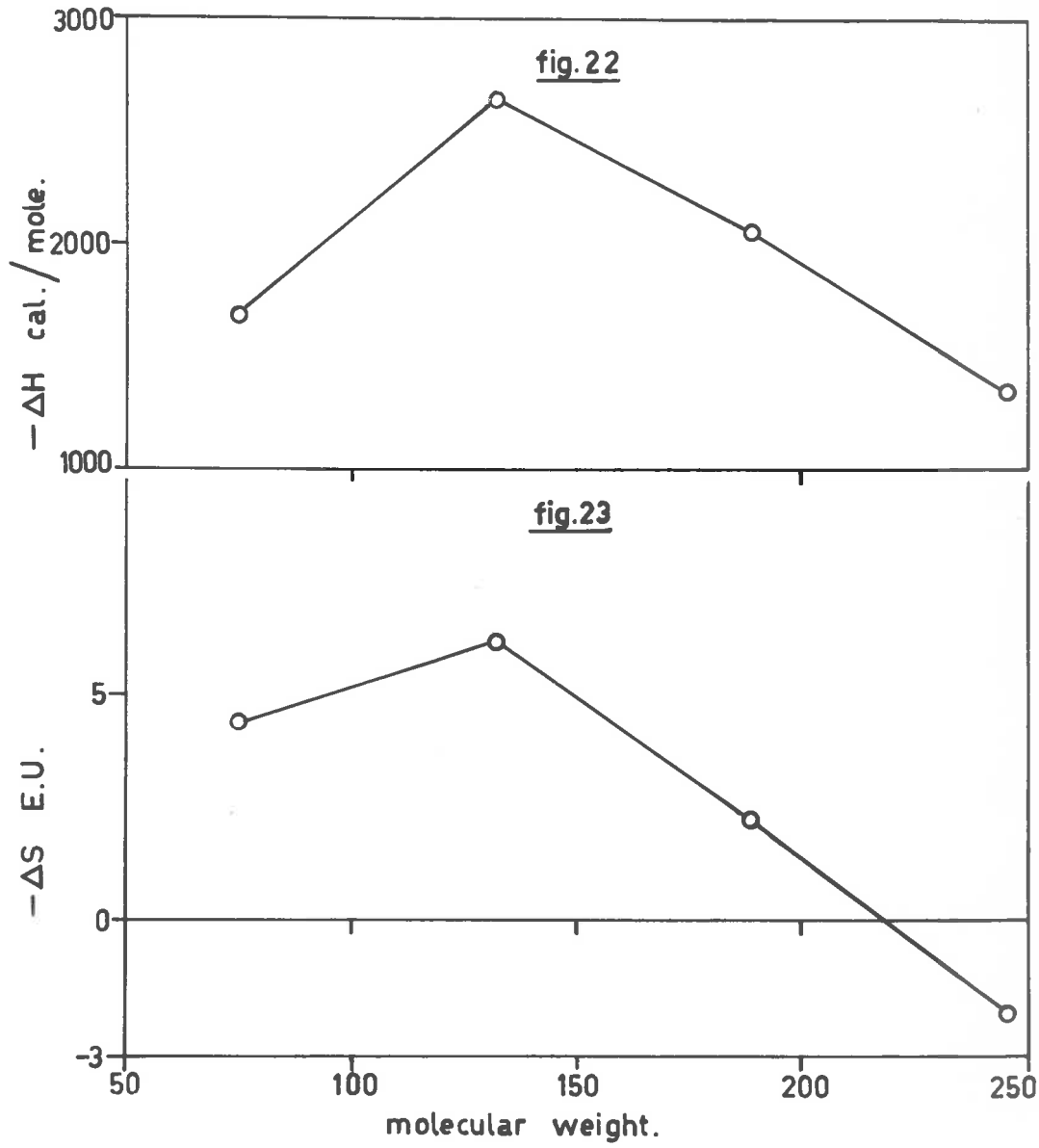


fig. 22
3000
2000
1000
−ΔH cal./mole.
fig. 23
5
0
-3
−ΔS E.U.
50
100
150
200
250
molecular weight.

mathematics to adsorption from aqueous solution allows useful correlations to be made between surface-solute affinity and molecular structure in some cases, but the real significance of the adsorbed solvent volume term is doubtful. In the present work, the three following experimental relationships provide evidence that the volume in which solute is adsorbed corresponds to the volume in which the exchangeable calcium occurs. As a result partition mathematics in its present application is valid:-

(1) The relationship between $-\Delta G^m$ and the dielectric increment of the adsorbate

Most amino-acids and peptides are more soluble in aqueous salt solutions than in pure water. This is closely related to the fact that the dielectric constant of an amino-acid or peptide solution is greater than that of pure water. The dielectric constant of these solutions is a linear function of the solute concentration (Cohn and Edsall, 1943).

$$D = D_o + \delta C$$

D = the dielectric constant of the amino-acid or peptide solution

D_o = the dielectric constant of water (80 at 20°C)

δ = the dielectric increment

C = the amino-acid or peptide concentration, mole./l.

The dielectric increment is positive for dipolar ions and negative for electrolytes and most other water-soluble organic compounds. It has been shown theoretically that the activity coefficients of each solute in a solution of a single non-electrolyte and a single

electrolyte are given by the relationships:

$$\ln\gamma_{salt} = \left(\frac{N\varepsilon^2 z_+ z_- \delta_n}{2RTb_s D_o^2 \rho_o}\right) m_n \qquad (1)$$

$$\ln\gamma_n = \left(\frac{N\varepsilon^2 z_+ z_- \delta_n}{2RTb_s D_o^2 \rho_o}\right) \nu\, m_{salt} \qquad (2)$$

(Falkenhagen, 1934; Cohn and Edsall, 1943).

γ_{salt} and γ_n = the activity coefficient of the salt and non-electrolyte respectively.

ε = the electronic charge.

N = Avogadro's number.

z_+ & z_- = the valency of the positive and negative ions respectively.

δ_n = the dielectric increment of the non-electrolyte.

b_s = a mean radius term for the salt.

ρ_o = the density of the solvent.

ν = the number of ions formed by the dissociation of one molecule of salt.

D_o = the dielectric constant of pure water.

m_{salt} and m_n = the molality of the salt and non-electrolyte respectively.

R and T = the gas constant and absolute temperature.

If δ_n is negative, then $\ln\gamma_n$ in equation (2) is positive since, of the other terms, only z_- is negative. $\ln\gamma_n$ increases with salt concentration and therefore the solubility of the non-electrolyte decreases with the addition of electrolyte. This is the commonly observed phenomenon of salting out. The reverse is true if δ_n is positive.

It is shown below that the standard free energy of physical adsorption ($-\Delta G^m$) determined from the partition constant is a direct linear function of the dielectric increment δ_n. There are three steps to this proof:

(i) From equation (2) $-\ln\gamma_n$ is a direct linear function of the dielectric increment δ_n for constant salt concentration.

$$-\ln\gamma_n = B m_{salt} \delta_n$$

$$\text{where } B = \frac{N\varepsilon^2 z_+ z_- \nu}{2RTb_s D_o^2 \rho_o}$$

(ii) By definition

$$-\ln\gamma_n = \ln S/S_o$$

where S = the solubility of the non-electrolyte in the salt solution

S_o = the solubility of the non-electrolyte in pure water where its activity coefficient is taken as unity (Schmidt, 1938).

(iii) The partition constant for the distribution of a solute between two immiscible solvents is approximately equal to the ratio of the solubilities in these two solvents (Glasstone, 1946).

For the present system:

$$K_m \cong S/S_o$$

Combining these three steps:

$$\begin{aligned} -\Delta G^m &= RT \ln K_m \\ &\cong RT \ln S/S_o \\ &= -RT \ln \gamma_n \\ &= RTB\, m_{salt} \delta_n \end{aligned}$$

Therefore, provided no change occurs in the distribution of the exchangeable cations with respect to the clay surface as adsorption proceeds, $-\Delta G^m$ is a linear function of δ_n, positive adsorption resulting from a positive dielectric increment.

Figure 21a shows the experimental relationship between $-\Delta G^m$ for the adsorption of glycine and its peptides by calcium montmorillonite and the dielectric increment of each adsorbate. A linear relationship is obtained and both $-\Delta G^m$ and δ are linear functions of the molecular weight (Figs. 20 and 21, and Cohn and Edsall, 1943, respectively).

In practice, there are deviations from this general theoretical relationship partly due to the fact that equation (2) which relates the activity coefficient and the dielectric increment of a non-electrolyte in an aqueous salt solution is over-simplified, since it does not include contributions due to solute and solvent separation by the electrostatic field of the ions or other dipolar interactions. In particular, the solubility (and therefore the activity coefficient)

of an amino-acid in an aqueous salt solution is determined by a combination of a "salting-in" effect due to the ion-dipole interactions determined by a positive dielectric increment and described by equation (2), and a salting-out effect due to the presence of organic groups (in particular hydrocarbon side-chains). This is clearly shown by the solubility studies of Pfeiffer and Würgler (1916) where the solubilities of glycine, aspartic acid and leucine (δ = 23, 28 and 25 respectively) were studied in solutions of sodium chloride, potassium chloride and calcium chloride. Although they all have approximately equal positive dielectric increments, aspartic acid and glycine, which carry large electric charges and small hydrocarbon residues are further dissolved by these salts while leucine, with its hydrocarbon side-chain is salted out by sodium and potassium chlorides (See Schmidt, 1938).

(2) The relationship between $-\Delta G^m$ and the solubility ratio S/S_o

If partition mathematics apply to the present system, then:

$$K_m \simeq S/S_o$$

where S is the solubility of the adsorbate in the volume of water containing the exchangeable cations, which for calcium clays is taken as the Stern layer.

The solubility of glycine and its peptides has been determined in water and in a calcium chloride solution in which the $\left[Ca^{++}\right]$ is made equal to that calculated for the Stern layer of montmorillonite.

For the experimental details and results of these solubility experiments, see Appendix VI.

The value of $\log_{10} S/S_o$ is calculated from this data for each solute and plotted against the molecular weight in Fig. 24. A linear relation is obtained for the peptides but the values of $\log_{10} S/S_o$ are well below the corresponding values of $\log_{10} K_m$ as shown in this figure.

Both the values of K_m and $[Ca^{++}]_{Stern}$ depend equally upon the calculated value of the adsorbed phase water volume and hence an incorrect estimate of this volume does not markedly effect the observed difference between corresponding values of $\log_{10} S/S_o$ (where S is an approximately linear function of $[Ca^{++}]$ of the $CaCl_2$ solution used) and $\log_{10} K_m$.

A more important criticism of the interpretation of this data is that it is the ionic strength and not simply the concentration of an electrolyte solution which determines the value of S. It is difficult to assign an ionic strength to the Stern layer since the extent to which the negatively charged alumino-silicate layers influence the ionic strength is unknown. However, it is probable that the ionic strength of the Stern layer is less than that of a $CaCl_2$ solution of equivalent $[Ca^{++}]$ and therefore the quantity $\log_{10} S/S_o$ determined with this solution would perhaps be expected to show a closer agreement with $\log_{10} K_m$ than is illustrated in Fig.24. However, the observed values clearly illustrate that the Stern layer is a far more effective solvent for glycine and its peptides than the "equivalent" $CaCl_2$ solution. This is supported by comparison of the solubility data

Fig. 24. The variation in $\log_{10} S/S^{o}$, △ $\log_{10} K_m$, O , and $\log_{10} K_m - \log_{10} S/S^{o}$, □ , with molecular weight for glycine and its peptides.

Fig. 25. The increase in the equilibrium basal spacing of moist Ca^{++}montmorillonite, (d(001)-19)Å with increase in the dielectric constant of the interlamellar solution, given by $\delta \cdot [RH^{\pm}]_{Stern}$, for the adsorption of glycine, □ , glycyl glycine, × , diglycyl glycine, △ , and triglycyl glycine, O .

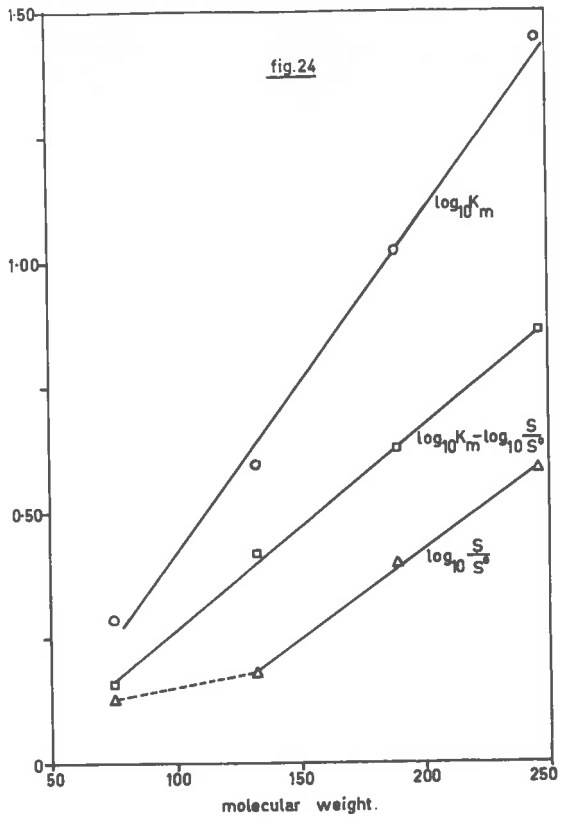


fig.24
1·50
1·00
0·50
0
50
100
150
200
250
molecular weight.
$\log_{10} K_m$
$\log_{10} K_m - \log_{10} \frac{S}{S^b}$
$\log_{10} \frac{S}{S^a}$

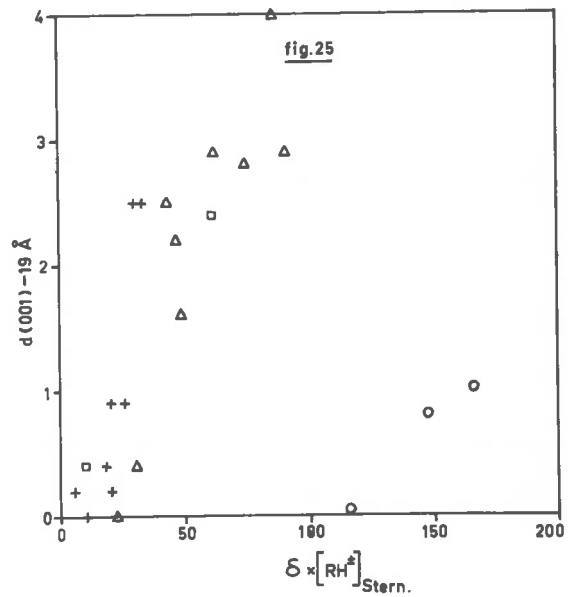


fig.25
4
3
2
1
0
0
50
100
150
200
d(001) − 19 Å
$\delta \times [RH^{\neq}]_{Stern.}$

of Pfeiffer and Würgler (1916) with the adsorption data of the present work for glycine and leucine. The values of $\log_{10}K_m$ for the adsorption of these amino-acids by both calcium montmorillonite and calcium illite are more than twice the corresponding values of $\log_{10}{}^{S}/_{S_o}$.

By tentatively assuming that $RT \ln {}^{S}/_{S_o}$ represents an upper limit to the exchangeable ion-dipole contribution to $-\Delta G^m$ it is seen that other interactions (in particular, surface-dipole interactions and van der Waals forces) represented by the difference ($\log_{10}K_m - \log_{10}{}^{S}/_{S_o}$) in Fig. 24 are at least as important as ion-dipole interactions. Probably the effects of exchangeable ion-dipole and surface-dipole interactions cannot be experimentally differentiated, because of their common dependence upon the dipole moment of the adsorbate.

(3) The relationship between $-\Delta G^m$ and the surface density of charge.

A comparison between Figs. 20 and 21 and between columns 5 and 7 of Table 8 shows that $-\Delta G^m$ is greater for the adsorption of each solute by calcium illite than by calcium montmorillonite. The free energies given in Table 8 for adsorption by illite have been determined using the external surface area. The use of the total (external and internal) area yields $-\Delta G^m$ values 205 cal./mole. less than those quoted, but which are still greater than the corresponding free energies for montmorillonite. The important difference between these two substrates is that illite has more than twice the surface density of charge (as determined by using the external surface area) and therefore a Stern layer $\left[Ca^{++}\right]$ more than twice that of montmorillonite (5.67 N cf. 2.49 N). It would therefore be expected to have higher free energies of

physical adsorption as determined from the partition equilibrium constant. The concentration of calcium ions in the adsorbed phase of illite based on the total surface area is 4.00 N. The illites generally have surface densitites of charge closer to the micas (1 charge per 45 $\mathring{A}^2$) than the montmorillonites (1 charge per 120 to 140 $\mathring{A}^2$). The differences in $-\Delta G^m$ values observed in the present instance between the sample of illite used and montmorillonite is therefore probably a general phenomenon. With adsorbates which have a negative dielectric increment, the free energy of physical adsorption may be expected to be less for illites than for montmorillonites, due to the increased importance of the salting-out effect for illite suspensions.

e. <u>The contribution of dipolar interactions, van der Waals forces and the salting-out effect of hydrocarbon groups to the physical adsorption of amino-acids and peptides.</u>

It has been shown that the adsorption of amino-acids and peptides is determined by three molecular properties:

(1) The dielectric increment δ (or the dipole moment, which is related to δ) which determines the magnitude of the exchangeable ion-dipole and surface-dipole interactions.

(2) The molecular weight, which largely determines the magnitude of van der Waals forces.

(3) The salting-out effect of hydrocarbon side-chains.

The significance of each of these properties can be seen by a consideration of the free energies of physical adsorption for each of the amino-acids and peptides studied (Figs. 20 and 21).

Glycine and its peptides substantiate experimentally the theoretical linear relationship between the free energy of physical adsorption and the dielectric increment because the successive addition of a glycyl- peptide residue to glycine maintains a structural similarity for the series which prevents marked changes in the magnitude of the salting-out effect with increasing molecular weight. Therefore the lines joining the points for glycine and its peptides in Figs. 20 and 21 are used as references to which the free energies of adsorption of the remaining amino-acids can be compared.

The points for leucine and phenyl alanine lie below this line, showing the influence of the salting-out effect of the hydrocarbon groups on their physical adsorption. The solubility data of Pfeiffer and Würgler (1916) show that the value of $\log_{10} S/S_o$ for glycine is nearly double that for leucine for a 2.49 N $CaCl_2$ solution whereas the free energies of physical adsorption of glycine and leucine by both montmorillonite and illite are approximately equal. This suggests that the salting-out effect of the iso-butyl group of leucine is approximately compensated for by the increase in van der Waals forces. All α-amino-acids have dielectric increments approximately equal to 25 M^{-1} and therefore the dipolar interactions for both glycine and leucine are similar in magnitude.

