Physico-Chemical Studies on the Interaction of Proteins and Dyes with Clay Minerals

Thesis submitted for the award
of the Degree of
Doctor of Philosophy
in
Chemistry

by

SURESH KUMAR SRIVASTAVA

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF ROORKEE
ROORKEE

October, 1968

V

PHYSICO-CHEMICAL STUDIES ON THE INTERACTION OF PROTEINS AND DYES WITH CLAY MINERALS

THESIS SUBMITTED FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY

By

SURESH KUMAR SRIVASTAVA

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF ROORKEE,
ROORKEE.

OCTOBER, 1968.

C_E_R_T_I_F_I_C_A_T_E

Certified that the thesis entitled''Physico-chemical studies on the interaction of proteins and dyes with clay minerals'',which is being submitted,by Mr.Suresh Kumar Srivastava,for the award of the degree of Doctor of Philosophy,in Chemistry,of the University of Roorkee,is a record of his own work,carried out under my guidance and supervision. The matter embodied in this thesis has not been submitted for the award of any other degree of any university.

This is further to certify that he has worked for a period of three years and two months in this department to prepare this thesis.

Wahid U. Malik

(Wahid U.Malik)
Ph.D.,D.Sc.,F.N.A.Sc.,
F.R.I.C.(London)
Professor and Head of the
Department of Chemistry,
University of Roorkee.

Roorkee.
October 8,1968.

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

It has been a privilege to work under the learned guidance and kind supervision of Prof.Wahid U.Malik, Ph.D.,D.Sc.,F.N.A.Sc.,F.R.I.C.(London),Head of the Chemistry Department,University of Roorkee,Roorkee,for which I gratefully acknowledge my indebtedness to him.

I am also obliged to my colleagues and friends for their help and assistance in carrying out these investigations.

S. K. Srivastava
(Suresh Kumar Srivastava)

Department of Chemistry,
University of Roorkee,
Roorkee.

C_O_N_T_E_N_T_S

—

GENERAL INTRODUCTION

The process of soil formation is primarily a process of disintegration or 'weathering'. In the course of geological ages nearly 4% of the igneous rocks of the eaths crust have been weathered to clays,shales and surface soils. All these substances are made up of finely divided components exhbiting properties of association and dispersion,flocculation and peptisation or of undergoing surface reactions-properties which rightly categorise them under the well known form of matter named colloid. The four components of soil occuring in the state of fine sub-division are minerals, organic matter,water and air.

The last thirty years or so have witnessed great advancement in the field of soil science. Scientists from different disciplines have contributed to the overall development which has been possible with the help of new research tools like X-ray,differential thermal analysis, electron microscopy,infra red spectroscopy etc. The growing economic importance of clays has also been responsible for the rapid progress in this direction.

Mineral species.

According to Pauling',who was the first to elucidate the structure of clay minerals,each plate like clay particle consists of a stack of parallel unit layers. The principal building elements of the clay minerals are two-

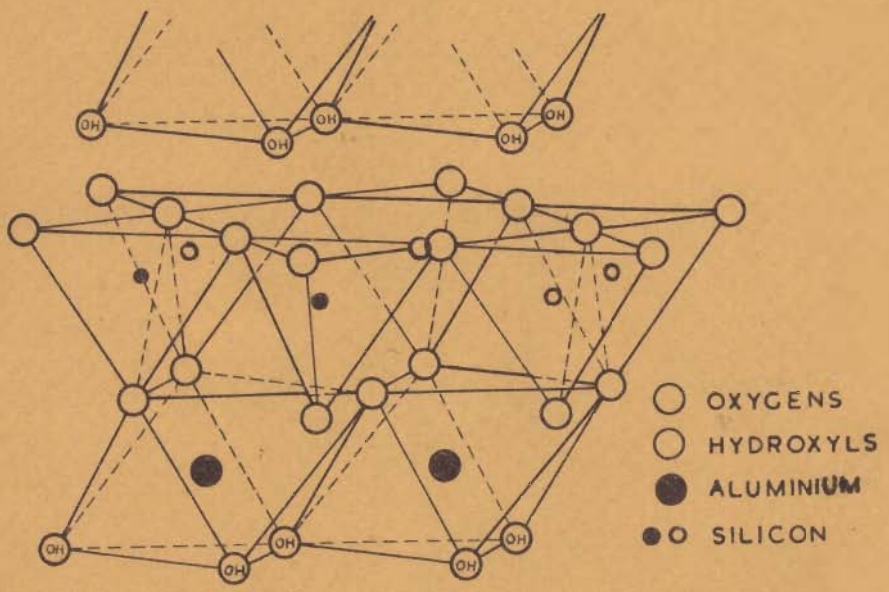


FIG. I. DIAGRAMMATIC SKETCH OF THE STRUCTURE OF KAOLINITE.

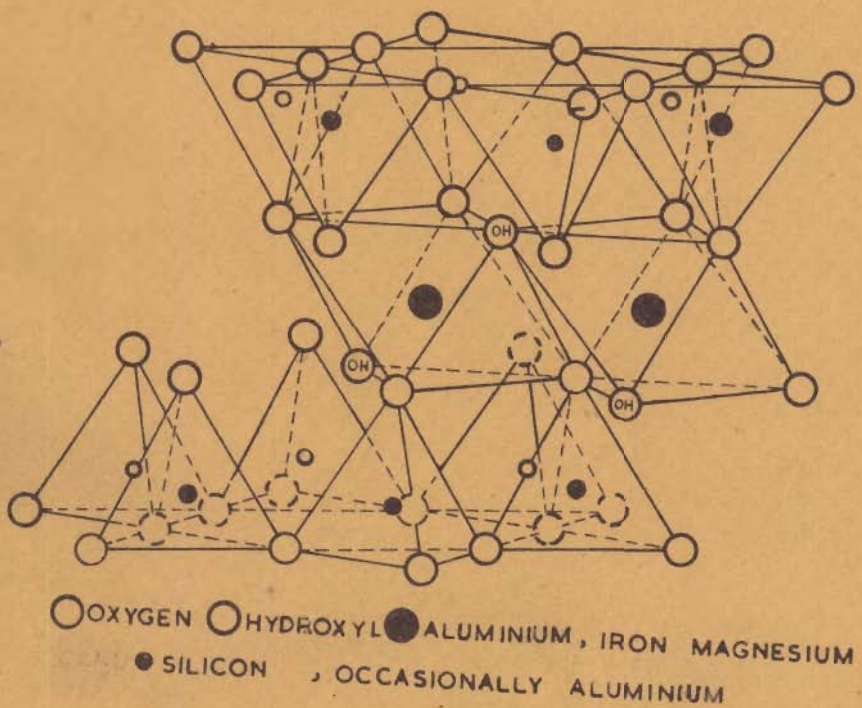


FIG. II. DIAGRAMMATIC SKETCH OF THE STRUCTURE OF MONTMORILLONITE.