On the other hand, β-alanine, with a dielectric increment of 35 M^{-1} and a relatively low molecular weight, has free energies situated above the reference lines. Serine, a typical α-amino-acid, without large hydrocarbon groups gives points relatively close to the reference

lines.

α-Alanine has a dielectric increment normal for an α-amino-acid and also a methyl group so that the standard free energies would be expected to be slightly below the reference lines. In fact they are coincident with the β-alanine values for adsorption by both montmorillonite and illite and therefore appear to be anomalous.

f. The complementary nature and common origin of the two interpretations of isotherm linearity.

Two interpretations of the linearity of the isotherms have been presented:

(1) The mechanistic interpretation, that the solute molecules act as a swelling agent for the clay, thereby effectively creating the sites that they occupy. This interpretation is supported by the observed swelling of calcium montmorillonite on intercalation of glycine and its peptides, but this observation of itself is of no direct assistance in explaining the linearity of the isotherms observed when calcium illite is the substrate.

(2) The non-mechanistic, thermodynamic interpretation that constant partition of solute occurs between the bulk and the adsorbed layer solvents.

These interpretations are complementary and are now considered together. The expansion of montmorillonite on intercalation of amino-acids illustrates the conditions necessary for constant partition to apply. If the adsorbed phase volume were strictly constant, the partition isotherm could be linear only when concentrations in the

adsorbed layer were expressed in molalities or mole fractions, since the total amount of solvent in a fixed volume decreases as solute is desorbed. In the present systems, the adsorbed phase for both calcium montmorillonite and calcium illite expands to accomodate the incoming solute molecules without displacing solvent into the bulk solution and constant partition is obtained with the concentrations in the adsorbed phase expressed in molarities. The mechanism envisaged for the expansion of the adsorbed layer is, in qualitative terms, as follows. Water within the hydration shells of the exchangeable cations is partly displaced by the stronger amino-acid or peptide dipoles on adsorption, thus increasing the average dielectric constant of the solution surrounding the cations and decreasing the electrostatic attractive forces which determine the distribution of the cations with respect to the surface. The cations therefore move further from the clay surface to a new equilibrium distribution.

The complementary interpretations of isotherm linearity are therefore dependent upon a common molecular parameter of the adsorbate, the positive dielectric increment. The significance of the dielectric increment has been illustrated both theoretically and experimentally in the thermodynamic interpretation. However, in the mechanistic interpretation, the increase in the dielectric constant of the adsorbed phase remains a postulate, proposed to explain the linearity of the isotherms and to account for the observed swelling of calcium montmorillonite. Experimental verification of this postulate has therefore been sought.

g. The relationship between the swelling of calcium montmorillonite and the dielectric increment of intercalated amino-acids or peptides.

The basal spacings of moist montmorillonite complexes obtained directly from the separation stages of the equilibrium experiments for the adsorption of glycine and its peptides were determined. The increase in basal spacing beyond 19 Å on intercalation of the solutes was measured and compared with the increase in the average dielectric constant of the interlamellar solution which was assumed to be $\delta\left[RH^{\pm}\right]_{Stern}$. The dielectric increments were obtained from Cohn and Edsall (1943) and the Stern layer concentrations were calculated from the relevant isotherms. Fig. 25 shows that with the exception of triglycyl glycine, the basal spacing is directly related to the average dielectric constant of the interlamellar solution, independently of the solute adsorbed. The basal spacing of moist triglycyl glycine complexes increases with the Stern layer concentration, but is much less than would be predicted from the value of the dielectric increment. The linear relationship between $-\Delta G^{m}$ and δ (Fig. 21a) siggests that the dielectric increments of glycine and its peptides bear the same relationships to each other in the Stern layer as in the bulk solution (i.e. there is no anomalous decrease in the dielectric increment of triglycyl glycine on adsorption by calcium montmorillonite). It is therefore suggested that the swelling of calcium montmorillonite in the presence of triglycyl glycine is partly restricted by the operation of van der Waals or surface-dipole interactions which tend to bind the adjacent surfaces together, and that the smaller molecules of the glycine peptide series are too short

to achieve a similar inter-surface binding.

The increase in the basal spacing on intercalation of water is not continuous, but occurs in a stepwise manner. The basal spacings shown in Fig. 25 are the result of interstratification of a 19 Å with a 22 Å (and perhaps higher) basal spacing. The number of lamellae with higher spacings increases with the amount of solute adsorbed and the resultant increase in d(001) values given in Fig. 25 is apparently continuous. It is seen however, that the values tend to occur in two groups, at 19-20 Å and 21-22 Å.

h. The enthalpy and entropy change on physical adsorption of glycine and its peptides by calcium montmorillonite.

In considering this data for adsorption from solution it must be emphasised that the quantities determined are the resultant of the heat or entropy of adsorption of solute and of desorption of solvent on an approximately volume-for-volume basis. In the present system however, solvent desorption is largely restricted to the movement of the water molecules from lower to higher energy sites within the adsorbed layer, since it has been shown (Table 7) that at the upper limits of linearity of the isotherms, adsorption of solute is accompanied by a transport of solvent from the bulk to the adsorbed phase as a result of swelling (triglycyl glycine excepted, since water is displaced from the interlamellar volume to the extent of approximately 50% of the volume of solute adsorbed). This further complication of the present system due to solvent transport on adsorption prevents the quantitative interpretation of the enthalpy and entropy data given in Figs. 22 and 23

and Table 9. However, the enthalpy change is least negative for triglycyl glycine, the only adsorbate for which there is a net desorption of water. Similarly the change in entropy on adsorption is expected to be negative as the result of the loss of one or more degrees of translational freedom and the gain of an equivalent number of degrees of "soft" vibration, (Barrer, 1958). Where adsorption is accompanied by transport of water to the adsorbed phase, the entropy is in fact found to be negative (Table 9, page 74).

According to the Sackur-Tetrode equation:

$$-\Delta S = R \ln (MT)^{\frac{1}{2}}$$

the entropy decrease with the loss of one degree of translational freedom is proportional to one half the log. of the molecular weight and $-\Delta S$ therefore increases only slowly with molecular weight.

It is readily seen that the net entropy change on adsorption of the larger solute molecules is largely determined by the movement of solvent molecules. For example, one mole of triglycyl glycine displaces nearly six moles of water from the adsorbed phase, or more than nine moles from the lower energy sites within the Stern layer that are ultimately occupied by solute molecules. Even if the entropy change per mole of solvent transported is quite small, it constitutes a significant contribution to the net entropy change when multiplied by the number of moles involved in the transport. The estimation of the number of water molecules transported on adsorption of a molecule of glycine or its peptides is dependent upon the X-ray data for the moist complexes and is therefore of insufficient accuracy to permit

the determination of the type of adsorption model that represents the adsorbed phase in the present system.

ii. The adsorption of neutral amino-acids and peptides by sodium montmorillonite and sodium illite.

The adsorption isotherms for most of the substances studied (Figs. 4,9,13) are non-linear. Since partition of solute between the bulk and adsorbed-phase solvents probably applies to the adsorption by sodium clays, it must consequently be a non-constant partition, the solubility in the adsorbed phase (defined by the volume in which the exchangeable sodium ions effectively operate) changing as adsorption progresses. With sodium ions an extensive diffuse double layer may develop and hence it is not possible to assign a definite volume to the adsorbed layer. Also the adsorption of amino-acids and peptides would profoundly alter the distribution of sodium ions with respect to the clay surface by virtue of the consequent changes in the magnitude of the electrostatic forces operating in the electric double layer. Therefore both the effective volume of the adsorbed phase and the resultant solubility of the amino-acids and peptides in this volume will alter as adsorption progresses, and hence non-constant partition is to be expected.

The concept of a definite adsorbed phase volume which contains both the exchangeable cations and the adsorbed amino-acid or peptide dipolar ions is clearly an over simplification. A more precise description of the adsorbed phase is that the exchangeable cations

increase in concentration approximately exponentially as the surface is approached and the dipolar ions, by virtue of the influence of an electrolyte on their solubilities, will have a somewhat similar variation in concentration with respect to the clay surface. Their "concentration" will rise sharply in the immediate vicinity of the surface because of the action of surface-dipole and van der Waals forces. Consequently, the variation in the dielectric constant of the adsorbed phase on approaching the surface, and the influence of the dipolar ions on the distribution of the exchangeable cations in the diffuse layer will be complex. The isotherms (Figs. 4, 9) show qualitatively the importance of those interactions which increase with molecular weight (including van der Waals forces and the dipolar forces dependent upon the magnitude of the dielectric increment). The importance of surface-dipole forces is illustrated by the isotherm for the adsorption of leucine by sodium illite (Fig. 13). Since leucine is salted out of sodium chloride solutions as a result of the influence of the isobutyl side chain (see Schmidt, 1938) the positive adsorption by sodium illite shows that the salting-out effect is more than compensated for by interactions of the leucine molecules with the clay surface.

iii. Adsorption by hydrogen montmorillonite.

The isotherms for the adsorption of amino-acids by montmorillonite reported in the literature have been determined at pH 2 (McLaren et al., 1958; Sieskind, 1960). Sieskind emphasised that adsorption is largely determined by the basicity of the amino-acids and deduced that they occur in the adsorbed phase as cations. No attempt was

made in the present work to maintain the suspensions at constant pH as it was considered preferable to keep the ionic strength of the system as low as possible. The pH of each suspension at equilibrium was determined and was observed to increase with increasing amino-acid or peptide concentration.

The hydrogen montmorillonite suspensions were prepared by reaction with the hydrogen form of a cation exchange resin. The method of preparation and subsequent characterisation of the suspensions are given in Appendix II. No montmorillonite suspension could be prepared with more than 77 m equiv./100 g of titrateable hydrogen, either by equilibration of the clay with successive batches of exchange resin or by passing the suspension down a column, or a combination of these two proeedures. The same result was obtained with both sodium and calcium montmorillonite. The reaction time did not exceed 90 min. and the hydrogen montmorillonite was used immediately after preparation. The amount of contamination of the surface by aluminium is probably less than 2 m equiv./100 g and the reason for the low values reported for titrateable hydrogen remains uncertain.

The hydrogen form of sulphonated polystyrene resins hydrolyse peptides more rapidly than the "equivalent" concentration of HCl at 104°C (Whitaker & Deatherage, 1955). When the reaction rates obtained by these workers are considered in terms of both the entropy of activation expected for peptide hydrolysis and the lower surface concentration of hydrogen ions on montmorillonite (in comparison with the exchange resin they used) it is apparent that the hydrolysis half

lives of the glycine peptides by hydrogen montmorillonite at 20°C will probably be much greater than 60 h. Therefore the peptide hydrolysis which occurs during overnight shaking has been neglected.

The adsorption isotherms are given in Figs. 6 (glycine and its peptides), 12 (α-alanine, β-alanine, leucine and serine) and 15 (aspartic and glutamic acids, phenyl alanine and p-aminobenzoic acid). Much more amino-acid or peptide is adsorbed by hydrogen- than by sodium- or calcium-montmorillonite. The maximum adsorption of both diglycyl and triglycyl-glycine is in excess of the titrateable hydrogen content (Fig. 6). The increased adsorption by hydrogen montmorillonite is due to proton transfer from the surface hydrogen ions to the amino-acid or peptide dipolar ion in an analogous manner to salt formation:

$$RH^{\pm} + \text{clay } H_3O \rightleftharpoons \text{clay } RH_2 + H_2O \qquad (1)$$

This mechanism is closely similar to that reported for the adsorption of neutral amino-acids by the hydrogen form of cation exchange resins (Seno and Yamabe, 1960).

The equilibrium constant for this reaction is:

$$K_a = \frac{a'_{RH_2^+}}{a'_{H_3O^+} \cdot a_{RH^{\pm}}}$$

$$= \frac{N_{RH_2^+}}{N_{H_3O^+}} \cdot \frac{1}{[RH^{\pm}]} \times \frac{\gamma'_{RH_2^+}}{\gamma'_{H_3O^+}} \cdot \frac{1}{\gamma_{RH^{\pm}}}$$

$$= K_n \cdot K_\gamma$$

where N_{RH_2} is the amount of amino-acid or peptide adsorbed at equilibrium and $N_{H_3O^+}$ the amount of titrateable hydrogen remaining at equilibrium, expressed in m equiv./100g and $a'_{RH_2^+}$, $a'_{H_3O^+}$ and $\gamma'_{RH_2^+}$, $\gamma'_{H_3O^+}$ the corresponding activities and activity coefficients respectively.

In common with other exchange reactions, the isotherms are Langmuir type. Since the values of the activity coefficients are unknown, K_γ is assumed to be constant and the equilibrium (1) is discussed in terms of K_n. By plotting the ratio $\frac{N_{RH_2^+}}{N_{H_3O^+}}$ against the equilibrium concentration for each isotherm, a straight line of slope K_n should result. These graphs may conveniently be described as mass action plots. The data are expressed in this manner in Figs. 26 (glycine and glycyl glycine), 27 (diglycyl glycine and triglycyl glycine) 28 (α-alanine, β-alanine, leucine and serine) and 29 (aspartic acid, glutamic acid, phenyl alanine and p-amino benzoic acid).

It must be emphasised that considerable error is involved in some instances when the results are expressed in this manner. For those isotherms with high plateau adsorptions (for the peptides in particular, Fig. 6) the amount of titrateable hydrogen remaining, $N_{H_3O^+}$ is small and the estimation of its value involves a very large error. The subsequent substitution of this quantity in $\frac{N_{RH_2^+}}{N_{H_3O^+}}$ means that the relative error in this ratio increases with its magnitude.

It is therefore considered that the accuracy of estimation of

Mass action plots for proton transfer adsorption by H_3O^+-montmorillonite.

Fig. 26. The adsorption of glycine, □, and glycyl glycine, +.

Fig. 27. The adsorption of diglycyl glycine, △, and triglycyl glycine, ○.

Fig. 28. The adsorption of α-alanine, +, β-alanine, ×, leucine, ○, and serine, □.

Fig. 29. The adsorption of aspartic acid, ○, glutamic acid, □, phenylalanine, ×, and p-aminobenzoic acid, +.

fig. 26

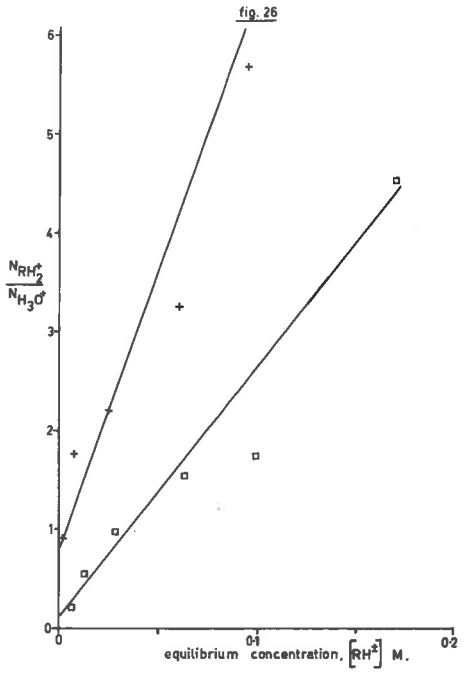


fig. 27

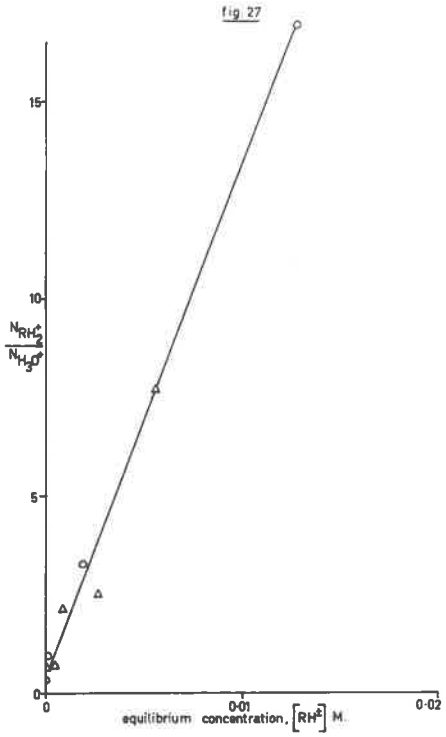


fig. 28

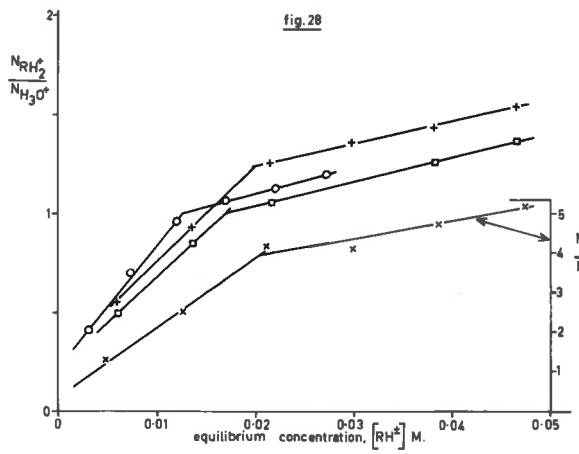
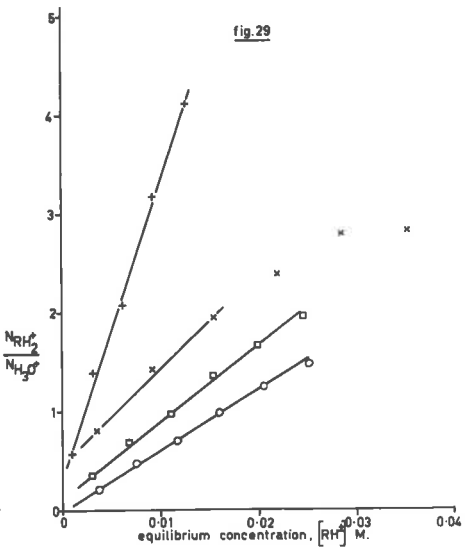


fig. 29



the values of K_n from Figs. 26 to 29, while sufficient for the purposes of the following discussion, does not warrant the calculation of standard free energies of proton transfer. The greatest error for a single point is probably not greater than 100% and the results are therefore correct to better than an order of magnitude. The plots for glycine and its peptides and β-alanine involve the greatest error since the magnitude of $\frac{N_{RH_2^+}}{N_{H_3O^+}}$ is greater than 1 for most points. The following discussion of K_n values is therefore largely confined to the remaining compounds studied.

The curves for glycine, α-alanine, β-alanine, leucine, serine and phenyl alanine decrease in slope above an equilibrium concentration of approximately 0.02M. Several reasons can be advanced for this observation:

(1) the adsorption sites are not equivalent.

(2) K_γ varies with increasing surface coverage.

(3) steric and other forms of adsorbed cation-cation interactions increase with surface coverage.

(4) the collapse of the interlamellar space on intercalation of the amino-acid cations restricts surface migration to the extent that the isotherms were not determined under equilibrium conditions.