dimensional array of silicon-oxygen tetrahedra and two-dimensional arrays of aluminium or magnesium-oxygen-hydroxyl octahedra. In the sheet, three oxygen atoms are shared by the neighbouring tetrahedra and the fourth oxygen atom protruding from the tetrahedral sheet is shared by octahedral sheet. In the octahedral sheet Al or Mg atoms are coordinated with six oxygen atoms or oH groups which are located around the Al or Mg atom with their centres on the six corners of a regular octahedron. The symmetry and almost identical dimensions in the tetrahedral and octahedral sheets allow the sharing of oxygen atoms between these sheets. This sharing may occur between one silica and one alumina sheet, as in a two layer mineral like kaolinite or between alumina and two silica sheets giving rise to a three layer mineral like montmorillonite, illite etc. The combination of one octahedral and one or two tetrahedral sheets is called a unit layer. Within each unit layer a certain unit of structure repeats itself in a lateral direction and is known as unit cell. Such unit layers stacked parallel to each other constitute various clay minerals.

Montmorillonite clays.

(Fig.2) These are three layer minerals wherein a tetravalent silica is replaced by trivalent Al or Al

in the octahedral sheet is partly replaced by divalent Mg without the third vacant position being filled. Such substitutions by elements of lower valence results in an excess of negative charge on the lattice. The adsorption of cations, both on the interior and the exterior surfaces of the stack takes place to compensate the net negative charge on the lattice. These compensating cations may be exchanged for other cations and are known as exchangeable cations of the clay.

The most typical property of montmorillonites is the phenomenon of interlayer swelling with water. Water penetrates between the unit layers and pushes them apart a distance equivalent to 1-4 monomolecular layers of water, which increases the c spacing from 10 A° to 12.5-20 A°.

Illites.

These have the same basic structure as montmorillonites. The total amount of lattice substitution is larger than that for montmorillonites and is predominantly that of Si by Al in the tetrahedral sheet. The striking feature of these clays is that the compensating cations are mainly potassium ions. These minerals do not show interlayer swelling which is attributed to the strong electrostatic attraction between the potassium

ions and the two charged unit layers on each side. In the absence of interlayer swelling these cations are not available for exchange.

Kaolinites.

It is a two layer mineral consisting of sheet units (of the type already described) continuous in the a and b directions and stacked one above the other in the c direction. The exchangeable ions are quite low and are situated on the broken edges of the kaolinite plates where they would compensate charge deficiencies owing to broken bonds. The charge deficiency owing to isomorphous substitution is absent in this case.

Phenomenon of ion-exchange.

The clay minerals have the property of sorbing certain cations and anions and retaining them in an exchangeable position. These ions are held around the outside of the silica-alumina clay-mineral structural unit,and the exchange reaction generally does not affect the structure of the silica-alumina packet.The exchange reaction is stoichiometric and the exchange capacity is measured in terms of milliequivalents per 100 gm. of clay. This property is of fundamental importance in the study of clay minerals.

In agricultural soils,plant foods are frequently

held in the soils as exchangeable ions,and consequently their persistence in the soil and their availability for plant growth depends on exchange reactions.The retention and availability of potash added in fertilizers depends on cation exchange between the potassium salt and the clay mineral of the soil. The replacement of the Na^{+} by another ion,usually Ca^{++}will make the soil more suitable for agriculture.

Similarly in the field of geology,during weathering process the liberation of alkalies may or may not be retained in the secondary material depending on exchange reactions.

In ceramics the plastic properties of clay can be adjusted according to needs,by changing the exchangeable cations.

There are a number of factors on which the exchange adsorption normally depends:
(i) valency of the ion,(ii) hydration of the ion,(iii) ionic radii and (iv) structural configuration of the clay micelle.

The valence and hydration of ions are the most important factors in determining the energy of adsorption and release. Ion exchange will be difficult when the adsorbed ions have got a higher valency or are weakly hydrated. It is so,because ions of higher valency

are adsorbed more strongly than those of the lower valency and weakly hydrated ions are more tightly bound up than those containing a large water hull. Weigner and Jenny[2,3] and Alten and Kurmies[4] have overemphasised the influence of hydration. Hendricks[5] and his colleagues, after careful dehydration studies have shown that Na^+, H^+ and K^+ are not hydrated when adsorbed by clay minerals while Ca^{++}, Mg^{++} and Li^+ undergo hydration.

Another factor, which controls exchange phenomenon, is the size of the exchanging ion. It is normally observed, that ions of smaller ionic radii are easily replaced by ions of larger ionic radii and vice versa.

Apart from this the nature of the clay surface, according to Gieseking and Jenny[5], Jerusov[6] etc., guides the energy with which a given ion is held.

Basically there are three causes of the cation exchange phenomenon:

1. Substitution within the lattice structure of silicon and aluminium by ions of lower valence results in unbalanced charges in the structural units of some clay minerals.
2. Broken bonds around the edges of the silica-alumina units would give rise to unsatisfied charges, which would be balanced by adsorbed cations.
3. The hydrogen of the exposed hydroxyls may be replaced

by a cation which would be exchangeable. Some oH groups would be exposed around the broken edges of all the clay minerals, and cation exchange due to these would be through the replacement of hydrogen.

Hydrogen clays.

When clays are subjected to acid leaching, or are electrodialysed, or passed through an ion exchange column of resin like Amberlite I.R.120 (labelled with H^+) hydrogen ions are preferentially adsorbed by clay particles resulting in the formation of H - clays. Baver and Marshall[6], Chatterjee and Paul[7], Mukherjee and others[8], have shown that hydrogen montmorillonites and hydrogen kaolinites are in reality hydrogen-aluminium systems. According to them it is impossible to prepare a clay in which all the exchange positions are occupied by H^+ since Al^{3+} moves from the lattice to exchange positions before saturation with H^+ becomes complete. The conclusions are applicable to other clay minerals as well and apply to a greatest degree to the expanding-lattice minerals. Kaolinite is effected to a least degree with the illite showing an intermediate effect. Aldrich and Buchanan[9] have developed methods of preparing clays containing varying amounts of Al and H ions. The clays with a minimal Al/H ratio are named as hydrogen clays,

while those having a higher ratio are called Al-clays.

Colloid-chemical behaviour and the nature of the electrical double layer.

Clay suspensions can neither be classified as purely lyophilic or lyophobic colloids. A large influence of other factors like adsorption, hydration etc., apart from charge of the particle, leads one to place them as intermediate between the two well defined classes.

Because of the inherent structure the minerals readily pass over to colloidal state when brought in contact with water. The clay particles have a highly negative surface constituting the inner part of the double layer, the outer layer being made up of a swarm of loosely held cations surrounding the particle. When such particles are dispersed in water, the cations get hydrated and then dissociate to a certain distance from the surface, finally leading to the formation of a diffused electrical layer[10]. These loosely held cations which act as counter ions are exchangeable. This exchange capacity of the double layer is an outstanding property of clay and has a high value.

The charge of the electrical double layer on the surface, solely depends on the type and degree of isomorphous lattice substitutions and so has a constant value

and is independent of the presence of electrolytes in suspension. Such a double layer is uncommon in hydrophobic colloids where the charge varies with bulk electrolyte concentration. There are indications that specific adsorption forces play between the lattice of a mineral and the counter ions with the result that a larger fraction of counter ions will be located on the surface and a smaller fraction will be in the diffuse layer[11,12]. Hence the Stern-Gouy model would be more applicable to the clay double layer than the Gouy model alone.

Existence of positively charged double layer.

Apart from the double layer at the flat surface, a possibility of the existence of a positive double layer at the surface of broken edges has been postulated by van Olphen[13]. His view point finds support in the work of Thiessen[14] on the interaction of kaolinite suspension with negatively charged gold sol. At the edges of the plates the tetrahedral silica sheets and the octahedral alumina sheets are disrupted,and primary bonds are broken. The part of the edge surface at which octahedral sheet is broken may be compared with the surface of an alumina particle and would carry a positive double layer in acid solutions with

Al as potential determining ions and a negative double layer in alkaline medium with oH as potential determining ions[15]. The edge surface of a tetrahedral sheet may be compared with the surface of silica particle. Though silica surface carries a negative double layer its charge is known to become positive in presence of even very small quantities of Al ions in suspension. Owing to slight solubility of clay such small concentration of Al ions would always be there. Moreover it is possible that silica sheets are always broken at the places where Al ions have substituted silicon. Hence a positive double layer would always exist at broken edges.

Soil surface and adsorption.

Chemically, soils are alumino-silicate systems and as such are characterised by possessing exposed surface layers rich in oxygen atoms and hydroxyl groups. These surfaces are basically highly polar in character and possess an intense residual force. Generally these forces are responsible for holding the other ions or molecules at the surface temporarily or permanently depending on the nature of the forces operating at it. Residual valence forces, dipolar attractions or any other physical forces of the van der Waal type lead to adsorption, where the molecules are held temporarily; while

chemical or electrostatic forces give rise to a permanent binding and molecules stay permanently on the surface. From soil engineering point of view, physical forces play a dominant role in determining the soil characteristics in relation to water and other materials added. In the present work dyes and proteins have been used, which are preferentially adsorbed at the surface giving rise to physical adsorption.

Clay-mineral-organic reactions.

Investigations in the field of agriculture have presented evidence for some sort of inorganic-organic combination in many soils. Demolin and Barbier[16] showed a definite fixation of humic acid and protein by clay and Mattson[17] has demonstrated a reduced base exchange capacity by complexing clay with organic compounds. Sedletsky[18] investigated the matter in considerable detail and concluded that many soils contain clay mineral-organic complexes.

Further evidence for such reactions is provided by the color reactions produced when clays are mixed with organic compounds[19]. The development or change in colour requires some kind of interaction between the clay and the organic compound added.

The early work of Hofmann[20] et al. showed that polar compounds get adsorbed on the surface of the clay mineral and the c axis dimension of montmorillonite varied following treatment with alcohol, acetone etc. Bradley[21,22] and MacEwan[23,24] at the same time quite independently showed definitely that the non-ionic organic molecules could also be adsorbed on the clay surface.

Hendricks[25] has indicated that the organic ions are held by Van der Waals forces in addition to the Coulombic force. In general the larger ions are more strongly adsorbed because of the greater Van der Waals forces. Grim et al.[26] have shown that small ions are adsorbed only upto cation-exchange capacity whereas larger ions may be adsorbed in excess. Bradley and Grim[27] beleive that the coulombic forces are further supplemented by C - H ..... O bonds between organic molecule and clay mineral surface.

Uses of clay-organic reactions.

1. X-ray studies: The montmorillonite mineral commonly gives diffuse reflections and a nonintegral series of basal reflections. Organo-montmorillonites comparatively give high degree of regularity in c spacing and sharp reflections. As such the identification of

montmorillonite becomes simple if organic treated clay is taken for X-ray studies.

2. Optical methods: The adsorption of organic molecules between the basal plane surfaces of montmorillonite cause a definite change in their indices of refraction. This characteristic can be used for identification of minerals.

3. Surface-area determinations: The total surface, the external surface and by difference the internal surface of a clay mineral can be determined by measuring the retention or adsorption of polar molecules like glycol, glycerol etc..

Besides the above mentioned applications the clay -organic reactions have been used as a basis of many other techniques for determining certain properties of clay minerals themselves, and for the determination of the geometry and properties of organic molecules.

Clay mineral-dye interactions:

The investigations on the clay mineral-dye interactions start from the development of staining methods of identifying clays[19]; besides this the work of Bossazza[28] and Emodi[29] is worth mentioning. They investigated the adsorption studies of kaolinite and bentonite for a few dyes and corelated the quantity adsorbed with mineralogical composition. They obtained

low adsorption capacities,since the samples used by them were not pure. White and Cowen[30] and Worrell[31] worked on adsorption of methylene blue on fire clays in a bid to develop a rapid method of measuring exchange capacity but the results obtained were quite abnormal. Systematic and critical studies on these reactions are,however,lacking. Colorimetric and polarographic studies can prove useful in understanding the mechanism of adsorption and form a part of the present work.

Clay-protein interactions.

Numerous explanations of the ''Storehouse'' of nitrogen in soils have been advanced. The possibility that complex formation can occur between clay minerals and soil nitrogeneous components is an extremely attractive hypothesis.

Although the existence of protein-clay complexes in the soil has not been shown,all evidence points to the fact that the components necessary for their formation are available and the conditions necessary for their synthesis are present in the soil.

Work done[32-34] in this direction has shown that one of the principal factors responsible for enhanced retention of organic compounds (proteins,amino acids etc.)

is its ability to form complexes with clay minerals. Waksman[35] separated the humus complex into two fractions alpha and beta humus and recognised the second as a chemical compound between protein and clay constituent of the soil.