The fourth reason may be discounted immediately because the basal spacings for the moist complexes (Appendix VII, 5) show that the montmorillonite collapses only to within 0.2 to 2.0 Å of the value for the corresponding dried complexes, and preliminary adsorption rate studies

showed that equilibrium was reached in less than four hours. The suspensions were shaken overnight for all experiments.

The initial slope portions of Figs. 26 to 29 correspond to the regions of lower surface coverage, when contributions from (2) and (3) are at a minimum. The K_n values given in Table 10 were determined from the initial slopes.

Table 10

The mass action quotients for proton transfer adsorption of amino-acids and peptides by hydrogen montmorillonite together with the first dissociation constants of the cations and the product $K_n K_1$.

Solute	K_n	$K_1 \times 10^3$	$K_n K_1$
glycine	26	4.60	0.12
glycyl glycine	56	0.85	0.05
diglycyl glycine	1270	0.55	0.70
triglycyl glycine	1270	0.89	1.13
α-alanine	49	4.48	0.22
β-alanine	178	0.25	0.05
leucine	60	4.70	0.28
serine	45	6.17	0.28
aspartic acid	62	7.95	0.49
glutamic acid	78	7.95	0.62
phenyl alanine	97	2.63	0.25
p-amino benzoic acid	302	4.38	1.32

To determine the significance of physical adsorption mechanisms which operate in addition to proton transfer, the equilibrium (1) is compared with the first dissociation constant of the amino-acid or peptide cation (K_1) *.

Equilibrium		Equilibrium constant
$RH_2^+ + H_2O \rightleftharpoons RH^\pm + H_3O^+$	. . .(2)	K_1
proton transfer reaction		
$RH^\pm$ + clay $H_3O \rightleftharpoons$ clay $RH_2 + H_2O$	. . .(1)	K_a
adding (1) and (2)		
RH_2^+ + clay $H_3O \rightleftharpoons$ clay $RH_2 + H_3O^+$	. . .(3)	K_aK_1

An increase in the product K_aK_1 (or in practice K_nK_1) reflects an increase in the stability, or decrease in dissociation of the montmorillonite - organic cation complex (clay RH_2) due to the action of physical adsorption forces in addition to proton transfer. These additional forces tend to move the hypothetical equilibrium (3) to the right. Therefore the product K_nK_1 has been calculated for each compound studied (see Table 10) the value of K_1 being obtained from the literature (Heilbron, 1934; Cohn and Edsall, 1943; Hodgman, 1957). K_nK_1 is plotted against molecular weight in Fig. 30. This figure shows that a general relationship exists between the contribution by physical forces to the adsorption of amino-acids and peptides (denoted by the variation in K_nK_1) and the molecular weight.

* K_1 is related to the base dissociation constant k_b discussed by Sieskind (1960) by the equation $K_1 = {}^{k_w}/_{k_b}$.

Fig. 30. The variation in $K_n M_1$ with molecular weight for the adsorption of amino-acids and peptides by H_3O^+ montmorillonite.

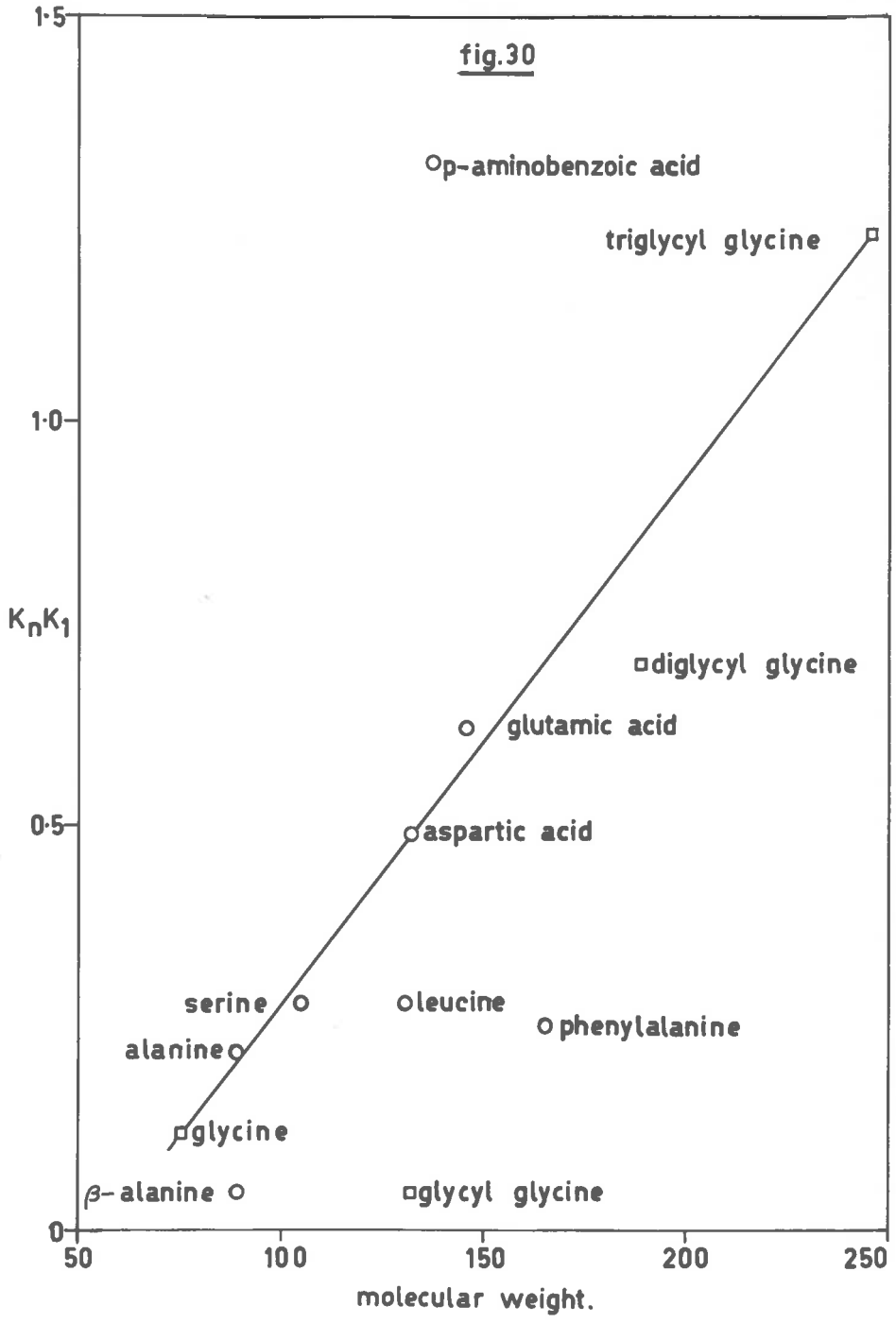


fig.30
1·5
1·0
0·5
0
K_nK_1
p-aminobenzoic acid
triglycyl glycine
diglycyl glycine
glutamic acid
aspartic acid
serine
leucine
phenylalanine
alanine
glycine
β-alanine
glycyl glycine
50
100
150
200
250
molecular weight.

The points for both leucine and phenyl alanine lie below the general line for the remainder of the compounds studied showing that a property of the hydrocarbon groups of these two compounds tends to oppose van der Waals and other forces in determining the resultant physical adsorption contribution to their adsorption by hydrogen montmorillonite. This property is not strictly a salting-out effect, but is closely analogous to it since the hydrocarbon groups oppose the increase in the amino-acid cation concentration at the clay surface. The product $K_n K_1$ for p-aminobenzoic acid is very much larger than is predicted from the molecular weight of this compound. The large physical adsorption contribution to proton transfer is almost certainly due to the interaction of the benazene ring with the clay surface intimate contact being possible because of the planar nature of the p-aminobenzoic acid molecule. Haxaire and Bloch (1956) showed that significant physical adsorption occurred with planar aromatic molecules which could associate closely with the clay surface. Since phenyl alanine is not a planar molecule, this type of interaction is of little significance in determining the physical adsorption contribution to proton transfer for this compound. The comparison of $K_n K_1$ for phenyl alanine (0.25) with that for p-amino benzoic acid (1.32) shows that the shapes of the molecules play an important part in determining their physical adsorption, and that van der Waals forces are effective over very short distances only. Assuming that the phenyl group of intercalated phenyl alanine is placed centrally in the interlamellar space, and parallel to the surfaces, the distance between the tops of

the surface oxygen atoms and the faces of the phenyl group is approximately 0.6 Å. This distance is too great for van der Waals attraction to overcome the repulsive forces that the phenyl group experiences in the aqueous environment of the adsorbed phase.

iv. The adsorption of basic amino-acids

Hendricks (1941) showed that organic cations are intercalated by montmorillonite and Talibudeen (1951) emphasised the importance of cationic groups in the adsorption of basic amino-acids and proteins. It was anticipated that the application of thermodynamics to the cation exchange reaction (Cowan and White, 1958) involving the naturally occurring basic amino-acids might supply important information on the adsorption of these compounds. The investigation may also increase the understanding of the factors determining the relative accumulation of basic amino-acids in soil organic matter over extended periods of soil cultivation (Stevenson, 1956, 1957).

The adsorption of the following compounds from solutions of their hydrochlorides has been studied:

(1) the basic amino-acids arginine, histidine and lysine,

(2) the basic dipeptide carnosine (β-alanyl histidine) and

(3) glycine (prepared by addition of the theoretical quantity of standard HCl solution to solid glycine and drying over NaOH and P_2O_5 at room temperature in vacuo.)

The adsorption isotherms are given in Figs. 16 to 19.

Adsorption was invariably accompanied by the liberation of an approximately equivalent amount of the exchangeable cation. The

cation exchange equilibria and equilibrium constants are as follows:

$$RH_2^+ + \text{clay Na} \rightleftharpoons \text{clay } RH_2 + Na^+ \qquad (1)$$

$$K_a = K_r K_Y$$

$$K_r = \frac{N_{RH_2^+}}{N_{Na^+}} \cdot \frac{[Na^+]}{[RH_2^+]}$$

$$2RH_2^+ + \text{clay Ca} \rightleftharpoons \text{clay } 2RH_2 + Ca^{++} \qquad (2)$$

$$K_s = \frac{N^2_{RH_2^+}}{N_{Ca^{++}}} \cdot \frac{[Ca^{++}]}{[RH_2^+]^2}$$

The adsorption of glycine from its hydrochloride solution by calcium montmorillonite differs in detail from (2) above. Since glycine hydrochloride is the salt of a strong acid and a very weak base, it undergoes considerable hydrolysis in solution:

$$RH_2^+ + H_2O \rightleftharpoons RH^{\pm} + H_3O^+$$

and the overall exchange reaction is :

$$RH_2^+ + H_3O^+ + \text{clay Ca} \rightleftharpoons \text{clay } H_3O, RH_2 + Ca^{++} \qquad (3)$$

$$K'_s = \frac{N_{RH_2^+} \cdot N_{H_3O^+}}{N_{Ca^{++}}} \cdot \frac{[Ca^{++}]}{[RH_2^+][H_3O^+]}$$

The adsorption of glycine is accompanied by a marked decrease in pH and the liberation of Ca^{++} equivalent to the amino-acid cation and H_3O^+ adsorbed (Fig. 16).

The symbols above are as defined in section 4,iii, (The adsorption by hydrogen montmorillonite). However, the units of

concentration and surface concentration are g ion/l. and mg ion/ 100 g respectively. K_γ is assumed to be constant. The values of K_r, K_s, and K'_s were determined by the method of Cowan and White (1958) whereby the ratio of concentrations in the solution phase (e.g. $\frac{[Na^+]}{[RH_2^+]}$) is plotted against the corresponding ratio in the adsorbed phase ($\frac{N_{Na^+}}{N_{RH_2^+}}$). A straight line of slope K_r, K_s or K'_s is expected (Figs. 31, 32, 33 respectively). This procedure differs from that employed to determine K_n for proton transfer, (section 4, iii) in that the points corresponding to the plateau adsorptions are closest to the origins of the graphs. The initial slope portions of the isotherms are consequently emphasised. The important error is therefore in the determination of the equilibrium concentration $[RH_2^+]$ and the relative error in the determination of the ratio $\frac{[\text{exchangeable cation}]}{[RH_2^+]}$ increases with its magnitude. The exchangeable cation remaining in the adsorbed phase at equilibrium is determined from the amount of exchangeable cation liberated into the solution phase. The error in estimation of this quantity is therefore less than the corresponding error in $N_{H_3O^+}$ for proton transfer. The initial slope portions of the isotherms for the adsorption of carnosine by sodium illite and of histidine by sodium montmorillonite (second experiment, Fig. 18) are not determined in sufficient detail to warrant this type of analysis. For the remainder of the isotherms the error probably does not exceed 60% for a single point.

Mass action plots for cation exchange.

Fig. 31. The adsorption of arginine, +, histidine, O, lysine, ×, and carnosine, □, by Na^{+} montmorillonite and of histidine, ●, by Na^{+} illite.

Fig. 32. The adsorption of DL-arginine, +, L-arginine, +, and histidine, O, by Ca^{++} montmorillonite and of histidine, ●, by Ca^{++} illite.

Fig. 33. The adsorption of glycine by Ca^{++} montmorillonite.

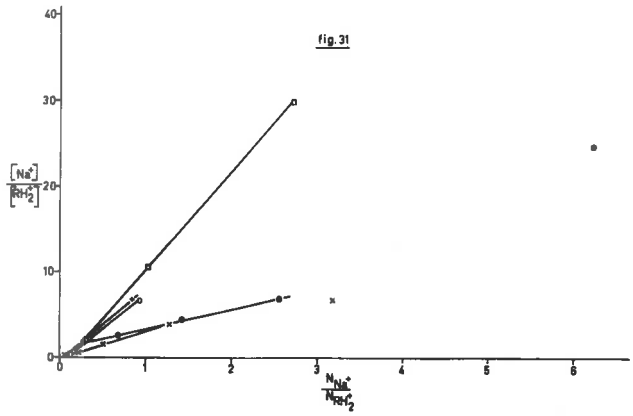


fig. 31

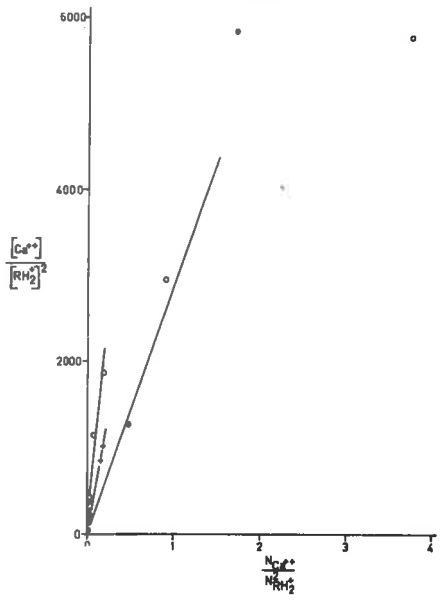


fig. 32

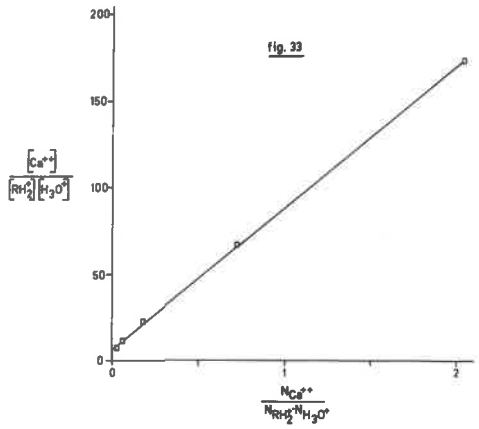


fig. 33

The determination of K'_s for the adsorption of glycine by calcium montmorillonite requires the values of $N_{H_3O^+}$ and $[H_3O^+]$. These are obtained by subtraction of $N_{RH_2^+}$ from the amount (in m equiv./100 g) of Ca^{++} liberated, and from the pH of the supernatant solution respectively.

In addition, the standard free energies of cation exchange have been calculated from

$$-\Delta G = RT \ln K - RT \ln J$$

where the standard state (J) for each reaction is unity. The results of the calculations are given in Table 11.

Table 11

The mass action quotients and free energies for adsorption by cation exchange of amino-acid and peptide cations

	Adsorbate									
Molecular weight	Arginine 174		Histidine 155		Lysine 146		Carnosine 226		Glycine 76	
Substrate	K	-ΔG cal./g ion	K	-ΔG cal./g ion	K	-ΔG cal./g.ion	K	-ΔG cal./g ion	K	-ΔG cal./g ion
Na^+ montmorillonite	8.1	1240	8.2	1250	3.4	720	12.0	1470		
Ca^{++} montmorillonite	5350	5080	10^4	5460					83	2600
Na^+ illite			2.3	490						
Ca^{++} illite			2860	4700						

a. Adsorption by sodium montmorillonite

Cowan and White (1958) studied the adsorption of a homologous series of n-alkylammonium cations by sodium montmorillonite. They found that the n-octylammonium ion (molecular weight 130) has a standard free energy of cation exchange in this system of 1290 cal./ g ion and that it is the first of this series to be adsorbed in excess of the exchange capacity. The excess amine is present as the uncharged molecule, adsorption being accompanied by a decrease in the pH due to the hydrolysis:

$$RNH_3^+ + H_2O \rightleftharpoons RNH_2 + H_3O^+$$

All the amino-acid cations adsorbed by sodium montmorillonite have molecular weights greater than that of the n-octylammonium ion, but only carnosine (M.W. = 226) has a larger standard free energy of cation exchange (Table 11). Also, ~~although~~ the hydrolysis of the amino-acid and peptide cations is more facile than that of the n-alkyl ammonium cations. The pK values fall between 6.0 and 9.0 for the former and are approximately 10 for the latter group of cations, (Cohn and Edsall, 1943; Hodgson, 1957). None of the amino-acids are adsorbed in excess of the exchange capacity (Figs. 17 and 18).

There are three main reasons which may be suggested to explain why the amino-acid and peptide cations have a smaller adsorption affinity than the n-alkylammonium ions of comparable molecular weight:
(1) The shape effect. The n-alkyl chains can pack closely together on adsorption, probably in a highly ordered manner (Weiss et al., 1957) thus allowing the greatest possible van der Waals interaction. The amino-acid and peptide cations are much less symmetrical and

therefore do not have the same efficient van der Waals interactions. This is illustrated quantitatively in the following comparisons:

(i) For the adsorption of n-alkylammonium cations above pentylammonium there is a constant increment in $-\Delta G^m$ of 400 cal./CH_2 group (M.W. = 14) for the homologous series (Cowan and White, 1958). By comparison, the increment in $-\Delta G^r$ for histidine and its β-alanyl peptide carnosine, is only 240 cal./β-alanyl group (M.W. = 71).

(ii) The lysine cation (M.W. = 146) has approximately the same standard free energy of cation exchange as the heptylammonium ion (M.W. = 116).

(2) The swelling effect. n-Propyl- and n-butyl-ammonium-montmorillonite complexes swell readily in water (Walker, 1960). Above n-butylammonium, the adsorbed cations have stable configurations in which the axes of their carbon chains form an angle of 56° to the clay surface (Weiss et al., 1957). The basal spacings of these complexes are larger than the collapsed amino-acid- or peptide- montmorillonite complexes and the internal surfaces are therefore more readily accessible to incoming adsorbate cations.

With the exception of the lysine complex the amino-acid or peptide montmorillonite complexes show very little intra-crystalline swelling (Table 12).

(3) The precipitation effect. n-Octyl- and n-decyl- amines are only slightly soluble in water while the higher amines may be regarded as insoluble. Adsorption above the exchange capacity may therefore be facilitated by a process analogous to precipitation. On the other hand, the amino-acid dipolar ions that would result from

Table 12

Basal spacings of both collapsed (dried) and fully expanded (moist) amino-acid and peptide complexes with montmorillonite.

	Basal spacing (Å)	
Adsorbed cation	Dry†	Moist†
arginine	13.6	16.6
carnosine	13.5	13.7
histidine	13.5	13.7
lysine	13.6	>100

† The moist samples correspond to complexes taken directly from the separation stage of the relevant isotherms. They were subsequently washed with water until chloride free, and dried over P_2O_5 *in vacuo* at room temperature. All figures are for complexes from the plateau region of the isotherms.

cation hydrolysis are soluble in water.

b. The comparison of montmorillonite and illite substrates

Inspection of Table 11 reveals that the ease of exchange of the histidine cation with sodium montmorillonite is greater than with sodium illite. This is also true of the corresponding calcium clays. Since a histidine cation in the interlamellar region of montmorillonite interacts with both surfaces, exchange is energetically more favourable than on the external surface of illite. Whereas $-\Delta G^r$ for cation

exchange of histidine is less for illite than for montmorillonite, $-\Delta G^m$, the free energy of physical adsorption of neutral amino-acids and peptides (section 4, i) is greater. It must be remembered, however, that the basal spacings of moist Ca^{++} montmorillonite complexes with the neutral amino-acids and peptides are too great for efficient contact of a physically adsorbed molecule with both internal surfaces of montmorillonite simultaneously, as occurs with the histidine cation. Therefore, on comparison of physical adsorption by montmorillonite with that by illite, the interaction of physically adsorbed molecules with adjacent internal surfaces of montmorillonite simultaneously is expected to be less than the influence of the increased surface density of charge on adsorption by illite. Exchange involving montmorillonite becomes kinetically less favourable as adsorption progresses because of the steric restriction to cation diffusion on the interlamellar surfaces, but this does not effect the value of $-\Delta G^r$.

c. <u>The comparison of sodium and calcium clays</u>.

Inspection of Table 11 reveals that the ease of exchange of amino-acid cations with calcium clays is apparently greater than with the corresponding sodium clays. Closer comparison could be achieved by referring all results to a common standard state, but, since the precise significance of the various standard states involved is not at present clear, closer comparison is not justified. Inspection of the initial slope portions of the relevant isotherms (Figs. 16, 17, 18 19) shows that arginine and histidine are more strongly adsorbed by sodium clays than by the corresponding calcium clays, and can replace

sodium more readily than calcium at the exchange sites.

d. The desorption of amino-acid and peptide cations.

It might be expected that the small amount of intra-crystalline swelling of the carnosine, histidine and arginine complexes with montmorillonite (Table 12) would result in a very low rate of surface migration of cations because of steric restrictions. The amino-acid or peptide cations may therefore desorb only very slowly in the presence of an electrolyte solution. The following desorption experiments were therefore carried out.

(1) Amino-acid-montmorillonite complexes corresponding to the regions of maximum adsorption were washed free of chloride ion on a grade 4 sintered glass funnel and then leached with a 10-fold excess of 0.01 N NaCl over a period of 2 to 4 hr. The quantities removed by, and retained against this washing procedure determined by Kjeldahl digestion of the dried clays are given in Table 13.

(2) Samples of these clays after leaching with 0.01 N NaCl were taken without drying and shaken with a 100 fold excess of N KCl for 20 hr. and then centrifuged. The dried clays were analysed by the Kjeldahl technique. The quantity retained against this washing procedure is given in Table 13, column 4.

(3) Experiment (2) was repeated with complexes obtained from the separation stages of the following adsorption experiments.

Carnosine adsorbed by sodium illite and sodium montmorillonite

Histidine adsorbed by sodium illite, calcium illite and calcium montmorillonite. The results are given in Table 14.

Table 13.

The amounts of adsorbed amino-acids retained against extraction with electrolyte solutions, montmorillonite substrate.

Adsorbed cation	Amount present initially	Amount retained against 0.01 N NaCl m mole./100g	Amount retained against 1 N KCl
arginine	76	28	21
histidine	66	44	22
lysine	75	19	1

Table 14

The amounts of adsorbed histidine and carnosine retained against extraction with KCl solution, montmorillonite and illite substrates.

Substrate	Adsorbed cation	Amount present initially	Amount retained against 1 N KCl
		m mole./ 100g	
Na^+ illite	carnosine	16	1.4
Na^+ mont-morillonite	"	75	33
Na^+ illite	histidine	17	2.0
Ca^{++} illite	"	18	0.7
Ca^{++} mont-morillonite	"	45	26

It is clear from these three experiments that carnosine, histidine and to a slightly lesser extent, arginine, are not readily desorbed from the interlamellar surfaces of montmorillonite because of the difficulty of cation migration within the collapsed interlamellar spaces. This is in agreement with the results of Mortland (1961) who showed that the adsorption of K^+ by vermiculite was restricted in the presence of some organic cations including those of the basic amino-acids. Histidine is the most effective of the amino-acids in inhibiting K^+ adsorption and is almost as effective as NH_4^+. Mortland concluded that the inhibition is due to a restriction of cation migration on the vermiculite surface, possibly because the cation sites become "clogged" (sic) with organic cations which cannot be displaced by K^+.

It is probable that both the higher value of $-\Delta G^r$ for the adsorption of histidine by sodium montmorillonite and the lower basal spacings of moist histidine-montmorillonite complexes compared with the corresponding values for arginine and lysine are due to stronger interactions between the polar groups of the organic cations with each other and with the clay surface. These dipolar interactions will be a function of molecular structure. This may be related to the fact that, of these three amino-acids, histidine forms the most stable coordination complexes with transition metal cations. The stability of these complexes is also dependent upon the molecular structure of the ligand (Chaberek and Martell, 1959).

5. Conclusion to Part II.

i. A general theory for physical adsorption from aqueous solution by clays.

It has been found that, provided no significant proton transfer or cation exchange occurs, the adsorption can be considered as the formation of a solution in the water containing the exchangeable cations. The properties of a solute that determine its solubility in the adsorbed phase solvent, which can be considered as an electrolyte solution, are the dielectric increment, the molecular weight and the salting-out effect of organic groups. Two types of adsorption can be distinguished, depending on the sign of the dielectric increment.

(1) When the dielectric increment is positive, the van der Waals forces and the dipolar interactions (determined by the dielectric increment) act together and in opposition to the salting-out effect to determine the resultant change in free energy of physical adsorption, $-\Delta G^m$. $-\Delta G^m$ is increased by:

(i) increasing dielectric increment

(ii) increasing surface density of charge

(iii) increasing molecular weight. If the molecular weight is increased by the addition of an organic group which also increases the salting-out effect (e.g. the phenyl- and iso-butyl- groups of phenylalanine and leucine respectively) then the increased van der Waals force will be partly offset. The magnitude of the van der Waals interactions is largely dependent upon the efficiency of contact between the adsorbed molecules and the surface and between the

adsorbed molecules themselves. The more symmetrical molecules (e.g. planar aromatic and n-alkyl compounds) allow efficient van der Waals contact.

(iv) decreasing electrolyte concentration in the bulk phase. As this concentration decreases the activity coefficient of the non-electrolyte is increased, thereby increasing $-\Delta G^m$.

(2) When the dielectric increment is negative, the dipolar forces and the salting-out effect are identical and they act in opposition to the van der Waals forces. Therefore no physical adsorption can occur unless the molecular weight is sufficiently large to overcome the opposing contribution. When this condition is fulfilled, the free energy of physical adsorption is increased by :

(i) decreasing magnitude of the dielectric increment.

(ii) increasing electrolyte concentration in the bulk phase (The non-electrolyte is then salted-out of the bulk phase into the adsorbed phase).

The physical adsorption by clays will also be influenced by other factors not directly investigated in the present work. These factors include:

(a) The polarising power of the exchangeable cation. It is expected that the magnitude of ion-dipole interactions will increase with increasing valency and decreasing radius of the exchangeable cation. However, the comparison between Na^+ and Ca^{++} clays in the present work suggests that the higher polarising power of Ca^{++} is offset by other factors not clearly understood (but related to the lower adsorbed

phase volume) since both sodium and calcium clays have approximately equal adsorption maxima for the same adsorbate.

(b) Hydrogen bonding. It has been shown that water-to-surface hydrogen bonding is not significant in comparison to ion-dipole interactions. However, solute-to-water and solute-to-solute hydrogen bonds may influence the adsorption of some types of compound.

(c) Coordination complex formation. This is likely to be less important when the exchange sites are occupied by the cations normally present in a soil system (e.g. Na^{+}, K^{+}, Ca^{++}, $Mg^{++}Al^{3+}$) than for the transition metal cations. However, it is clear that further work is necessary, using clays saturated with a wide range of cations, before the significance of polarising power or coordination complex formation can be evaluated.

(d) Monolayer saturation. When the monolayer is saturated, further adsorption occurs at lower energy. Also, additional work is required to expand montmorillonite and similar minerals on intercalation of molecules above the monolayer capacity. These related effects cause steps to appear in the adsorption isotherms. However, for physical adsorption from aqueous solution, these complications would only be observed either at very high solute concentrations or for the adsorption of large molecules.

The general theory is supported for adsorption of compounds with negative dielectric increments by the data of Hoffmann and Brindley (1960) who studied the adsorption of a range of straight-chain aliphatic 1:3 diketones, 1:4 diketones, ethers, ether-nitriles,

ether-alcohols, ether-esters and dihydric alcohols from aqueous solution by calcium montmorillonite. All these compounds have negative dielectric increments (in comparison with simple alcohols, ethers and ketones, probably ranging between 0 and -5 M^{-1}) and, since all are straight chain molecules, the influence of molecular weight on the extent of adsorption is clearly evident. A minimum chain length of 6 atoms (M.W. between 100 and 120) is required for adsorption to be observed, and thereafter, the extent of adsorption as shown by the slopes of the published isotherms is strongly dependent on the molecular weight. The adsorption of 1:3 diketones is apparently anomalous and of these, acetyl acetone is by far the most extensively adsorbed. However, these compounds have almost certainly polymerised on the clay surface since the complexes were observed to darken with time. It is of interest to note that the first of the n-alkylamines to be adsorbed in excess of the exchange capacity by sodium montmorillonite (Cowan and White, 1958) is n-octylamine (M.W. = 129).

Bradley (1945) showed from X-ray data that ethylene glycol (M.W. = 62) is adsorbed from aqueous solution by montmorillonite. This is apparently contradictory to the conclusion of Hoffmann and Brindley (1960) that a minimum chain length of 6 atoms is required for adsorption. However, the extent of adsorption and the concentration of the aqueous glycol solution were not stated.

ii. Proton transfer and cation exchange reactions.

The data given in the present work show clearly that although proton transfer and cation exchange respectively are the dominant

mechanisms involved in these two types of adsorption, physical adsorption has some secondary importance. The influence of the shape of the adsorbed molecule on the van der Waals contribution to physical adsorption was illustrated. The carboxyl groups of aspartic and glutamic acids do not restrict the adsorption of these compounds by proton transfer (Fig. 30). Adsorption of organic acids at higher pH values, where dissociation of the carboxyl groups is significant, is expected to be restricted by repulsion of COO^- groups from the clay surface. Diglycyl glycine (M.W. = 189) and triglycyl glycine (M.W. = 246) are adsorbed in excess of the titrateable hydrogen content of hydrogen montmorillonite, but no similar excess adsorption was observed with the cation exchange of the basic amino-acids or carnosine (M.W. = 226). With the exception of lysine, the intercalation of amino-acid cations by montmorillonite causes the collapse of the interlamellar space, with the result that the surface migration of the cations (and therefore the rates of adsorption and desorption) are restricted. Since work is required to expand the lattice, adsorption of arginine, carnosine and histidine may be restricted to monolayer coverage. It is shown in Part III that only diglycyl glycine and triglycyl glycine have been adsorbed in excess of the monolayer capacity. These observations are in agreement with the data of Pinck et al., (1961 a, b) who studied the adsorption and desorption of a range of acidic, amphoteric and basic antibiotics by hydrogen and calcium montmorillonite. The basal spacings of the resultant complexes revealed that two layers of amphoteric antibiotic molecules were inter-

calated by both cationic forms of montmorillonite, but only single layer complexes were formed with the basic compounds. The amphoteric but not the basic antibiotics were desorbed by citrate and phosphate buffer solutions. It is apparent that the intercalation of large organic cations which causes a collapse of the interlamellar space results in a very low rate of desorption (and perhaps a fixation of some cations) whereas the adsorption of dipolar ions gives rise to a more mobile film. The availability of neutral amino-acids for microbial or enzyme catalysed decomposition is therefore to be expected. On the other hand, the relative resistance of the basic amino-acids and amino-sugars of soil organic matter to microbial decomposition may be partly explained by the adsorption and immobilisation of decomposition products of the soil organic matter which are cationic in nature. It is therefore suggested that further micro-biological studies should be carried out on montmorillonite complexes with basic amino-acids (particularly histidine) both before and after partial desorption by sodium and potassium chloride. It would be important in these studies to show that the basic amino-acid cations were intercalated and not merely adsorbed on the external surfaces of montmorillonite.

Part III. X-RAY INVESTIGATION AND INFRA-RED SPECTRA

1. Introduction

The earlier parts of this thesis have shown that the mechanisms invloved in bonding organic compounds to montmorillonite or illite do not include C-H. . .O hydrogen bonds or similar interactions. However, the apparent contraction observed on intercalation of organic compounds by montmorillonite has previously been interpreted in terms of C-H. . .O hydrogen bonding (MacEwan, 1948). In an endeavour to reconcile the present work with earlier investigations, the dried (over P_2O_5 at room temperature) amino-acid complexes have been studied by X-ray diffraction. The X-ray data for the moist complexes have been discussed in Part II. Also, the infra-red spectra of clay organic complexes have been studied, since interactions of the C-H. . .O hydrogen bonding type involving methylene groups would lead to a depression in the C-H stretching frequencies.

2. Description of apparatus and experimental details.

i. X-ray investigations

Iron-filtered CoKα radiation obtained from a Philips PW 1010 X-ray generator was used with a model PW 1005 diffractometer. X-ray diffraction diagrams were obtained from the complexes by drying the centrifuged samples over P_2O_5 at room temperature and then grinding and spreading the powders so obtained on recessed aluminium plates. Some slight orientation of the clay platelets in the direction of the beam was obtained by smoothing with a glass slide. When measurements were made on the suspensions, the clay was centrifuged in a tube with a detachable base similar to that described by Farrin (1955). After centrifuging, the supernatant was decanted and the base of the tube with the clay paste mounted in the diffractometer. Alternatively, moist complexes obtained after separation of the supernatant during

the equilibrium experiments were spread onto the recessed aluminium plates. The complete X-ray data are given in Appendix VII. The moist complexes contain a small amount of entrained solution which increases in concentration on drying, and a slight increase in the amount adsorbed would have occurred. Therefore, the amounts of amino-acid or peptide adsorbed (obtained from the equilibrium experiments) given in Appendix VII are slightly low for the dried complexes.

ii. Infra-red investigations

A Perkin-Elmer model 112 single beam, double pass spectrometer fitted with a calcium fluoride prism was used to determine the spectra of sodium montmorillonite complexes with methyl alcohol, ethylene glycol and p-dioxane. The investigations were confined to the region of the spectrum between 3800 and 2700 cm^{-1} where the fundamental O-H and C-H stretching vibrations occur.

The complexes prepared by equilibrating 1 g. of clay with 0.25 g of organic liquid for 30 min. in a stoppered glass tube, were supported as thin films between two sodium chloride plates separated by a 0.008 mm lead washer. The resultant spectra were compared with those of the pure liquids supported for investigation in a similar manner. No changes in frequency or relative intensity of the absorption bands for the C-H or O-H stretching vibrations were observed on adsorption of the organic liquid. The complete results are given in Appendix VIII.

Dried montmorillonite-amino-acid or peptide complexes proved unsatisfactory for investigation as the spectrum of the montmorillonite itself largely concealed the absorption bands for the adsorbed species.

3. Discussion

i. X-ray investigations

The results of the X-ray diffraction studies of the dried complexes (Appendix VII) show that, with the exception of diglycyl glycine and triglycyl glycine, the amino-acids and peptides formed complexes with only a single layer of molecules between adjacent alumino-silicate lamellae, with basal spacings within the range 12.5 to 14.8 Å. Diglycyl glycine and triglycyl glycine gave both single and double layer complexes, with basal spacings of approximately 12.8 Å and 16.0 Å respectively.

Since the discussion is largely concerned with the significance of the apparent contraction in van der Waals thickness of adsorbed molecules, the Δ-values (MacEwan, 1948) were calculated by subtracting 9.5 Å (the assumed thickness of the alumino-silicate layer, Greene-Kelly, 1955) from the basal spacing. Δ-values for dried complexes are given in Appendix VII and are collected in Table 15 (for complexes showing the most rational series of (001) reflections). The minimum molecular thicknesses given in Table 15 were derived from Catalin molecular models. It is necessary to correct the molecular thicknesses obtained from these models, since the van der Waals radii are lower than the generally accepted values (Pauling, 1960) for some atoms. The corrections applied in the present work are given in Table 15.

Table 15

The van der Waals radii or half-thickness of methyl, methylene, hydrogen and phenyl, calculated from Catalin models and according to Pauling (1960).

Atom or group	van der Waals radius or half-thickness, Å	
	Catalin	Pauling
hydrogen	0.95	1.2
methyl and methylene	1.75	2.0
phenyl	1.4	1.7

The minimum molecular thicknesses for glycine and its peptides were derived from the X-ray data of Albrecht and Corey (1939) and Hughes and Moore (1949) for single crystals of glycine and glycyl. glycine.

It is shown in Table 16 that, for all amino-acids and peptides studied, the apparent contraction in the van der Waals thickness of the adsorbed molecules ranges from 0 to 1.2 Å. The maximum extent to which keying of the molecules into the hexagonal depressions in the surface oxygen sheets may occur with the amino-acids and peptides studied has been determined using molecular models. The montmorillonite surfaces were prepared to a scale of 1 cm. to 1 Å using the dimensions and rotations of the upper oxygen atoms of the silica tetrahedra given by Radoslovich (1960 and private communication). The amino-acid and peptide molecules were made with Catalin models. It was found

Table 16

MacEwan's Δ values, the minimum molecular thicknesses (derived from Catalin models and corrected to accord with Pauling's values of van der Waals radii) and the apparent contraction of amino-acid and peptide molecules on adsorption by montmorillonite.