Ensminger and Giesking[36] and Maclaren[37] investigated the adsorption of proteins like lysozyme and enzymes on bentonite and kaolinite and showed the formation of interlamellar clay-protein complexes. Their studies were,however,limited to proteins of low molecular dimensions.Other works[38,39] reported in the field concern with the formation of clay-protein complexes and a study of their microbial degradation in natural conditions.

From what has been said above it is evident that the problem of protein-clay interaction has not been comprehensively studied and need much more work to get a better understanding of the phenomenon. To achieve this, reactions of fibrillar as well as globular protein on different clay minerals were studied employing u.v. spectrophotometric and viscometric studies. This approach has proved quite useful in appreciating the possible mechanism of protein-clay interaction.

Investigations carried out.

(i) The binding of the dye to the clays has been investigated at various dye concentrations and at different pH.

Results have been explained on the basis of area per exchange site and area covered by the dye molecule on the surface of clay mineral.

(ii) Polarographic technique has been used to identify clays with the help of dyes and to determine their base exchange capacity.

(iii) Adsorption of proteins on clays has been investigated spectrophotometrically at various protein concentrations, keeping in view the stability of the clay-protein complex on changing the pH of the medium.

Viscosimetric constants of aqueous suspensions of bentonite-protein complexes have been calculated and used to determine the extent of particle-particle interaction, hydrodynamic interaction and the dissymmetry of suspended particles.

(iv) In the appendix experimental evidence for the existence of positive double layer at the edge surface has been provided with the help of radio-isotopic technique.

—

R E F E R E N C E S

1. Pauling,L.,Proc. Natl.Acad.Sci. U.S.,16,123,1930.
2. Wiegner,G. and Jenny,H.,Kolloid-Z.43,269,1927.
3. Wiegner,G.,Trans.Intern.Congr.Soil Sci.,3,5,1935.
4. Alten,F. and Kurmies,B.,Handbuck der Bodenlehre.Vol.8.
5. Hendricks,S.B., Nelson,R.A. and Alexander,L.T., J.Am. Chem.Soc.,62,1457,1940.
6. Baver,H. and Marshall,C.E., J.Soc.Chem.Ind.,53,750,1934.
7. Chatterjee,B. and Paul,M., Indian J.Agri.Sci.,12,113, 1942.
8. Mukherjee,J.N. and Chatterjee,B., J.Ind.Chem.Soc.,19, 40,1942.
9. Aldrich,D.G. and Buchanan,J.R.,Soil Sci.Soc.Am.Proc. 22,281,1958.
10. Freundlich,H., Kapillar Chemie,Vol.I,p.356.
11. Jenny,H. and Reitemeier,R.F., J.Phys.Chem.,39,593,1935.
12. Weigner,G., 4th Intern.Conf.Soil Sci.,Rome.1925.
13. van Olphen,H., Clay Colloid Chemistry. Interscience Publishers.p. 90.
14. Thiessen,P.A., Z.Elektrochem., 48,675,1942.
15. Van Schuylenborgh Rec. Trav.Chim. 79,985,1951.
16. Demolin,A. and Barbier,G., Compt.rend., 188,654,1939.
17. Mattson,S., Soil Sci.,23,41,1932.
18. Sedletsky,I.D.,''X-ray Studies of Soils'' Moscow(1939).
19. Faust,G.F.,U.S.Bureaw.Mines.Rept.Invest.3522(1940).
20. Hofmann,U.,Endell,K. and Wilm,D.,Angew.Chem.47,539,1934.
21. Bradley,W.F., J.Am.Chem.Soc.,67,975,1945.
22. Bradley,W.F., Am.Mineral.,30,704,1945.

23. MacEwan,D.M.C., Nature,154,1944.

24. MacEwan,D.M.C., J.Soc.Chem.Ind.,65,298,1946.

25. Hendricks,S.B.,J.Phys.Chem.,45,65,1941.

26. Grim,R.E.,Allaway,W.H., and Cuthbert,F.L.,J.Am.Ceram. Soc.,30,137,1947.

27. Grim,R.E.,Bradley,W.F., J.Phys.Chem.,52,1404,1947.

28. Bossazza,V.L.,Amer.Mineral.,29,335,1944.

29. Emodi,B.S.,Brit.Clay.Min.Bull.,3,76,1949.

30. White,D. and Cowen,C.T., Trans.Brit.Cer.Soc.,59,16, 1950.

31. Worrell,W., J.Pharm.Pharmacol.,3,27,1951.

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

33. Allison,F.E.Doetsch,J.H., Soil Sci.,75,373,1953.

34. Ensminger,L.E. and Giesking,J.E.,Soil Sci.481,467, 1939.

35. Waksman,S.A., Soil Sci.78,118,1954.

36. Ensminger,L.E. and Giesking,J.E.,Soil Sci.,48,467, 1939.

37. MacLaren,A.D., Peterson,G.H. and Barshad,I.,Soil.Sci. Soc.Am.Proc.,22,239,1958.

38. Pinck,L.A. and Allison,F.E.,Science 114,130,1951.

39. Pinck,L.A. Dyal,R.S. and Allison,F.E.,Soil Sci.78,189.

—

CHAPTER I.

Adsorption of dyes with clay minerals.

INTRODUCTION

The clay minerals provide interesting materials for the study of adsorption phenomenon. Of the different types of adsorption possible at the solid liquid interface, the clay minerals predominantly exhibit exchange adsorption which can take place through variety of materials, both inorganic and organic. Exchange reaction with inorganic ions is stoichiometric and is of great fundamental and practical importance in all the fields in which clay minerals are studied and used. It is with the help of these exchange reactions that the base exchange capacity of different clays can be determined and the original clay material can be changed into different cationic clays having only one kind of counter ions in its double layer.

The organic cations also enter into exchange reactions with clays like inorganic ions although the mechanism is slightly different. Here the adsorption is not limited to the exterior surfaces only but interlamellar adsorption is also observed. The phenomenon is accompanied by an increase of the basal spacing of the clay resulting in the formation of what may be called as clay-organic complexes. These complexes are important from the basic and applied view point. Their existence besides providing a quicker method for the determination of base exchange capacity with reasonable accuracy allow the determination of surface area

of clays. For instance the uptake of glycerol or ethylene glycol[1] affords an excellent method of determining the total internal and external surface area of clay minerals and soil systems. Recent investigations on clay-organic complexes have led to the discovery of several commercial clay products[2,3] which can be used as gelling agents, lubricants, fillers etc..

X-Ray studies reveal that the organic molecules arrange themselves in parallel layers between the structural sheet of clay mineral and lie as flat as possible. Bradley[4] has proposed a CH-O bond formation between the methylene groups and the oxygen ion layer of the clay surface.

The investigations on the adsorption of organic compound also go to show that besides exchange adsorption other types of adsorption can exist in clay minerals. Hendricks[5] with the help of montmorillonite complexes of organic bases, showed that the organic cations are held to the surface of silicate layers by van der Waal forces also. In this process exchange sites are made in accessible and the exchange capacity is consequently decreased. Allaway and Cuthbert[6] found that small organic molecules replace the exchangeable cations quantitatively but with larger molecules the exchange is incomplete due to cover up effect. Large amounts of the adsorbate are taken up due to multilayer adsorption. To quote is the case of the adsorption of quaternary ammonium compounds on montmorri-

lonite where about two and a half times more adsorption than the amount equivalent to the base exchange capacity is adsorbed.

Besides the adsorption of the organic molecule as such on the clay mineral there is another aspect worth considering. It concerns with the adsorption of organic compounds by clays in water suspensions. Very few references on this aspect are available in the literature. Recent studies by Brindley and Coworkers[7,8], Badder and Smith[9] have shown that water acts in competition with organic material for sites on the clay surface. In such cases the pH of the suspension should also influence the adsorption characteristics of clays.

Doehler and Young[10] on the basis of their studies on the adsorption of quinoline by clay suspensions have shown that the adsorption of the organic molecule depends on its concentration,pH, salinity and temperature. According to them both exchange and molecular adsorptions are operative. It has been shown by Mackenzie[11] that montmorillonite adsorbs many organic compounds like monohydric and dihydric alcohols,nitriles etc. but when the clay is immersed in a dilute aqueous solution of these compounds preferential adsorption of solvent occurs.

From what has been discussed above it is evident that very little has been done so far on the adsorption of organic compounds by clay suspensions. This is specially true for dyes where the available references[13-18] are either of qualitative nature (dealing with the development of some staining methods of identifying clays) or describe results of semi-quantitative nature obtained from faulty and unsystematic experimentation applied to impure clay samples. Worrell[19] drew attention to an important fact based on the investigations on the adsorption of methylene blue on fire clays, that although adsorption occurred mainly by cation exchange, it may not be the sole mechanism in the case of dye adsorption phenomenon.

Dye adsorption from aqueous solution has its practical importance also. The adsorption of methylene blue is employed for measuring the cation exchange capacity of petroleum reservoir formations. This method offers a considerable saving in operation time over that required for the method based on the exchange adsorption of inorganic ions[20,21].

The problem of the adsorption of dyes by clay suspensions thus needs a thorough and systematic investigation. Investigations in this direction were initiated by studying the adsorption isotherms of some basic dyes

using pure bentonite, kaolinite and illite (obtained from Ward's Natural Science Est. New York) as adsorbents. These isotherms were then analysed to elucidate the mechanism of adsorption and to know how this type of adsorption is related to base exchange capacity of the clay. These studies were extended to suspensions of varying pH to differentiate between the face and the edge adsorption in clays as has been postulated by some workers.

—

E_X_P_E_R_I_M_E_N_T_A_L

Reagents.

Bentonite, kaolinite and illite used in these studies were obtained from Ward's Natural Science Establishment, New York.

Three basic dyestuffs used in these studies were methylene blue, crystal violet and rhodamine 6G. These were obtained from B.D.H.

Preparation of H-clay.

The minerals were ground to pass through a 100 mesh sieve. The clays were then treated with hydrogen peroxide for few hours at room temperature and then in an oven at 60°C, with occasional stirring till all the organic matter was removed. The excess of hydrogen peroxide was removed by heating the samples over a water bath for sometime . These minerals were then dried and suspended in double distilled water. The suspension was passed through an Amberlite I.R.120 ion-exchange column to convert it into H-clay. The process was repeated till the conversion into H-clay was complete. The concentration of the stock suspension was determined by drying it in air at 120°C for few hours.

Stock solutions of dyes were also prepared in double distilled water.

Apparatus.

Bausch and Lomb 'Spectronic 20' was used for

observing the optical density of dyes before and after adsorption. Beckmann model H-2 pH meter was used for recording the pH values of various solutions.

Procedure for adsorption measurements.

Calibration curves of the dyes were first prepared (Fig.1.).

Adsorption isotherms were run by taking different concentrations of dyes in tubes. Definite amount of clay was mixed in each tube and the total volume was made up to 20 c.c. with double distilled water. The effective concentration of clay was kept 30 mg. throughout. The tubes were mechanically stirred for sometime and the readings were taken after sufficient time of mixing till the equilibrium was attained. The concentration of dye in equilibrium solution was obtained by measuring the absorbance of the solution obtained after centrifuging the contents of each tube for 20 minutes.

To study the effect of pH on adsorption varying amounts of either HCl or NaOH were added to known mixtures of H-clay and dye. Adsorption was determined colorimetrically as described above.

Surface area and cation exchange capacity measurements.

Surface area was determined by glycerol retention method[I]. The results are tabulated in Table No.1. The cation exchange capacity of each mineral was determined

by the standard ammonium acetate method[22]. The values are summarised in Table No.1. Tables No.2 to 10 give the adsorption data while Tables No.11 to 13 depict the variations in the adsorption of methylene blue with pH.

Table No.1

Clay mineral	Percentage of glycerol retention of clay sample.	Percentage of glycerol retention of the same after heating the sample to 300°C.	Percentage of glycerol retention due to external surface.	Percentage of glycerol retention due to internal surface.	External area $m^2/gm.$ +	Internal area $m^2/gm.$	Total surface area m^2/gm	C.E.C. of the mineral as determined by standard ammonium acetate method in m.e./100 gm. clay.
Bentonite	26.3	10.0	10.0	16.3	175.0	575.39	750.39	99
Illite	5.35	5.3	5.3	-	94.0	-	94.00	26
Kaolinite	1.03	1.03	1.03	-	18.12	-	18.12	6.3

+ It has been computed by X-Ray studies that 1% glycerol retention corresponds to an external surface are of 17.55 m^2/g. and internal surface area of 35.3 $m^2/gm.$

Adsorption of methylene blue by bentonite, illite and kaolinite.

Table No.2

Amount of bentonite added=30 mg. in each case.