Adsorbate	Δ d(001)-9.5 Å	Minimum molecular thickness Catalin Å	Corrected Å	Apparent Contraction Å
glycine	3.2		4.1	0.9
glycyl glycine	3.5		4.3	0.8
diglycyl glycine	3.5, 3.7		4.3	0.8, 0.6
triglycyl glycine	3.6, 3.8		4.3	0.7, 0.5
α-alanine	4.2	4.2	4.7	0.5
β-alanine	3.5	3.5	4.0	0.5
leucine	4.8, 5.3	4.7	5.3	0.5, 0
serine	3.7	4.2	4.7	1.0
aspartic acid	3.4	4.3_5	4.6	1.2
glutamic acid	3.7	4.2	4.7	1.0
phenylalanine	4.4	4.4	4.9	0.5
p-aminobenzoic acid	3.4, 3.5	3.0	3.4	0
arginine	4.1	4.2	4.7	0.6
carnosine	4.0	4.2	4.7	0.7
histidine	4.0	4.2	4.7	0.7
lysine	4.1	4.2	4.7	0.6

possible to key the molecules into the surface to give contractions greater by about 0.2 Å than those actually observed (Table 16). The smaller hydrogen atoms of Catalin models (c.f. Pauling's values) can key into the depressions to a greater extent than true molecular representations. However, this fact could not account for more than 0.1 to 0.2 Å of the contraction determined from the models, and it seems that keying of the adsorbed molecules can account for the observed contraction without considering possible molecular distortion, C-H ⋮ ⋮ O bond formation or an incorrect estimate of the alumino-silicate layer thickness (Part I).

Random interstratification patterns were observed when both single and double layer complexes were present, and not two rational series of reflections from the single and double layer spacings. This is in contrast to the findings of Greenland (1956) for sugars adsorbed by montmorillonite and Hoffmann and Brindley (1960) for various polyfunctional alcohols, ethers and ketones. It is difficult to suggest a satisfactory explanation for the difference between these groups of compounds. The interlamellar separation in aqueous suspension is greater than that of the two layer complex, so that no work has to be done in separating the lamellae. Therefore this cannot be advanced as an explanation.

ii. Infra-red investigations

The chief experimental difficulty encountered in determining the infra-red spectra of adsorbed compounds is to obtain a sufficient number of adsorbed molecules in the incident beam without masking the

resultant spectrum with that of the substrate or by scatter of radiation at the solid surfaces. However, montmorillonite and other expanding lattice minerals have the important advantage that their high specific surface area and the resultant high monolayer capacity tend to minimise the significance of the spectra of the substrates (which have their most intense bands at about 1100 cm^{-1}). Also, scatter of radiation can be reduced if the adsorption complex is sufficiently stable to allow their support for investigation as pressed discs in a matrix of KCl or KBr.

Hoffmann and Brindley (1960) suggested that the adsorption of aliphatic compounds containing a high proportion of methylene groups adjacent to electron withdrawing atoms is explained in part by C-H. . . O interactions of a hydrogen bonding type (see Part I). This suggestion has been investigated by means of infra-red spectroscopy. The infra-red spectra of methyl alcohol, p-dioxane and ethylene glycol between 3800 and 2700 cm^{-1} show no change in frequency or relative intensity on adsorption by sodium montmorillonite (Appendix VIII). In particular, there is no decrease in the C-H stretching frequencies which would be expected to accompany the type of interaction between CH_2 groups and the surface oxygen atoms as described by Hoffmann and Brindley. The spectrometer used is capable of detecting a change in frequency of less than $1cm^{-1}$ in the region of the spectrum studied. It is therefore concluded that the CH_2 groups of these and similar compounds are not involved in C-H . . .O hydrogen bonding to the clay surface on adsorption. Neither is there any weak interaction which might be expected to lower the C-H stretching frequency, and augment adsprption

of polyfunctional aliphatic compounds containing a high proportion of α-CH_2 groups.

4. Summary of Part III.

The apparent contraction in the van der Waals thickness of adsorbed amino-acids and peptides (and probably all other aliphatic molecules) is due to keying of methyl and methylene groups into the hexagonal cavities of the clay surface.

The infra-red spectra of adsorbed methyl alcohol, p-dioxane and ethylene glycol further confirm that both C-H• • • O hydrogen bonding and similar interactions that are too weak to be regarded as true bond formation are of no significance in the adsorption process. The adsorption of poly-functional alcohols, esters, ethers and ketones containing a high proportion of α-methylene groups (Hoffmann and Brindley, 1960) is readily explained in terms of the general theory of physical adsorption from aqueous solution by clays proposed in the conclusion to Part II (page 109). The more detailed study of montmorillonite complexes with organic compounds by means of infra-red spectroscopy could yield important information on the types of bonding interaction between adsorbed molecules.

GENERAL CONCLUSION

The three basic aims of the present work were:

(1) To elucidate the bonding mechanisms involved in amino-acid- or peptide-clay adsorption complexes.

(2) To determine the affinities or free energies of adsorption of the compounds studied.

(3) To study the dependence of (1) and (2) upon the molecular properties of the adsorbates.

To a large extent, these aims have been realized and a general theory for physical adsorption of organic compounds from aqueous solution by clay minerals has been proposed (Part II, section 5, i). The general theory, together with the conclusions regarding the proton transfer and cation exchange reactions (Part II, section 5,ii) allow the qualitative prediction of adsorption affinities of water-soluble organic compounds from their relevent molecular parameters. This can be illustrated by considering separately the bonding mechanisms involved.

(i) Proton transfer. The extent of adsorption by this mechanism is determined by the number of Brønsted acid sites on the mineral surfaces and the basicity of the adsorbed compound. The number of acid sites increases with decreasing pH.

(ii) Cation exchange. Adsorption by cation exchange depends upon the number of cation sites available and on the proportion of the adsorbate present in solution in a cationic form.

Mechanisms (i) and (ii) give rise to strong electrostatic

forces. Surface migration of cations intercalated by montmorillonite is restricted if adsorption leads to a collapse of the interlamellar space (e.g. adsorption of histidine and carnosine cations). The extent of adsorption decreases with increasing concentration of inorganic salts because of competition for cation sites. In addition to the electrostatic bonding which results from proton transfer or cation exchange, weaker but, for some cations, significant physical adsorption or van der Waals forces act. These forces increase with molecular weight and are influenced by the shape of the cation. Planar aromatic and straight chain aliphatic cations which permit an intimate contact with the surface and with each other are more strongly adsorbed than irregularly shaped cations of comparable molecular weight. The increase in van der Waals attraction of a cation due to hydrocarbon groups may be partly offset by an effect that is somewhat analogous to a salting out from the adsorbed phase.

(iii) Ion-dipole and related surface-dipole interactions. These yield weaker bonding forces than (i) and (ii) above and are dependent upon the sign and magnitude of the dielectric increment of the adsorbate and on the surface density of charge of the substrates. According to the general theory of physical adsorption, a positive dielectric increment leads to positive adsorption which increases with the magnitude of δ and with increasing surface density of charge. The extent of adsorption decreases with increasing electrolyte concentration in the bulk phase. A negative dielectric increment opposes positive adsorption to an extent which increases with the magnitude of δ and with increasing surface density of charge. Increasing electrolyte

concentration in the bulk phase favours the positive adsorption of compounds with negative dielectric increments by tending to salt the molecules out of the bulk phase into the adsorbed phase.

(iv) van der Waals interactions. Adsorption due to these interactions increases with molecular weight and is influenced by the shape of the adsorbed molecules in the same manner as described for the physical adsorption which supplements cation exchange and proton transfer.

Mechanisms (iii) and (iv) probably give rise to a mobile film of adsorbed molecules.

(v) The salting-out effect of hydrocarbon side-chains. This phenomenon is only distinguishable for molecules with positive dielectric increments and tends to oppose positive adsorption.

(vi) Hydrogen bonding. Adsorbate-to-substrate hydrogen bonding probably does not contribute significantly to adsorption from aqueous solution by ~~these~~ clay minerals whose available surfaces ~~that~~ are predominantly sheets of oxygen atoms.

The chemical implications of the general theory of physical adsorption and other conclusions in the present work are self evident but their application to the understanding of soil nitrogen and organic matter transformations requires further consideration.

It is possible that, in many soils, surfaces and micro-pores exist which are inaccessible to microbes and extra-cellular enzymes. It is therefore of value to discuss conditions which would cause adsorption or desorption of the water-soluble intermediates of soil organic matter transformations. These compounds are the nutrients

by means of which soil micro-flora largely effect organic matter transformations. The intermediates envisaged include amino-acids, plant phenols, sugars, uronic acids, purine and pyrimidine bases, and compounds derived from these.

Any set of conditions which brings about an increase in the ionic strength of the soil solution, e.g. the drying of a soil or the influx of salts, will promote the desorption of organic cations that are adsorbed by cation exchange or proton transfer. In addition, uncharged intermediates which have positive dielectric increments (e.g. amino-acid dipolar ions) will tend to be salted out of the adsorbed phase by the increasing electrolyte concentration of the soil solution. On the other hand, those intermediates with negative dielectric increments, e.g. plant phenols and sugars, will tend to be salted onto the mineral surfaces. Since organic cations and dipolar ions commonly contain nitrogen atoms in their structures, conditions which favour an increase in the ionic strength of the soil solution will also tend to increase the availability of water-soluble organic nitrogen for microbial utilization. A decrease in the ionic strength of the soil solution will tend to decrease the equilibrium concentration of organic cations and dipolar ions. These ideas are supported by the experimental observations of Paul and Schmidt (1961) who studied the extraction of amino-acids from soil. They found that aqueous salt solutions not only liberate the cationically bound basic amino-acids in greater quantities than does aqueous ethanol, but also a large number of neutral and acid amino-acids are more extensively extracted.

It was shown that physical adsorption affinity increases with molecular weight, which suggests that physically adsorbed polymers might constitute a significant proportion of the organic matter of a soil. This would be possible only if the shape of the polymer were sufficiently uniform to permit an adequate number of points of van der Waals contact with the mineral surfaces, a condition one might expect to be fulfilled if chemical polymerisation of the adsorbed monomers is a significant reaction in the soil.

The retention of histidine cations by montmorillonite against a 20 hr. extraction period using 1N KCl suggests that a significant proportion of the nitrogen in a soil may exist as intercalated organic cations whose surface migration is restricted by the collapse of the interlamellar space.

The following experiments are suggested to augment the results of the present work.

(1) Experiments designed to evaluate the quantitative relationships between adsorption affinities and the relevent molecular parameters, e.g.

(i) The adsorption of amino-acids and peptides in the presence of different concentrations of electrolyte could be studied to establish the quantitative relationship between adsorption affinity and ionic strength of the bulk phase for compounds with positive dielectric increments. Glycine and its peptides would be useful adsorbates to study.

(ii) The adsorption of compounds with negative dielectric increments may be investigated with the aim of separating the contributions to the adsorption affinity that are dependent upon the dielectric

increment, the molecular weight and the shape of the molecule. The influence of electrolyte should also be studied. The adsorption of plant phenols, sugars and other known intermediates of soil organic matter transformations would maintain the relevance to soil science of this type of work.

(iii) The influence of surface density of charge of the adsorbent may be evaluated in a quantitative manner by studying the adsorption of glycine and its peptides by a range of clay minerals from very low (hydrated halloysite) to very high (vermiculite and illites) charge densities.

(iv) The influence of the polarizing power of exchangeable cations could be investigated along with (iii) by saturating selected minerals with a range of cations of differing sizes and charge.

Experiments (i) to (iv) would be largely concerned with the interpretation of adsorption isotherms.

(v) The adsorption of planar aromatic cations and uncharged molecules by montmorillonite should be systematically studied to evaluate the significance of the interaction of the π-electrons of the aromatic nuclei with the alumino-silicate surface (Haxaire and Bloch, 1956). As a supplement to the study of adsorption isotherms, the use of infra-red spectroscopy would be of value in this type of investigation, since the frequencies and intensities of the aromatic bands would vary significantly if this type of interaction occurred.

(2) Experiments designed to relate the present results to the understanding of soil organic matter transformations with a greater

degree of probability.

(i) An investigation of the availability of histidine adsorbed by montmorillonite for bacterial utilization, particularly after preliminary extraction with KCl. Depending on the results of this investigation, it may be of value to study further complexes in a similar manner.

(ii) Extraction of the soil clay fraction with suitable solutions and solvents and subsequent detailed organic chemical investigations of the solutions may reveal the extent of adsorbed water-soluble intermediates in the soil. The amounts of these compounds in the adsorbed phase is likely to be small.

Appendix I

Preparation and analytical characterisation of clay suspensions

1. Preparation

(i) Calcium clays

The less than 2 μ e.s.d. fraction of montmorillonite or illite was separated by sedimentation, the top 17.5 cm of 2% (or less) suspensions being syphoned from the remainder after a settling time of 16 h. The resultant suspensions were rendered 1M with $CaCl_2$ and then adjusted to pH3 with HCl. Illite suspensions were kept at pH3 until all $CaCO_3$ impurity was removed. The clays were then filtered on a Buchner funnel, leached with 3 l. of 1M $CaCl_2$ and then with distilled water until no chloride could be detected in the leachate. The resultant calcium clays were stored at 5°C as suspensions in distilled water.

(ii) Sodium clays

Sodium clays were prepared and stored in a similar manner to calcium clays except that the electrolyte concentration of the suspensions was reduced by dialysis against distilled water. Before use, the dialysis tubing was boiled in successive lots of distilled water until constant conductivity of the leachates was achieved.

(iii) Hydrogen montmorillonite

Hydrogen montmorillonite was prepared from approximately 2% sodium or calcium clay suspensions either by passing them down a column of the hydrogen form of Amberlite I.R.120 cation exchange resin, or by equilibrating the suspensionswith 3 successive batches of resin (a six fold excess in each batch).

2. Characterisation

(i) Cation exchange capacity

Cation exchange capacities were determined by saturation of the clay with ammonium ion and subsequent steam distillation of portions of the ammonium clay suspension with MgO in a Markham still. The ammonia was collected in 5 ml. of 4% boric acid solution and estimated by titration against 0.02 N HCl using a mixed brom-cresol green-methyl red indicator.

Two procedures were used to saturate the clay with ammonium (Cowan and White, 1958). (a) The 2% clay suspension was passed down a column of the NH_4^+ form of Amberlite I.R.120 (b) The clay was shaken with three successive portions of 1 N ammonium acetate at pH7 centrifuging and decanting between shakings. The clay was washed free of ammonium acetate with 1:1 distilled water-alcohol mixture. There was no difference in the results obtained using these procedures. The supernatants, without the washings, were combined for subsequent estimation of the exchangeable cation initially present.

(ii) Calcium estimation

Calcium was estimated in the ammonium acetate leachates by titration at pH10 with ethylenediaminetetra-acetic acid, disodium salt solution using Eriochrome Black T indicator. It was found that none of the compounds interfered with the estimation.

(iii) Sodium estimation

An Eel flame photometer was used.

(iv) Titrateable hydrogen

Titrateable hydrogen was estimated by titration of the hydrogen montmorillonite suspensions to pH7 with 0.02N NaOH solution.

The pH of the clay suspension was checked after shaking overnight and was readjusted to pH7 with 0.02N NaOH if necessary. The titrations were also repeated in the presence of 0.5N NaCl. No difference was detected. A Cambridge portable pH meter and glass electrode were employed for the pH determinations.

(v) Electrolyte concentrations of suspensions

Since chloride salts were used in the clay saturations, the chloride ion concentration of the suspensions was determined by potentiometric titration using 0.01N $AgNO_3$.

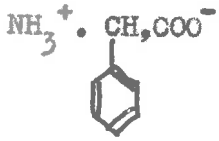
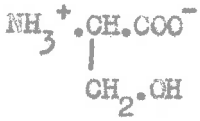
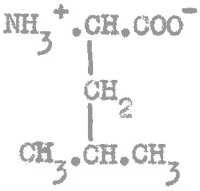
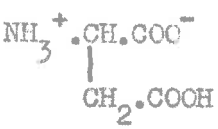
3. Characteristics of the clay suspensions

Substrate	Amount of clay in suspension g/100 ml.	pH	$[Cl^-]$ N.	Cation exchange capacity m.equiv./ 100g.	Titrateable H_3O^+ or exchangeable cation, m.equiv./ 100g.
Montmorillonites:					
Sodium	2.6	6.16	3.5×10^{-5}	88	87.3 (incl. 5 of Ca^{++})
Calcium	6.4	6.60	5×10^{-5}	90	89
Hydrogen* a	2.2	3.81			77.1
b	1.2	2.61			70.2
c	2.0	2.86			72.0
d	2.1	2.95			76.4
Illites:					
Sodium	16		$< 10^{-5}$	29.3	29.2
Calcium	8		$< 10^{-5}$	29.2	29.0

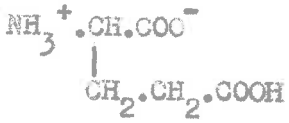
* Used for the adsorption of (a) glycine and glycyl glycine (b) diglycyl glycine and triglycyl glycine (c) α-alanine, β-alanine leucine and serine (d) aspartic, glutamic and p-aminobenzoic acids and phenyl alanine

Appendix II

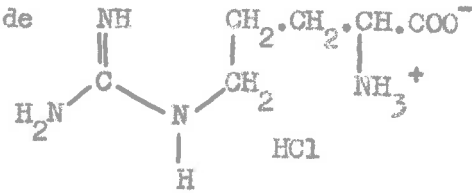
Sources and formulae of compounds studied

1.	Glycine	$NH_3^+.CH_2COO^-$	British Drug Houses (AnalaR)
2.	Glycyl glycine	$NH_3^+.CH_2.CO.NH.CH_2COO^-$	British Drug Houses
3.	Diglycyl glycine	$NH_3^+(CH_2.CO.NH.)_2CH_2.COO^-$	Nutritional Biochemicals Co.
4.	Triglycyl glycine	$NH_3^-(CH_2.CO.NH)_3CH_2.COO^-$	Nutritional Biochemicals Co.
5.	DL-α-Alanine	$NH_3^+.CH(CH_3).COO^-$	British Drug Houses
6.	β-Alanine	$NH_3^+.CH_2.CH_2.COO^-$	British Drug Houses
7.	β-Phenyl-DL-α-Alanine		British Drug Houses
8.	DL-Serine		Aldrich Chemical Co. Inc.
9.	DL-Leucine		L. Light and Co.
10.	p-Aminobenzoic acid		British Drug Houses
11.	DL-Aspartic acid		British Drug Houses

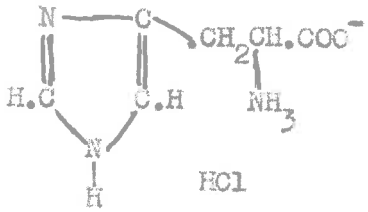
12. L-Glutamic acid — British Drug Houses



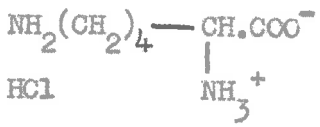
13. DL- and L-Arginine hydrochloride — Nutritional Biochemicals Co. and British Drug Houses respectively



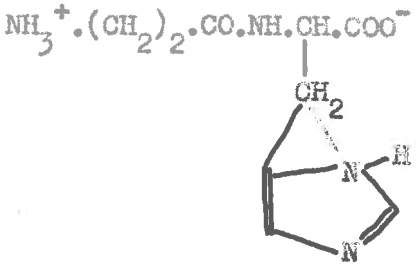
14. DL-Histidine hydrochloride — California Biochemical Research Corp.