Initial conc. of dye. x 10^{-5}M	O.D. of dye in equili-brium.	Equilibrium conc. of dye C_s. x 10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm. of clay (x).	$\frac{C_s}{x}$ x 10^5
2.00	0.12	0.13	1.80	60.0	0.021
2.40	0.14	0.15	2.25	75.0	0.025
3.00	0.45	0.50	2.49	83.0	0.060
3.55	0.90	1.00	2.55	85.0	0.110
4.30	1.40	1.60	2.70	90.0	0.170
5.30	1.80	2.00	3.30	110.0	0.180

Fig.2

Table No.3

Amount of illite added=30 mg. in each case.

Initial conc. of dye. x 10^{-5}M	O.D. of dye in equili-brium.	Equilibrium conc. of dye C_s. x 10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm. of clay (x).	$\frac{C_s}{x}$ x 10^5
0.350	0.18	0.200	0.650	21.6	0.009
1.180	0.45	0.500	0.680	22.7	0.022
1.710	0.90	0.996	0.714	23.8	0.042
2.220	1.35	1.500	0.720	24.1	0.062
2.420	1.60	1.694	0.726	24.2	0.070
3.100	1.90	2.200	0.900	28.0	0.073

Fig.3

Table No.4

Amount of kaolinite added = 30 mg.in each case.

Initial conc.of dye. x 10^{-5}M	O.D.of dye in equili-brium.	Equilibrium conc.of dye C_s. x10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm.of clay (x).	$\frac{C_s}{x}$ x 10^5
0.250	0.10	0.120	0.130	4.3	0.027
0.350	0.18	0.210	0.140	4.5	0.046
0.530	0.34	0.390	0.140	4.6	0.085
0.650	0.45	0.500	0.150	5.0	0.100
0.950	0.72	0.794	0.156	5.2	0.158
1.250	0.98	1.090	0.160	5.3	0.218
1.300	1.30	1.140	0.160	5.3	0.218

Fig.4

Adsorption of crystal violet by bentonite,illite and kaolinite.

Table No.5

Amount of bentonite added = 30 mg.in each case.

Initial conc.of dye. x 10^{-5}M	O.D.of dye in equili-brium.	Equilibrium conc.of dye C_s. x 10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm. of clay (x).	$\frac{C_s}{x}$ x 10^6
1.760	0.15	0.110	1.650	55.0	0.020
2.340	0.24	0.180	2.160	72.0	0.022
2.920	0.31	0.250	2.670	89.2	0.028
3.310	0.72	0.580	2.730	91.0	0.062
3.450	0.96	0.690	2.760	92.0	0.075
4.000	1.12	0.890	3.110	102.0	0.086

Fig.5.

Table No.6

Amount of illite added=30 mg.in each case.

Initial conc.of dye. x 10^{-5}M	O.D.of dye in equilibrium.	Equilibrium conc.of dye C_s. x 10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm. of clay (x).	$\frac{C_s}{x} \times 10^6$
0.60	0.18	0.150	0.450	15.0	0.100
0.91	0.31	0.247	0.663	22.1	0.113
1.00	0.37	0.300	0.700	23.0	0.130
1.10	0.50	0.398	0.702	23.4	0.165
1.40	0.81	0.650	0.750	25.0	0.260
1.60	1.29	0.810	0.790	26.3	0.380

Fig.6

Table No.7

Amount of kaolinite added = 30 mg. in each case.

Initial conc.of dye. x 10^{-5}M	O.D.of dye in equilibrium.	Equilibrium conc.of dye C_s. x 10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm. of clay (x).	$\frac{C_s}{x} \times 10^5$
0.25	0.13	0.100	0.150	5.0	0.020
0.31	0.16	0.142	0.168	5.6	0.025
0.69	0.65	0.513	0.177	5.9	0.085
0.93	0.94	0.750	0.180	6.0	0.125
1.12	1.17	0.937	0.183	6.1	0.153
1.38	1.48	1.190	0.190	6.3	0.190

Fig.7

Adsorption of rhodamine 6G by bentonite, kaolinite and illite.

Table No.8

Amount of bentonite added=30 mg. in each case.

Initial conc.of dye. x 10^{-5}M	O.D.of dye in equili-brium.	Equilibrium conc.of dye C_s. x 10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm. of clay (x).	$\frac{C_s}{x} \times 10^5$
2.10	0.22	0.30	1.80	60.0	0.050
2.50	0.26	0.37	2.13	71.0	0.052
3.27	0.55	0.75	2.52	84.0	0.089
3.64	0.73	1.00	2.64	88.0	0.110
4.00	0.96	1.30	2.70	90.0	0.144
4.55	1.32	1.79	2.76	92.0	0.190
5.00	1.62	2.18	2.82	94.0	0.230

Fig.8

Table No.9

Amount of illite added = 30 mg.in each case.

Initial conc.of dye. x 10^{-5}M	O.D.of dye in equili-brium.	Equilibrium conc.of dye C_s. x 10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm. of clay (x).	$\frac{C_s}{x} \times 10^5$
1.10	0.54	0.73	0.37	12.5	0.056
1.53	0.73	1.00	0.53	17.5	0.057
1.87	0.96	1.30	0.57	19.0	0.065
2.10	1.10	1.50	0.59	19.5	0.076
2.40	1.32	1.80	0.60	20.0	0.090
3.10	1.80	2.47	0.63	21.0	0.110

Fig.9

Table No.10

Amount of kaolinite added = 30 mg. in each case.

Initial conc. of dye. x 10^{-5}M	O.D. of dye in equilibrium.	Equilibrium conc. of dye C_s. x 10^{-5}M	Amount of dye adsorbed x 10^{-5}M	Milli equiv. of dye adsorbed per 100 gm. of clay (x).	$\frac{C_s}{x} \times 10^5$
0.38	0.18	0.25	0.13	4.3	0.058
0.49	0.25	0.35	0.14	4.6	0.076
0.65	0.36	0.50	0.15	5.1	0.098
0.80	0.48	0.65	0.156	5.2	0.120
0.96	0.58	0.80	0.160	5.3	0.150
1.20	0.76	1.03	0.170	5.6	0.180

Fig.10

Effect of pH on the adsorption of methylene blue by bentonite, illite and kaolinite.

Table No.11

(Bentonite - Methylene blue)

pH	Milli equiv. of dye adsorbed per 100 gm. of clay	pH	Milli equiv. of dye adsorbed per 100 gm. of clay
1.5	80.0	4.5	110.0
1.8	85.0	5.0	115.0
2.0	87.0	5.5	116.0
2.2	90.0	6.0	117.0
2.4	92.0	7.0	119.0
2.8	94.5	8.0	121.5
3.2	96.2	9.0	123.0
3.8	98.0	10.0	124.0

Fig.11

Table No.12

(Illite - Methylene blue)

pH	Milli equiv. of dye adsorbed per 100 gm. of clay	pH	Milli equiv. of dye adsorbed per 100 gm. of clay
2.0	22.0	4.5	29.5
2.2	23.0	5.0	31.2
2.4	24.0	6.0	32.0
2.8	25.2	7.0	32.5
3.2	25.5	8.0	33.0
3.8	28.0	9.0	34.5
		10.0	35.0

Fig.12

Table No.13

(Kaolinite - Methylene blue)

pH	Milli equiv. of dye adsorbed per 100 gm.of clay.	pH	Milli equiv. of dye adsorbed per 100 gm.of clay
2.0	4.00	4.5	5.80
2.2	4.40	5.0	5.90
2.4	4.60	5.5	6.00
2.8	5.00	6.5	6.30
3.4	5.30	7.0	6.50
3.8	5.50	8.0	7.00
		9.0	7.50
		10.0	7.80

Fig.13.

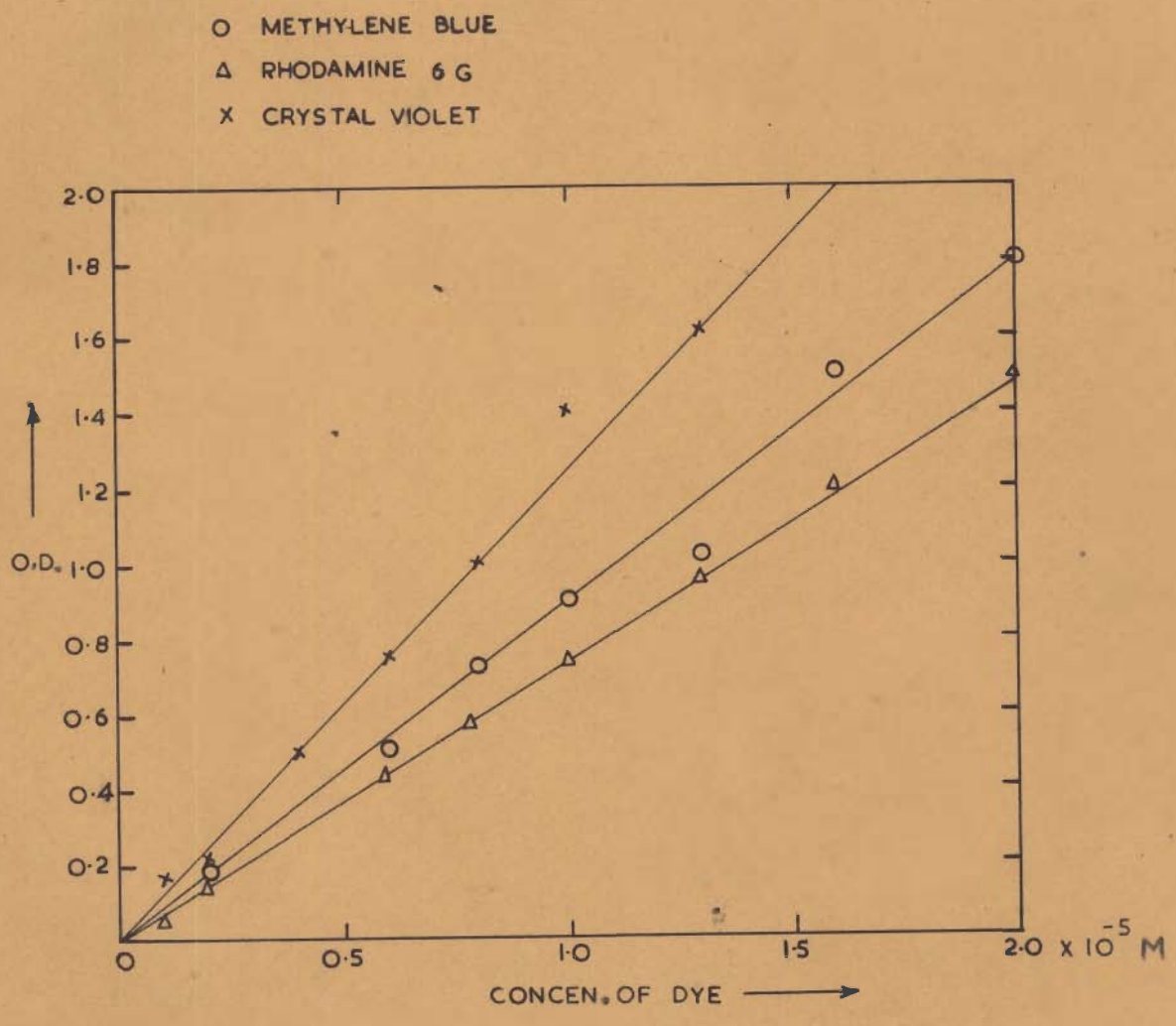

O METHYLENE BLUE
Δ RHODAMINE 6 G
X CRYSTAL VIOLET
O.D.
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
0·5
1·0
1·5
2·0 X 10⁻⁵ M
CONCEN. OF DYE

FIG. 1.

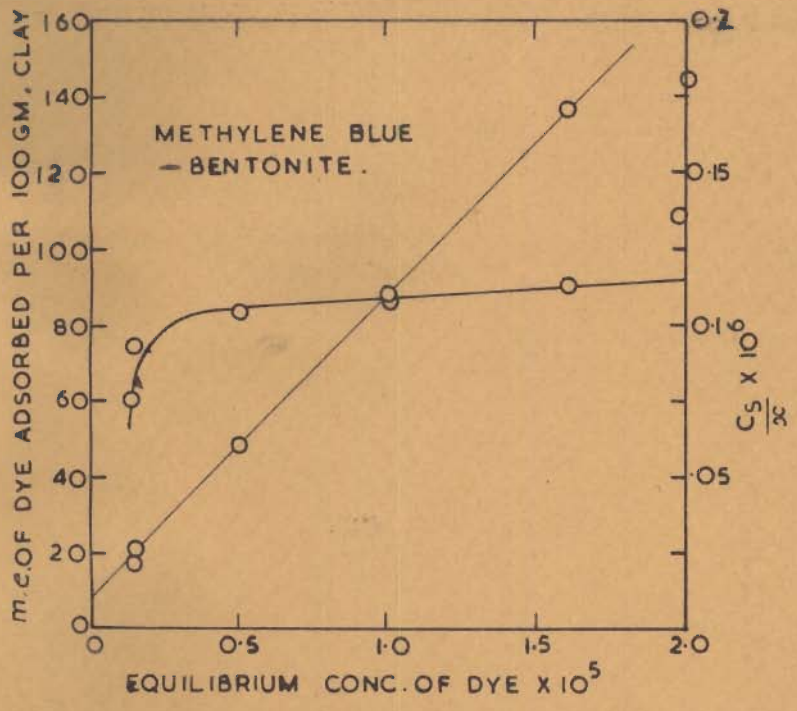


FIG.2.