15. L-Lysine hydrochloride — " " "



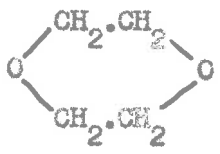
16. L-Carnosine (β-alanyl l-histidine) — L. Light and Co.



Sodium and calcium plus magnesium estimations were done on solutions of compounds 1 to 16. It was found that with the exception

of diglycyl glycine and triglycyl glycine, all compounds contained less than 0.015 equiv. ($Na^+ + Ca^{++} + Mg^{++}$) per mole. The two exceptions contained considerably more than this amount, but after four recrystallizations from distilled water, this quantity was reduced to less than 0.004 equiv./mole.

17. Methyl alcohol $CH_3.OH$

18. p-dioxane 

19. Ethylene glycol $HO.CH_2.CH_2OH$ British Drug Houses (AnalaR)

Compounds 17 and 18 were spectroscopically pure samples supplied by Mr. L. Sparrow, University of Melbourne.

Appendix III

The modified Kjeldahl technique used for nitrogen estimation.

Up to 10 ml. portions of the supernatant solutions were placed in 50 ml. Kjeldahl flasks to which were added 2 ml. of concentrated H_2SO_4 and 0.8g of catalyst mixture (1000g K_2SO_4 + 100g $CuSO_4$ + 10g Selenium) and the contents of the flasks were digested for 30 min. (glycine and its peptides) or 45 min. (the remainder of the compounds). These digestion times were found to be conveniently within the range of 100% recovery. Subsequent ammonia distillations were carried out in a Markham still, after addition of 10 ml. of 50% NaOH solution to the sample. The ammonia distilled was titrated with 0.02N or 0.1N HCl. The indicator used was a 3:1 mixture of methyl red: brom-cresol green (0.1% solution in alcohol).

Appendix IV

The final columns of the tables in this appendix are intended to give a qualitative estimate of the errors involved in each experiment. As discussed in Part II, section 3, ii, the maximum amount adsorbed per tube for each experiment (i.e., the maximum value of (c-d) for each experiment) is inversely related to the experimental error of determining the extent of adsorption (p.62).

Appendix IV

1. The adsorption of glycyl glycine, diglycyl glycine, triglycyl glycine by sodium montmorillonite, suspension pH = 6.16.

Adsorbate	Amount adsorbed m.mole/100g	Equil conc. M	pH	Maximum amount adsorbed per tube, m.mole
glycyl	1.3	3.19×10^{-3}	6.19	
glycine	2.7	6.36×10^{-3}	6.18	
	3.6	1.30×10^{-2}	6.16	
	10.0	3.23×10^{-2}	6.12	
	13.8	6.53×10^{-2}	6.06	
	19.4	9.82×10^{-2}	5.98	0.0744
diglycyl	1.7	3.15×10^{-3}	5.94	
glycine	5.2	6.05×10^{-3}	5.93	
	7.5	1.25×10^{-2}	5.82	
	20.0	3.11×10^{-2}	5.70	
	30.4	6.35×10^{-2}	5.63	
	39.4	9.61×10^{-2}	5.58	0.163
triglycyl	1.7	2.87×10^{-3}	6.08	
glycine	5.0	5.33×10^{-3}	6.02	
	9.1	1.12×10^{-2}	5.96	
	22.2	2.80×10^{-2}	5.82	
	39.8	5.66×10^{-2}	5.66	
	52.2	8.59×10^{-2}	5.58	0.200

Appendix IV

2. The adsorption of glycine, glycyl glycine, diglycyl glycine and triglycyl glycine by calcium montmorillonite, suspension pH = 6.60

Adsorbate	Amount adsorbed m.mole/100g.	Equil.conc. M	pH	Maximum amount adsorbed per tube, m.mole.
glycine	1.5	1.75×10^{-2}	6.54	
	1.7	3.57×10^{-2}	6.32	
	3.2	7.15×10^{-2}	6.20	
	4.9	1.07×10^{-1}	5.98	
	9.1	1.78×10^{-1}	5.92	0.087
glycyl	1.0	8.60×10^{-3}	6.44	
glycine	2.8	1.68×10^{-2}	6.28	
	5.4	3.38×10^{-2}	6.12	
	11.3	6.74×10^{-2}	5.94	
	17.3	1.01×10^{-1}	5.81	0.166
diglycyl	1.4	6.60×10^{-3}	6.10	
glycine	3.2	1.30×10^{-2}	5.97	
	7.8	2.54×10^{-2}	5.80	
	16.3	5.04×10^{-2}	5.64	
	25.0	7.54×10^{-2}	5.64	
	38.3	1.27×10^{-1}	5.65	0.368
triglycyl	1.8	2.52×10^{-3}	6.25	
glycine	4.0	4.98×10^{-3}	6.20	
	7.4	9.98×10^{-3}	6.11	

Appendix IV 2. contd.

triglycyl	19.9	2.45×10^{-2}	6.15	
glycine	39.3	4.91×10^{-2}	6.16	
	49.7	7.67×10^{-2}	5.95	0.477

Appendix IV

3. The adsorption of (i) glycine and glycyl glycine; (ii) diglycyl glycine and triglycyl glycine by hydrogen montmorillonite, clay suspension (i) pH = 3.81; (ii) 2.61.

Adsorbate	Amount adsorbed m.mole/100g.	Equil. conc. M	pH	Maximum amount adsorbed per tube, m.mole.
glycine	13.6	6.23×10^{-3}	4.02	
	27.4	1.24×10^{-2}	4.22	
	38.2	2.84×10^{-2}	4.46	
	46.8	6.32×10^{-2}	4.62	
	49.0	9.94×10^{-2}	4.69	
	63.2	1.70×10^{-1}	4.82	0.399
glycyl	36.9	1.71×10^{-3}	4.15	
glycine	49.2	7.65×10^{-3}	4.68	
	53.0	2.51×10^{-2}	5.02	
	59.0	6.02×10^{-2}	5.14	
	65.6	9.52×10^{-2}	5.16	
	77.3	1.66×10^{-1}	5.14	0.488
diglycyl	26.7	8×10^{-5}	3.07	
glycine	47.6	8.6×10^{-4}	3.66	
	62.1	5.61×10^{-3}	4.61	
	73.9	2.37×10^{-2}	5.18	
	75.8	5.58×10^{-2}	5.36	
	85.0	8.71×10^{-2}	5.42	0.298

Appendix IV 3. contd.

triglycyl	17.4	0	2.90	
glycine	34.1	1.3×10^{-4}	3.13	
	53.8	1.88×10^{-3}	4.04	
	66.3	1.28×10^{-2}	4.66	
	78.0	3.20×10^{-2}	4.80	
	89.8	5.12×10^{-2}	4.84	0.315

Appendix IV

4. The adsorption of glycine and glycyl glycine by calcium montmorillonite at 25° and 0°. In this experiment the shaking was carried out at 25°C in a water bath with variation of ± 0.5°C. At 0°C, temperature was controlled in a water-ice bath. No measurable temperature variation was observed here, or after centrifuging at either temperature. Triplicate analyses were performed on the supernatant solutions.

Adsorbate	Amount adsorbed m.mole/100g.	Equil. conc. M	Maximum amount adsorbed per tube, m.mole.
glycine, 0°C	2.2	2.70×10^{-2}	
	7.0*	5.32×10^{-2}	
	7.9	8.03×10^{-2}	
	10.2	1.08×10^{-1}	
	11.9	1.35×10^{-1}	
	14.5	1.62×10^{-1}	0.133
glycine, 25°C	1.2	2.72×10^{-2}	
	3.7	5.42×10^{-2}	
	5.8	8.09×10^{-2}	
	7.7	1.08×10^{-1}	
	7.1*	1.36×10^{-1}	
	12.0	1.64×10^{-1}	0.110

Appendix IV 4. contd.

glycyl	2.9	1.22×10^{-2}	
glycine,	5.2	2.45×10^{-2}	
0°C	8.1	3.67×10^{-2}	
	10.8	4.89×10^{-2}	
	15.3*	6.66×10^{-2}	
	15.4	7.37×10^{-2}	0.141
glycyl	2.2	1.24×10^{-2}	
glycine,	3.8	2.50×10^{-2}	
25°C	5.8	3.74×10^{-2}	
	7.4	5.00×10^{-2}	
	9.8	6.23×10^{-2}	
	11.1	7.51×10^{-2}	0.102

* Error incurred on transfer of suspension to centrifuge tubes.

Appendix IV

5. The adsorption of diglycyl glycine and triglycyl glycine by calcium montmorillonite at 25° and 0°. Temperature control and analysis as for previous experiments (4).

Adsorbate	Amount adsorbed m.mole/100g.	Equil conc. M	Maximum amount adsorbed per tube, m.mole
diglycyl glycine, 0°C	4.26	9.35×10^{-3}	
	8.80	1.86×10^{-2}	
	14.0	2.77×10^{-2}	
	18.7	3.69×10^{-2}	
	22.7	4.63×10^{-2}	
	26.3	5.59×10^{-2}	0.241
diglycyl glycine 25°C	3.36	9.63×10^{-3}	
	6.47	1.93×10^{-2}	
	10.0	2.89×10^{-2}	
	13.4	3.85×10^{-2}	
	17.9	4.78×10^{-2}	
	21.5	5.73×10^{-2}	0.197
triglycyl glycine	5.66	5.97×10^{-3}	
	12.3	1.17×10^{-2}	
	19.2	1.72×10^{-2}	
	26.2	2.27×10^{-2}	
	32.3	2.86×10^{-2}	
	37.6	3.47×10^{-2}	0.344

Appendix IV 5. contd.

triglycyl	4.22	6.41×10^{-3}	
glycine	9.48	1.25×10^{-2}	
25°C	15.3	1.84×10^{-2}	
	21.4	2.42×10^{-2}	
	27.4 *	3.01×10^{-2}	
	33.6	3.59×10^{-2}	0.308

* Error incurred on transfer of suspension to centrifuge tube

Appendix IV

6. The adsorption of glycine, glycyl glycine, diglycyl glycine and triglycyl glycine by sodium illite.

Adsorbate	Amount adsorbed m.mole /100g	Equil. conc. M	Maximum amount adsorbed per tube, m.mole.
glycine	5.5	2.80×10^{-2}	
	5.6	6.02×10^{-2}	
	6.6	9.17×10^{-2}	
	7.3	1.23×10^{-1}	
	7.3	1.56×10^{-1}	
	7.5	1.88×10^{-1}	0.171
glycyl glycine	3.5	1.41×10^{-2}	
	7.0	4.52×10^{-2}	
	7.6	6.17×10^{-2}	
	9.6	7.70×10^{-2}	
	11.1	9.28×10^{-2}	0.255
diglycyl glycine	4.1	1.13×10^{-2}	
	6.6	2.39×10^{-2}	
	8.7	3.68×10^{-2}	
	10.0	5.03×10^{-2}	
	11.8	6.34×10^{-2}	
	14.2	7.61×10^{-2}	0.326

Appendix IV 6 contd.

triglycyl glycine	2.7	3.70×10^{-3}	
	4.4	8.24×10^{-3}	
	5.6	1.31×10^{-2}	
	6.8	1.81×10^{-2}	
	7.7	2.32×10^{-2}	
	8.9	2.81×10^{-2}	0.204

Appendix IV

7. The adsorption of glycine, glycyl glycine, diglycyl glycine and triglycyl glycine by calcium illite.

Adsorbate	Amount adsorbed m.mole./100g.	Equil. conc. M	Maximum amount adsorbed per tube, m.mole.
glycine	1.6	3.16×10^{-2}	
	3.6	6.29×10^{-2}	
	4.3	9.47×10^{-2}	
	6.4	1.26×10^{-1}	
	6.9	1.58×10^{-1}	
	9.6	1.89×10^{-1}	0.107
glycyl	2.1	1.60×10^{-2}	
glycine	3.5	3.24×10^{-2}	
	4.8	4.87×10^{-2}	
	6.0	6.51×10^{-2}	
	7.2	8.14×10^{-2}	
	8.3	9.79×10^{-2}	0.092
diglycyl	3.1	1.33×10^{-2}	
glycine	4.4	2.73×10^{-2}	
	6.8	4.08×10^{-2}	
	8.3	5.48×10^{-2}	
	10.0	6.85×10^{-2}	
	10.9	8.27×10^{-2}	0.121

Appendix IV 7. contd.

Triglycyl	2.5	4.90×10^{-3}	
glycine	4.0	1.01×10^{-2}	
	5.3	1.55×10^{-2}	
	6.6	2.09×10^{-2}	
	7.9	2.61×10^{-2}	
	8.4	3.18×10^{-2}	0.093

Appendix IV

8. The adsorption of α-alanine, β-alanine, leucine and serine by calcium montmorillonite

Adsorbate	Amount adsorbed m.mole/100g.	Equil. conc. M	Maximum amount adsorbed per tube, m.mole.
α-alanine	1.5	8.03×10^{-3}	
	2.9	1.61×10^{-2}	
	4.3	2.41×10^{-2}	
	4.8	3.25×10^{-2}	
	6.0	4.06×10^{-2}	
	6.0	4.91×10^{-2}	0.055
β-alanine	1.9	8.36×10^{-3}	
	2.4	1.72×10^{-2}	
	4.6	2.54×10^{-2}	
	5.6	3.4×10^{-2}	
	6.4	4.28×10^{-2}	
	6.8	5.16×10^{-2}	0.063
leucine	0.15	5.13×10^{-3}	
	0.75	1.01×10^{-2}	
	0.85	1.53×10^{-2}	
	1.3	2.03×10^{-2}	
	1.2	2.55×10^{-2}	
	1.2	3.07×10^{-2}	0.011

Appendix IV 8. contd.

serine	0.7	8.25×10^{-3}	
	1.6	1.64×10^{-2}	
	2.6	2.46×10^{-2}	
	3.1	3.29×10^{-2}	
	3.2	4.13×10^{-2}	
	3.9	4.96×10^{-2}	0.036

Appendix IV

9. The adsorption of α-alanine, β-alanine, leucine and serine by hydrogen montmorillonite.

Adsorbate	Amount adsorbed m.mole./100g.	Equil. conc. M	Maximum amount adsorbed per tube, m.mole
α-alanine	25.5	5.82×10^{-3}	
	34.6	1.34×10^{-2}	
	40.0	2.13×10^{-2}	
	41.4	2.96×10^{-2}	
	42.3	3.80×10^{-2}	
	43.6	4.64×10^{-2}	0.135
β-alanine	40.6	4.72×10^{-3}	
	51.4	1.25×10^{-2}	
	58.0	2.09×10^{-2}	
	57.8	2.98×10^{-2}	
	59.4	3.86×10^{-2}	
	60.3	4.70×10^{-2}	0.196
leucine	20.7	3.02×10^{-3}	
	29.6	7.26×10^{-3}	
	35.1	1.19×10^{-2}	
	37.0	1.68×10^{-2}	
	38.0	2.19×10^{-2}	
	39.1	2.69×10^{-2}	0.121

Appendix IV 9. contd.

serine	23.7	5.99×10^{-3}	
	32.9	1.35×10^{-2}	
	36.9	2.15×10^{-2}	
	40.0	3.81×10^{-2}	
	41.5	4.65×10^{-2}	0.126

Appendix IV

10. The adsorption of α-alanine, β-alanine, leucine and serine by sodium illite. The two highest points on the leucine isotherm were repeated in duplicate.

Adsorbate	Amount adsorbed m. mole/100g.	Equil. conc. M	Maximum amount adsorbed per tube, m. mole.
α-alanine	3.9	1.17×10^{-2}	
	4.5	2.61×10^{-2}	
	5.0	4.04×10^{-2}	
	5.7	5.47×10^{-2}	
	5.5	6.97×10^{-2}	
	6.7	8.35×10^{-2}	0.155
β-alanine	3.4	1.37×10^{-2}	
	4.7	2.89×10^{-2}	
	5.6	4.49×10^{-2}	
	6.3	6.0×10^{-2}	
	6.8	7.63×10^{-2}	
	8.3	9.15×10^{-2}	0.192
leucine	0.80	4.67×10^{-3}	
	1.1	9.71×10^{-3}	
	1.6	1.99×10^{-2}	
	2.1	3.00×10^{-2}	0.049

Appendix IV 10. contd.

serine	2.3	1.37×10^{-2}	
	2.9	2.88×10^{-2}	
	3.5	4.39×10^{-2}	
	4.2	5.89×10^{-2}	
	4.3	7.44×10^{-2}	
	4.8	8.95×10^{-2}	0.110

Appendix IV

11. The adsorption of α-alanine, β-alanine, leucine and serine by calcium illite. All points on the leucine isotherm were repeated in duplicate.

Adsorbate	Amount adsorbed m.mole./100g.	Equil. conc. M	Average Maximum amount adsorbed per tube, m. mole.
α-alanine	0.6	1.44×10^{-2}	
	2.1	2.86×10^{-2}	
	2.4	4.32×10^{-2}	
	4.4	5.70×10^{-2}	
	5.0	7.15×10^{-2}	
	6.3	8.56×10^{-2}	0.071
β-alanine	0.9	1.59×10^{-2}	
	2.3	3.15×10^{-2}	
	3.1	4.74×10^{-2}	
	4.5	6.31×10^{-2}	
	5.8	7.88×10^{-2}	
	7.1	9.51×10^{-2}	0.079
leucine	0.3	1.04×10^{-2}	
	0.7	2.07×10^{-2}	
	1.1	3.10×10^{-2}	0.012
serine	0.9	1.51×10^{-2}	
	1.6	3.02×10^{-2}	

Appendix IV 11. contd.

serine	2.1	4.54×10^{-2}	
	3.4	6.04×10^{-2}	
	4.0	7.56×10^{-2}	
	5.0	9.06×10^{-2}	0.057

Appendix IV

12. The adsorption of aspartic acid, glutamic acid, phenyl alanine and p-amino benzoic acid by hydrogen montmorillonite.

Adsorbate	Amount adsorbed m. mole./100g.	Equil. conc. M	Maximum amount adsorbed per tube, m mole.
aspartic acid	13.0	3.65×10^{-3}	
	24.5	7.45×10^{-3}	
	31.5	1.17×10^{-2}	
	37.9	1.60×10^{-2}	
	42.5	2.05×10^{-2}	
	45.6	2.51×10^{-2}	0.133
glutamic acid	19.7	2.98×10^{-3}	
	31.2	6.77×10^{-3}	
	37.7	1.10×10^{-2}	
	44.0	1.54×10^{-2}	
	47.8	1.99×10^{-2}	
	50.6	2.45×10^{-2}	0.148
phenyl alanine	34.0	3.46×10^{-3}	
	45.0	9.19×10^{-3}	
	50.5	1.55×10^{-2}	
	53.9	2.20×10^{-2}	
	56.3	2.85×10^{-2}	
	56.5	3.53×10^{-2}	0.165

Appendix IV 12, contd.

p-amino benzoic	27.8	9.9×10^{-4}	
acid	44.4	3.10×10^{-3}	
	51.6	6.13×10^{-3}	
	58.1	9.23×10^{-3}	
	61.5	1.26×10^{-2}	
	66.4	1.59×10^{-2}	0.194

12a. The adsorption of phenyl alanine by calcium montmorillonite. The isothermal point given was repeated in duplicate and K_m was calculated on the assumption that this value lies on a linear isotherm passing through the origin.

phenyl alanine	5.3	3.29×10^{-2}	0.049

Appendix IV

13. The adsorption of DL- and L- arginine hydrochlorides and glycine hydrochloride by calcium montmorillonite. Clay suspension pH = 6.60

Adsorbate	Amount adsorbed m.mole/ 100g	Equil.conc. M	Ca^{++} liberated m.equiv./ 100g	Ca^{++} equil. conc., N x 10^{3}	pH	Maximum amount adsorbed per tube, m mole.
glycine hydrochloride	-0.8	3.70×10^{-3}	6.3	2.04	3.32	
	2.9	5.93×10^{-3}	9.6	3.09	2.83	
	5.5	1.20×10^{-2}	14.9	4.84	2.52	
	16.5	2.90×10^{-2}	27.1	8.78	2.18	
	34.2	5.77×10^{-2}	45.0	14.6	1.97	
	43.8	8.89×10^{-2}	57.1	18.5	1.85	0.420
DL-arginine hydrochloride	7.8	5.6×10^{-4}	6.9	2.23	6.06	
	14.6	1.45×10^{-3}	13.3	4.29	5.87	
	25.1	4.19×10^{-3}	24.0	7.76	5.90	
	49.6	1.48×10^{-2}	49.6	16.1	6.13	
	63.2	4.12×10^{-2}	66.0	21.4	5.92	0.638
L-arginine hydrochloride	8.7	5.2×10^{-4}	6.9	2.25	6.20	
	15.6	1.61×10^{-3}	13.6	4.40		
	26.7	4.67×10^{-3}	24.8	8.03		
	67.8	4.46×10^{-2}	65.9	21.4		
	70.0	7.72×10^{-2}	72.9	23.6	6.09	0.671

Appendix IV

14. The adsorption of arginine, histidine and lysine hydrochlorides by sodium montmorillonite. Clay suspension pH = 6.10.

Adsorbate	Amount adsorbed m.mole./ 100g.	Equil. conc. M	Na^{++} liberated m.equiv./ 100g.	Na^{++} equil. conc. N x 10^{3}	pH	Maximum amount adsorbed per tube, m mole.
L-arginine	24.7	1.2×10^{-4}	26.2	3.40	6.15	
hydro - chloride	44.5	8.7×10^{-4}	45.0	5.84	6.05	
	63.0	5.10×10^{-3}	65.7	8.51	5.94	
	77.4	2.31×10^{-2}	77.3	10.01	5.83	
	81.2	5.57×10^{-2}	77.8	10.07	5.68	
	75.8	8.95×10^{-2}	77.0	9.97	5.56	0.292
histidine	24.2	2.1×10^{-4}	25.5	3.30	5.97	
hydro- chloride	44.8	8.9×10^{-4}	45.9	5.95		
	68.4	4.52×10^{-3}	66.7	8.64		
	81.5	2.29×10^{-2}	76.3	9.88	4.53	
	86.8	5.56×10^{-2}	76.9	9.96	4.21	
	90.7	8.86×10^{-2}	77.1	9.99	4.10	0.349
lysine	17.6	5.0×10^{-4}	26.1	3.38	6.00	
hydro- chloride	31.8	1.44×10^{-3}	41.6	5.39	5.76	
	46.4	5.10×10^{-3}	58.8	7.62	5.60	
	53.9	2.09×10^{-2}	71.0	9.19	5.05	
	60.7	4.76×10^{-2}	74.7	9.67	4.92	
	56.8	7.72×10^{-2}	76.2	9.86	4.93	0.180

Appendix IV

15. The adsorption of carnosine and histidine hydrochlorides by sodium montmorillonite.

Adsorbate	Amount adsorbed m.mole/ 100 g.	Equil. conc. M	Na^{++} liberated m. equiv./ 100 g.	Na^{++} equil. conc. N x 10^3	Maximum amount adsorbed per tube, m mole.
carnosine	22.1	8.9×10^{-5}	20.8	2.83	
	41.8	5.0×10^{-4}	38.4	5.27	
	61.2	3.85×10^{-3}	63.3	8.05	
	22.0	1.99×10^{-2}	73.8	9.31	
	75.3	4.86×10^{-2}	76.0	9.61	0.189
histidine	19.4	3.1×10^{-4}	22.2	2.65	
	38.4	6.9×10^{-4}	41.3	4.90	
	62.7	3.15×10^{-3}	63.1	8.07	
	74.3	1.84×10^{-2}	73.0	9.41	
	76.0	4.64×10^{-2}	75.3	9.69	0.190

Appendix IV

16. The adsorption of histidine by calcium montmorillonite and by sodium and calcium illite. The adsorption of carnosine by sodium illite.

System	Amount adsorbed m.mole/100g.	Equil. conc. M	cation liberated m equiv./ 100 g.	cation equil. conc. N x 10^3	Maximum amount adsorbed per tube, m mole.
carnosine, sodium	2.5	2.7×10^{-5}	2.5	2.97	
illite	5.0	5.7×10^{-5}	5.1	6.08	
	9.7	4.6×10^{-4}	10.1	12.2	
	15.9	4.88×10^{-3}	16.3	19.6	0.365
histidine, sodium	2.4	2.3×10^{-5}	2.4	2.85	
illite	4.6	2.2×10^{-4}	4.6	5.53	
	7.4	1.47×10^{-3}	8.4	10.1	
	11.7	3.24×10^{-3}	11.9	14.4	
	17.4	7.83×10^{-3}	16.7	20.1	0.400
histidine, calcium	3.8	6.3×10^{-4}	4.0	2.35	
illite	6.8	1.73×10^{-3}	6.5	3.81	
	11.4	4.72×10^{-3}	11.1	6.98	
	14.7	8.53×10^{-3}	14.8	8.68	
	17.8	1.81×10^{-2}	17.8	10.4	0.201

Appendix IV 16, contd.

histidine,calcium	4.7	5.9×10^{-4}	4.3	2.01	
montmorillonite	9.4	1.19×10^{-3}	9.0	4.21	
	19.3	2.18×10^{-3}	19.0	8.89	
	28.9	3.33×10^{-3}	27.0	12.6	
	44.8	7.11×10^{-3}	44.8	21.0	0.410

Appendix V

Calculation of the ratio of the surface area covered by adsorbate to the total surface area available.

1. Montmorillonite

The surface area of montmorillonite has been determined by calculation from the area of the basal surfaces of the unit cell, 2×46 Å^2, and the unit cell weight, 765, as 725 m^2/g expressed on the basis of sodium clay free from interlamellar water. This value will be used to calculate θ, the surface covered when two layers of adsorbed molecules occupy the interlamellar regions. When there is only one layer of adsorbed molecules shared by adjacent clay faces the value for the surface area must be reduced by half, and allowance made for the basal area on the external surfaces of crystallites. The total external area can be measured by nitrogen adsorption, when the value obtained is 50 m^2/g. (Quirk and Aylmore, 1960). The value to be used in calculating θ', the surface coverage when only one layer of adsorbed molecules occupies the interlamellar positions, is therefore, $(725-50)/2 + 50 = 388$ m^2/g.

In both of these calculations it has been assumed that the edge areas of the montmorillonite crystals are negligible in comparison with the total basal areas, an assumption justified by observations made on electron micrographs. If the edge area is taken as 15% of the nitrogen surface, the values to be used in calculating θ and θ' are increased by only 1%. The manner in which the adsorbed molecules pack on the surface is not known. At low surface coverages where blocking of sites is the most important consideration, it is probably best to

assume that the adsorbed molecules occupy an area equivalent to the smallest containing rectangle. When a large proportion of the surface is covered, it is probable that some adjustment and possibly hydrogen bonding between adjacent molecules occurs as it does in the crystal. The area which should then be used is probably close to the precisely determined molecular projection. Values for these areas are given in table 1 below. Values of θ and θ' calculated on the basis of the rectangular projection are denoted by the subscript r and those calculated on the basis of the molecular projection by the subscript m. Part of the surface of the dried sodium or calcium montmorillonite complexes is probably covered by exchangeable cations and may not be available to the adsorbed molecules. The maximum value of θ'_r obtained for the adsorption of glycine and its peptides by these clays in the present work was 0.8 (appendix VII) while the maximum θ'_m values for the non-hydrated and hydrated cations is approximately 0.04 and 0.7 respectively. It is not known to what extent water molecules in the hydration shell of the exchangeable cations are replaced by adsorbate molecules in the dried complexes. Also, in suspension it is probable that the exchangeable cations will be dissociated to a large extent from the clay surface. Ion dipole interactions (found to be important in the present work, Part II) probably orient a significant proportion of the adsorbed molecules around the exchangeable cations and the concept of surface coverage is not strictly applicable to the clays in suspension. Therefore, no allowance has been made for surface coverage by the exchangeable cations.

The areas of the adsorbed molecules of glycine and its peptides

have been obtained from projections made assuming that they lie on the surface with their shortest axis perpendicular to the clay surface. Bond distances within the molecules have been obtained from X-ray analyses of glycine and glycyl glycine crystals (Albrecht and Corey, 1939; Hughes and Moore, 1949) and van der Waals' radii have been taken from Pauling (1960).

2. Illite

The surface area if the illite sample used was 106 m^2/g from the nitrogen adsorption isotherm and about 150 m^2/g when cetyl pyridinium bromide was used. The higher value for the total surface area probably indicates that the illite sample contains expanding lattice layers, presumably due to interstratified vermiculite. Since the use of cetyl pyridinium bromide to determine surface areas is still in the developmental stage (Greenland and Quirk, in press) the nitrogen surface area has been chosen in calculating the figures for illite in Table 1, it being recognised that the values of θ obtained using this value may be higher than the true value by up to 30%.

Table 1

Adsorbate	Projected molecular area, A^2		Calculated quantity required to give a monolayer of adsorbed molecules, m mole./100 g.					
	of containing rectangle	of molecule	Montmorillonite				Illite	
			In complete single layer complex		In complete double layer complex		complete single layer complex	
			$\theta'_r(1)$	$\theta'_m(2)$	$\theta_r(1)$	$\theta_m(2)$	$\theta_r(1)$	$\theta_m(2)$
glycine	30.8	24.8	207	258	390	483	57.1	70.7
glycyl glycine	57.4	41.9	112	154	208	288	30.4	42.1
diglycyl glycine	84.0	54.4	77	108	143	202	20.9	29.6
triglycyl glycine	101	76.8	63	84	118	157	17.3	23.0

(1) based on rectangular projection of molecule

(2) based on true molecular projection

The discussion of surface coverage in terms of θ values has been restricted to the adsorption of glycine and its peptides.

Appendix VI

The determination of saturation solubilities of glycine and its peptides in water and in $CaCl_2$ solution.

The solutes were recrystallized from water where necessary and carefully dried against P_2O_5 in a vacuum desiccator.

A calcium chloride solution of normality

$$\frac{0.90 \text{ m.equiv./g.}}{0.363 \text{ cm}^3/\text{g.}} = 2.49$$

was prepared and checked by titration with E.D.T.A. (see appendix I for method).

Four portions of this solution and four portions of distilled water were added to glass ampoules giving four pairs of ampoules to which were added in turn a calculated excess of the four solutes studied. The ampoules were sealed and sterilized at 121°C for 30 min. and then cooled to initiate crystallization. They were then placed in a water bath controlled at 25 ± 0.5°C and gently shaken for 14 days. At the end of that period they were opened and filtered at 25°C. 5 ml. portions were taken at 25°C, weighed and diluted to 25 ml. The diluted solutions were analysed by the Kjeldahl technique (appendix III). These experiments were not intended to produce solubility data of the accuracy of earlier workers (see Cohn and Edsall, 1943) but simply to obtain data for the solubility of these substances in a 2.49N $CaCl_2$ solution more accurately than could be obtained by interpolation from existing results. No recorded data could be found for the solubility of triglycyl glycine in $CaCl_2$ solutions and also the results were required in mole./l. rather than in the more usual molality.

Solute	Solvent	Density g./ml.	Concentration mole./l.	Solubility ratio $\frac{S}{S_o}$	$\log_{10} \frac{S}{S_o}$
glycine	H_2O	1.078	2.88	1.35	0.130
"	$CaCl_2$ soln.		3.88		
glycyl glycine	H_2O	1.083	1.51	1.51	0.178
" "	$CaCl_2$ soln.		2.28		
diglycyl glycine	H_2O	1.032	0.98	2.49	0.396
" "	$CaCl_2$ soln.		2.45		
triglycyl glycine	H_2O	1.007	0.106	3.84	0.584
" "	$CaCl_2$ soln.		0.408		

Appendix VII

Complete results of x-ray analyses.

1. Adsorption of glycine and its peptides by calcium montmorillonite. Data for moist complexes. The Stern layer concentration is obtained by dividing the amount of solute adsorbed (m mole./g.) by the volume of the Stern layer (0.36 cm^3/g).

Adsorbate	Amount adsorbed, m mole/ 100g.	Stern layer concentration, M	d(001) Å	Expansion in basal spacing, d(001)-19.3 Å
moist calcium montmorillonite	-	-	19.3	
glycine	14.5	0.4	19.7	0.4
glycyl glycine	3.0	0.08	19.5	0.2
	8.1	0.22	19.3	0
	9.5	0.26	19.7	0.4
	10.8	0.30	19.5	0.2
	11.0	0.30	20.2	0.9
	13.5	0.37	20.2	0.9
	16.0	0.44	21.8	2.5
diglycyl glycine	6.5	0.18	19.3	0
	8.8	0.24	19.7	0.4
	13.5	0.37	21.5	2.2
	14.0	0.39	20.9	1.6
	17.9	0.49	22.2	2.9
	21.5	0.59	22.1	2.8
	26.3	0.73	22.2	2.9

Appendix VII 1, contd.

triglycyl glycine	26.2	0.72	19.34	0.04
	33.4	0.92	20.1	0.8
	37.6	1.04	20.3	1.0
Earlier results				
moist calcium montmorillonite			20.0	
glycine	10	-	22.4	2.4
glycyl glycine	17	0.47	22.5	2.5
diglycyl glycine	12.5	0.35	22.5	2.5
	25	0.69	24.0	4.0

2. Adsorption of glycine and its peptides by calcium montmorillonite.

Dry complexes.

Adsorbate	Amount adsorbed m mole./100g	d(001) Å	d(003) Å	d(004) Å
glycyl glycine	12	13.15		3.12 (v.Br.) **
diglycyl glycine	24	13.20		3.17
triglycyl glycine	20	13.7 (Br.) *		3.20
	39	15.2 (Br.) *		3.24
	50	16.40	5.42	3.24

* Br. = Broad

** v.Br. = very broad

3. Adsorption of the glycine peptides by sodium montmorillonite.

Dry complexes.

Adsorbate	Amount adsorbed m mole./100g	d(001) Å	d(004) Å	Δ d(001)-9.5 Å	θ'_r
glycyl glycine	14	12.84	3.17	3.3	0.12
diglycyl glycine	8	12.80	3.16	3.3	0.10
	20	12.75	3.18	3.3	0.26
triglycyl glycine	9	12.75	3.16	3.3	0.14
	40	13.00	3.21	3.5	0.63

4. Adsorption of glycine and its peptides by hydrogen montmorillonite.

Dry complexes.

Adsorbate	Amount adsorbed m mole./100g.	d(001) Å	d(002) Å	d(003) Å	d(004) Å	d(005) Å	Δ d(001)-9.5 Å	θ'_r
glycine	47	12.70	6.24	4.30	3.13		3.2	0.23
	63	12.50			3.12		3.0	0.30
glycyl glycine	53	13.00			3.17		3.5	0.47
diglycyl glycine	27	12.70			3.16		3.2	0.35
	48	13.00	6.39		3.18		3.5	0.62
	62	13.20	6.31 (V.W.) +		3.20		3.7	0.81
	74	14.14 (Br.) *			3.23			0.96
	78	14.70			3.20			1.01
	85	16.33		5.43	4.10	3.25	6.8	1.10

+ V.W. = very weak * Br. = broad

Appendix VII, 4 contd.

triglycyl glycine	34	13.12	6.44	3.20	3.6	0.54
	54	13,30		3.21	3.8	0.86
	66	15.25		3.23		1.05
	78	16.0(Br.*)		3.24		1.24
	90	16.75(Br,*)		3.26		1.43

* Br. = broad

5. Adsorption of α-alanine, β-alanine, leucine, serine, aspartic acid, glutamic acid, p-amino benzoic acid and phenyl alanine by hydrogen montmorillonite. Both moist and dry complexes.

Adsorbate	Amount adsorbed m mole/ 100 g.	d(001) Å	d(002) Å	d(003) Å	d(004) Å	d(005) Å	Δ d(001)-9.5 Å
α-alanine (dry)	35	13.05			3.19		3.6
(moist)	41	13.9					
(dry)	44	13.70	6.55		3.20		4.2
(moist)	44	14.1					
β-alanine	60	13.00	6.50	4.35	3.22		3.5
(dry) (moist)	60	13.2(W)‡					
leucine	30	14.2			3.54		4.7
(dry)	35	14.30		4.79	3.50	2.99	4.8
(moist)	37	15.8					
(dry)	39	14.80		4.90	3.64	2.93	5.3
(moist)	39	15.3(m)*					
serine	42	13.20			3.24		3.7
(dry) (moist)	42	15.0(V.W.)					
aspartic acid (dry)	25	12.40			3.14		2.9
	38	12.80	6.38	4.24	3.19		3.3
(moist)	43	13.3					
(dry)	46	12.90	6.35		3.19		3.4
(moist)	46	13.5					

* m = medium ‡ W = weak † V.W. = very weak

Appendix VII, 5 contd.

glutamic acid (dry)	31	13.20	6.60		3.23		3.7
(moist	31	13.5 (V.W.)					
(dry)	44	13.23			3.25		3.7
(moist)	48	13.3					
(dry)	51	13.23	6.60	4.42	3.26		3.7
p-amino benzoic acid (dry)	44	12.90	6.36	4.28	3.17		3.4
(moist)	52	14.5					
	62	14.7					
(dry)	66	13.00	6.41		3.18		3.5
(moist)	66	14.9					
phenyl alanine (dry)	45	13.70	6.75	4.52	3.38	2.70	4.2
	54	13.85	6.80	4.53	3.39	2.72	4.4
(moist)	51	15.60		5.14		3.08	
	56	15.70	7.77	5.15		3.07	

6. Adsorption of arginine, carnosine, histidine and lysine by sodium montmorillonite. Both moist and dry complexes. The moist complexes were washed free of Cl^- before examination.

Adsorbate	Amount adsorbed m mole./ 100 g.	d(001) Å	d(002) Å	d(003) Å	d(004) Å	Δ d(001)-9.5 Å
arginine (dry)	63	13.35			3.28	3.9
	76	13.60		4.43	3.40	4.1
(moist)	76	16.6				
(dry)	81	13.60			3.37	
carnosine (dry)	75	13.52		4.38	3.32	4.0
(moist)	75	13.7				
histidine (dry)	68	13.35			3.30	3.9
	87	13.35		4.43	3.30	3.9
	91	13.52		4.38	3.32	4.0
(moist)	91	13.7				
lysine (dry)	46	13.31			3.30	3.8
	57	13.55			3.34	4.1
	61	13.60			3.34	4.1
(moist)	61	>100				

Appendix VIII

Infra red data

Frequencies (ν) expressed in cm^{-1}.

Adsorption of methyl alcohol, ethylene glycol and p-dioxane by sodium montmorillonite. No change was observed in the absorption band frequencies or relative intensities on adsorption of these liquids.

Methyl alcohol	3342 (νOH)	2965 ($\nu_{as}CH_3$)	2833 ($\nu_{s}CH_3$)
Ethylene glycol	3340 (νOH)	2933 ($\nu_{as}CH_2$)	2875 ($\nu_{s}CH_2$)
p-dioxane		2968, 2926, 2890 2861	

Acknowledgements

It is a pleasure to acknowledge the help and guidance of my supervisors Dr. J. P. Quirk and Dr. D. J. Greenland, Reader and Lecturer respectively in Soil Science of the Department of Agricultural Chemistry. There were many helpful discussions with other members of staff of this Department, in particular with Dr. A. M. Posner.

The technical assistance of Mr. R. M. Lilley in determining the surface areas of the illite sample used is also gratefully acknowledged.

Professor A. S. Buchanan of the Chemistry Department, the University of Melbourne, kindly granted me access to the Perkin Elmer model 112, infra-red spectrometer in that Department.

Finally, the present work would not have been possible but for the fact that the Victorian Department of Agriculture, Melbourne, granted me two and one half years study leave, during which the work was carried out.

REFERENCES

Albrecht, G. and Corey, R. B. 1939. J. Amer. chem. Soc. 61, 1087

Aldrich, D. G. and Buchanan, J. R. 1958. Proc. Soil Sci. Soc. Amer. 22, 281

Allison, F. E., Sherman, M. S. and Pinck, L. A. 1949. Soil Sci. 68, 463

Anderson, D. M. and Low, P. F. 1958. Proc. Soil Sci. Amer. 22, 99

Badger, R. M. 1940. J. chem. Phys. 8, 288

Barrer, R. M. 1958. Proc. 10th Symp. Colston Res. Soc., Bristol, p.6

Barrer, R. M. and Kelsey, K. E. 1961. Trans. Faraday Soc. 57, 452

Barrer, R. M. and Perry, G. S. 1961. J. chem. Soc. 850

Barshad, I. 1952. Proc. Soil Sci. Soc. Amer. 16, 176

Barshad, I. 1955. Proc. 1st Nat. Conf. Clays and clay Technol., Calif. Dept. Nat. Resources, Div. Mines Bull. 169, 70

Barshad, I. 1959. Proc. 8th Nat. Conf. Clays and clay Min., Norman, p.84

Berger, G. 1941. Chem. Weakbl. 38, 42

Bernal, J. D. and Fowler, R. H. 1933. J. chem. Phys. 1, 515

Bolt, G. H. and Warkentin, B. P. 1956. Trans. 6th Inter. Cong. Soil Sci., Paris, I, 33

Bradley, W. F. 1945. J. Amer. chem. Soc. 67, 975

Brand, J. C. D., Eglington, G. and Morman, J. F. 1960. J. chem. Soc. 2526

Bremner, J. M. 1950. Biochem. J. 47, 538

Bremner, J. M. 1952. J. Sci. Fd & Agric. 3, 497

Bremmer, J. M. 1955. J. agric. Sci. 46, 247

Bremmer, J. M. 1956. Soils & Fert. 19, 115

Brown, G. 1955. Clay Min. Bull. 2, 294

Caillere, S. and Henin, S. 1957. Bull. Gr. franc. Argiles 9, 77

Campbell, A. N. and Kartzmark, E. M. 1960. Can. J. Chem. 38, 652

Chaberek, S. and Martell, A. E. 1959. Organic Sequestering Agents. New York.

Chessick, J. J. and Zettlemeyer, A. C. 1961. J. phys. Chem. 65, 1672

Cohn, E. J. and Edsall, J. T. 1943. Proteins, Amino Acids and Peptides New York.

Coleman, N. T. and Craig, D. 1961. Soil Sci. 91, 14

Cowan, C. T. and White, D. 1958. Trans. Faraday Soc. 54, 691

Cummings, T., Garven, H. C., Giles, C. H., Rahman, S. M. K., Sneddon, J. G. and Stewart, C. E. 1959. J. chem. Soc. 535

Dadd, C. C., Fowden, L. and Pearsall, W. H. 1953. J. Soil Sci. 4, 69

Dahr, N. A., Timer, B. and Guha, R. 1955. Proc. nat. Acad. Sci. India A 24, 341

Damour, A. A. and Salvetat . 1847. Ann. Chim. (Phys.) (III) 21, 376

Deshpande, K. B. and Marshall, C. E. 1961. J. phys. chem. 65, 33

Dewel, H. and Huber, G. 1951. Helv. chim. Acta 34, 1697

Edelman, C. H. and Favajee, J. Ch. L. 1940. Z. Kristallogr. 102, 417

Ensminger, L. E. and Gieseking, J. E. 1939. Soil Sci. 48, 467

Ensminger, L. E. and Giesking, J. E. 1942. Soil Sci. 53, 205

Estermann, E. F., Peterson, G. H. and McLaren, A. D. Proc. Soil Sci. Soc. Amer. 23, 31

Everett, D. H. 1957. Proc. 2nd Inter. Cong. Surface Activity p.212

Everett, D. H. 1958. 10th Symp. Colston Res. Soc., Bristol, p. 28

Falkenhagen, H. 1934. Electrolytes. Oxford

Folman, M. and Yates, D. J. C. 1948. Trans. Faraday Soc. 54, 1684

Folman M. and Yates, D. J. C. 1958. Proc. roy. Soc. A 246, 32

Forslind, E. 1952. Acta Polytech. 3, No. 5

Frank, H. S. and Evans, M. W. 1945. J. chem. Phys. 13, 507

Freeguard, G. F. and Stock, R. 1961. Nature, 192, 257

Fripiat, J. J., Chaussidon, J. and Touillaux, R. 1960. J. phys. Chem. 64, 1234

Frohnsdorff, G. J. C. and Kington, G. L. 1958. Proc. roy. Soc. A 247, 469

Gieseking, J. E. 1949. Advanc. Agron. 1, 159

Giles, C. H. 1954. Disc. Faraday Soc. 16, 112

Giles, C. H. 1957. Hydrogen Bonding (Ed. D. Hadzi) New York p. 449

Giles, C. H., MacEwan, T. H., Nakhwa, S. N. and Smith, D. 1960. J. chem. Soc. 3973

Glaeser, R. 1951. C. R. Acad. Sci. Paris 232, 1496

Glasstone, S. 1946. Textbook of Physical Chemistry (2nd Ed.) New York

Goates, J. R. and Bennett, J. S. 1957. Soil Sci. 83, 325

Goldsmith, B. J. and Muir, J. 1960. Trans. Faraday Soc. 56, 1656

Greene-Kelly, R. 1955. Trans. Faraday Soc. 51, 412

Greene-Kelly, R. 1957. The Differential Thermal Investigation of Clays (Ed. R. C. MacKenzie) London p. 140

Greenland, D. J., Laby, R. H. and Quirk, J. P. 1962. Trans. Faraday Soc. 58, 829.

Greenland, D. J. and Russell, E. W. 1955. Trans. Faraday Soc. 51, 1300

Greenwood, D. J. and Lees, H. 1960a. Plant & Soil 12, 69

Greenwood, D. J. and Lees, H. 1960b. Plant & Soil 12, 175

Grim, R. E. 1953. Clay Mineralogy. New York

Grim, R. E. and Bradley, W. F. 1939. J. Amer. ceram. Soc. 22, 157

Gruner, J. W. 1935. Amer. Min. 20, 475

Hambley, A. N. 1961. Rev. pur. appl. Chem. 11, 212

Haxaire, A. and Bloch, J. M. 1956. Bull. Soc. franc. Mineral. crist. 79, 464

Heilbron, I. M. 1934. Dictionary of Organic Compounds. London

Heller, L. and Kalman, Z.H. 1961. Clay Min. Bull. 4, 213

Hendricks, S. B. 1941. J. phys. Chem. 45, 65

Hendricks, S. B. and Jefferson, M. E. 1938. Amer. Min. 23, 863

Hendricks, S. B., Nelson, R. A. and Alexander, L. T. 1940. J. Amer. chem. Soc. 62, 1457

Hockey, J. A. and Pethica, B. A. 1961. Trans. Faraday Soc. 57, 2247

Hodgman, C. D. 1957 (Ed.) Handbook of Chemistry and Physics (38th Ed.) Cleveland

Hoffmann, R. W. and Brindley, G. W. 1960. Geochim et cosmoch. Acta 20, 15

Hofmann, U., Endall, K. and Wilm, D. 1933. Z. Kristalbgr. 86, 340

Holzner, J. 1935. Chem. d. Erde 9, 464

Hughes, E. W. and Moore, W. J. 1949 J. Amer. chem. Soc. 71, 2618

Jackson, W. W. and West, J. 1930. Z. Kristallogr. 76, 211

Jackson, W. W. and West, J. 1933. Z. Kristallogr. 85, 160

Jura, G. and Hill, T. L., 1952. J. Amer. chem. Soc. 74, 1598

Jurinak, J. J. and Volman, D. H. 1961. J. phys. Chem. 65, 1853

Keay, J. and Wild, A. 1961. Clay Min. Bull. 4, 221

Kurosaki, S., Tsuchiya, T. and Kawai, R. 1957. Nippon Kagaku Zasshi 78, 1806 (from Chem. Abstr. 52, 12487 d)

Laby, R. H. M.Sc. Thesis, University of Melbourne

Lin, C. and Coleman, N. T. 1960. Proc. Soil Sci. Soc. Amer. 24, 444

Lippincott, E. and Schroeder, R. 1955. J. chem. Phys. 23, 1099

Lord, R. C. and Merrifield, R. E. 1953. J. chem. Phys. 21, 166

Low, P. F. 1955. Proc. Soil Sci. Soc. Amer. 19, 135

Low, P. F. 1959. Proc. 8th Nat. Conf. Clays and clay Min., Norman, p. 170

Low, P. F. 1961. Advanc. Agron. 13, 269

Low, P. F. and Anderson, D. M. 1958. Proc. Soil Sci. Soc. Amer. 22, 22

Lucchesi, P. J. and Glasson, W. A. 1956. J. Amer. chem. Soc. 78, 1347

Lyast, I. T. 1958. J. tech, Phys., Moscow 28, 827 (from Chem. Abstr. 53, 16623h)

MacEwan, D. M. C. 1948. Trans. Faraday Soc. 44, 349

MacKenzie, R. C. 1957. The Differential Thermal Investigation of Clays, Aberdeen.

McLaren, A. D. 1954. Proc. Soil Sci. Soc. Amer. 18, 170

McLaren, A. D. and Estermann, E. F. 1956. Arch. Biochem. Biophys. 61, 158

McLaren, A. D., Peterson, G. H. and Barshad, I. 1958. Proc. Soil Sci. Soc. Amer. 22, 239

Macey, H. H. 1942. Trans. Brit. Ceram. Soc. 41, 73

Marshall, C. E. 1935. Z. Kristallogr. 91, 433

Martin, A. E. 1947. Nature 159, 403

Martin, R. T. 1959. Proc. 8th Nat. Conf. Clays and clay Min., Norman, p. 102

Martin, R. T. 1960. Proc. 9th Nat. Conf. Clays and clay Min., Lafayette (in press).

Mathieson, A. McL. and Walker, G. F. 1954. Amer. Min. 39, 231

Molloy, M. W. and Kerr, P. F. 1961. Amer. Min. 46, 583

Mooney, R. W., Keenan, A. G. amd Wood, L. A. 1952a. J. Amer. chem. Soc. 74, 1367

Mooney, R. W., Keenan, A. G. and Wood, L. A. 1952b. J. Amer. chem. Soc. 74, 1371

Mortland, M. M. 1961. Nature, 192, 481

Muir, J. 1954. Trans. Faraday Soc. 50, 249

Ohlrogge, A. J. 1962. Soil Sci. 93,30

Olivier, J. P. and Ross, S. 1957. Proc. 2nd Inter Cong. Surface Activity II, 46

Orchison, H. D. 1955. Soil Sci 79, 71

Partington, J. R. 1954. An Advanced Treatise on Physical Chemistry Vol. 5. London

Paul, E. A. and Schmidt, E. L. 1960. Proc. Soil Sci Soc. Amer. 24, 195

Paul, E. A. and Schmidt, E. L. 1961. Proc. Soil Sci. Soc. Amer. 25, 359

Pauling, L. 1930. Proc. nat. Acad. Sci., Wash. 16, 123

Pauling, L. 1960. The Nature of the Chemical Bond (3rd Ed.) New York.

Payne, T. M. B., Toualt, J. W. and Katznelson, H. 1956. Soil Sci. 82, 521

Perrin, R. M. S. 1955. Clay Min. Bull. 2, 307

Pfeiffer, P. and Wurgler, J. 1916. Z. physiol. chem. 97, 128

Pinck, L. A. and Allison, F. E. 1951. Science 114, 130

Pinck, L. A., Dyal, R. S. and Allison, F. E. 1954. Soil Sci. 78, 109

Pinck, L. A., Holten, W. F. and Allison, F. E. 1961. Soil Sci. 91 22

Pinck, L. A., Soulides, D. A. and Allison, F. E. 1961. Soil Sci. 91, 94

Prelog, V. 1950. J. chem. Soc. 420

Prichard, H. O. and Skinner, H. A. 1955. Chem. Rev. 55, 745

Putnam, H. D. and Schmidt, E. L. 1959. Soil Sci. 87, 22

Quastel, J. H. and Scholefield, P. G. S. 1949. Nature 164, 1068

Quirk, J. P. and Aylemore, L. A. G. 1960. Trans. 7th Inter. Cong. Soil Sci. Madison, II, 378

Radoslovich, E. W. 1960. Acta cryst., Camb. 13, 919

Robins, J. S. 1952. Soil Sci. 74, 127

Robinson, R. A. and Stokes, R. H. 1959. Electrolyte Solutions (2nd Ed.) London p.18

Rosenqvist, I. Th. 1955, Norweg. Geotech Inst. Publ. No. 9

Russell, E. W. 1934. Phil. Trans. A 233, 361

Schmidt, C. L. A. 1938. The Chemistry of the Amino Acids and Proteins, Springfield.

Schmidt, E. L., Putnam, H. D. and Paul, E. A. 1960. Proc. Soil Sci. Soc. Amer. 24, 107

Shaduri, R. S. 1956. Trud. Inst. fiz. Akad. Nauk Gruzin. S.S.R. 4, 219 (from Chem. Abstr. 54, 23584c).

Sieskind, O. 1960a. C. R. Acad. Sci., Paris 250, 2228

Sieskind, O. 1960b. C. R. Acad. Sci., Paris 250, 2392

Sieskind, O. and Wey, R. 1958. C. R. Acad. Sci., Paris 247, 74

Sieskind, O. and Wey, R. 1959. C. R. Acad. Sci., Paris 248, 1652

Simonart, P. and Buysse, R. 1954. Trans. 5th Inter. Cong. Soil Sci., Leopoeldville, 3, 132.

Simonart, P. and Peeters, F. 1954. Trans. 5th Inter Cong. Soil Sci., Leopoeldville, 3, 136

Slabaugh, W. H. 1952. J. phys. Chem. 56, 748

Slabaugh, W. H. 1959. J. phys. Chem. 63, 436

Spencer, W. F. and Gieseking, J. E. 1952. J. phys. Chem. 56, 751

Stevenson, F. J. 1956. Proc. Soil Sci. Soc. Amer. 20, 2048

Stevenson, F. J. 1957. Soil Sci. 83, 113.

Takizawa, M. 1960. J. sci. Res. Inst., Tokyo, 54, 313.

Talibudeen, O. 1950. Nature 166, 236

Talibudeen, O. 1955. Trans. Faraday Soc. 51, 581

Tensmeyer, L. G., Hoffmann, R. W. and Brindley, G. W. 1960. J. phys. Chem. 64, 1655

Terenin, A. and Filimonov, V. 1959. Hydrogen Bonding (Ed. D. Hadzi) New York p. 545

Theng, K. G. B. 1961. Honours Thesis (B. Ag.Sc.) University of Adelaide.

Thompson, A. C. and Culbertson, J. L. 1959. J. phys. Chem. 63, 1917

Verhoogen, J. 1958. Amer. Min. 43, 558

Verwey, E. J. W. and Overbeek, J. Th. G. 1948. Theory of the Stability of Lyophobic Colloids. New York.

Walker, G. F. 1955. Proc. 4th Nat. conf. Clays and clay Min. Pennsylvania, p.101

Walker, G. F. 1960. Nature 187, 312

Walker, G. F. and Garrett, W. G. 1961. Nature, 191, 1389

Weiss, A., Michel, E. and Weiss, A. 1957. Hydrogen Bonding (Ed. D. Hadzi) p. 495

Whalen, J. W. 1961. J. phys. Chem. 65, 1676

Whitaker, J. R. and Deatherage, F. E. 1955. J. Amer. chem. Soc. 77, 5298

Young, G. J. 1958. J. Colloid Sci. 13, 67

Zettlemoyer, A. C., Young, G. J. and Chessick, J. J. 1955. J. phys. Chem. 59, 962