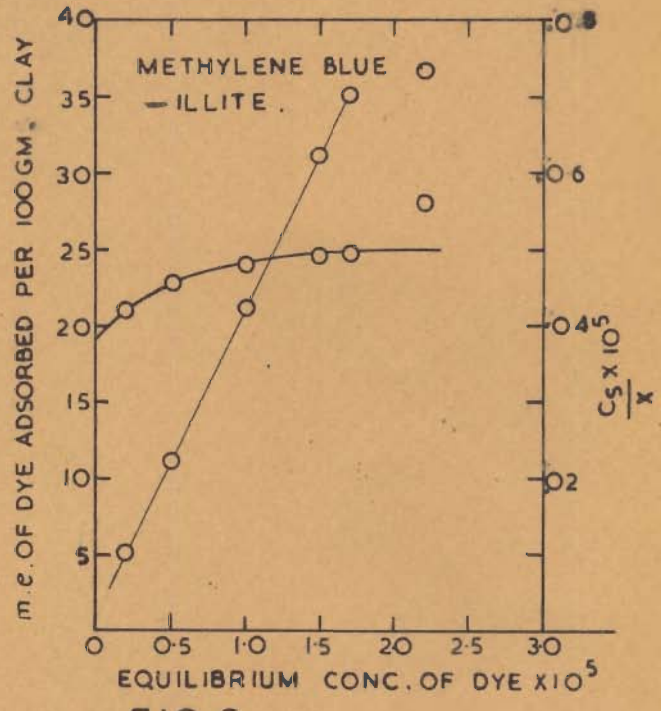


FIG.3.

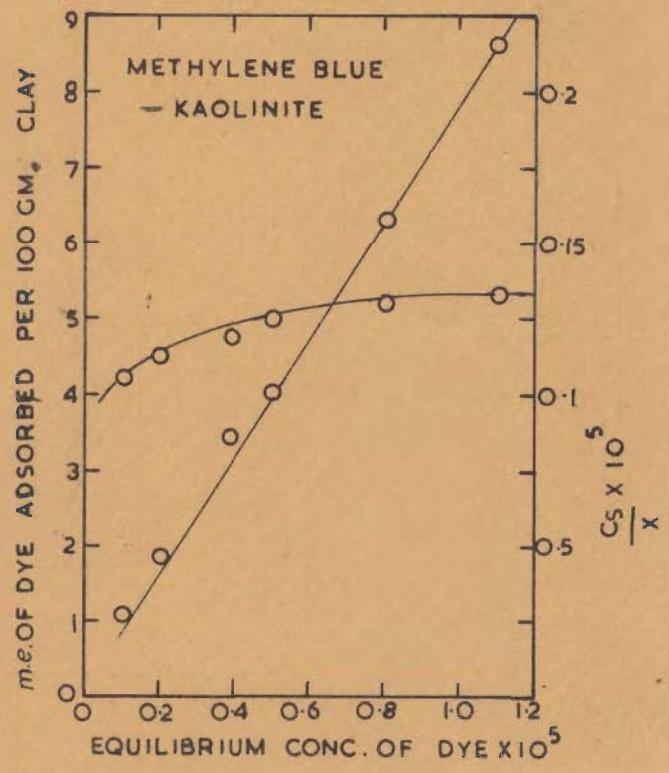


FIG.4.

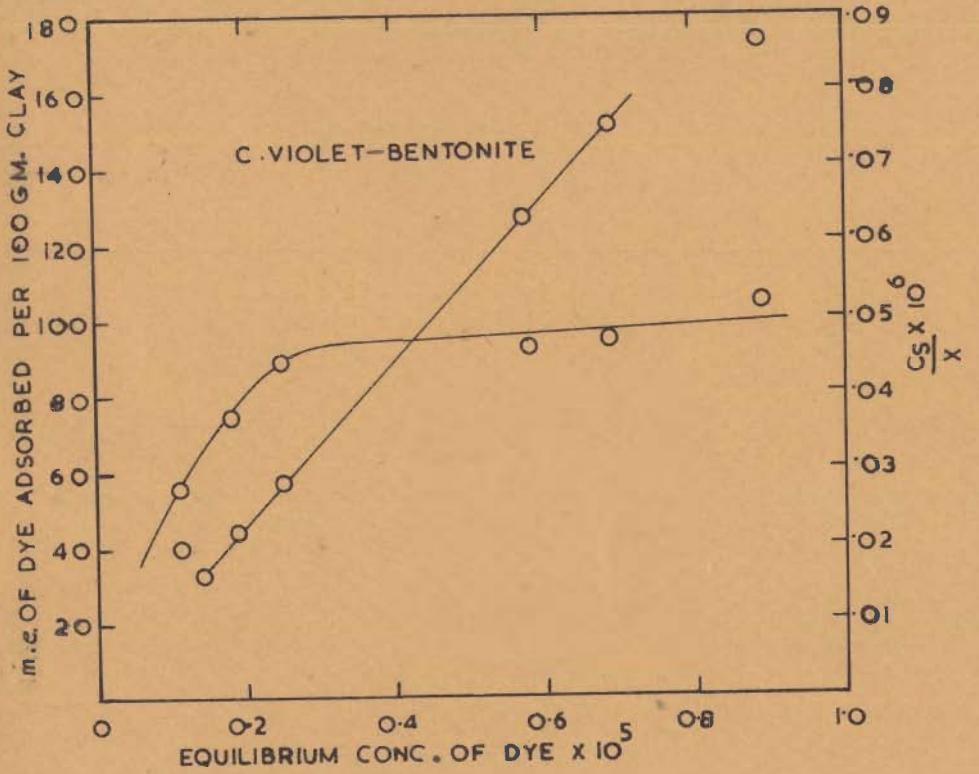

C.VIOLET–BENTONITE
m.e.OF DYE ADSORBED PER 100 GM. CLAY
$\frac{C_S}{X} \times 10^6$
EQUILIBRIUM CONC. OF DYE X 10^5
0
0·2
0·4
0·6
0·8
1·0
20
40
60
80
100
120
140
160
180
·01
·02
·03
·04
·05
·06
·07
·08
·09

FIG 5

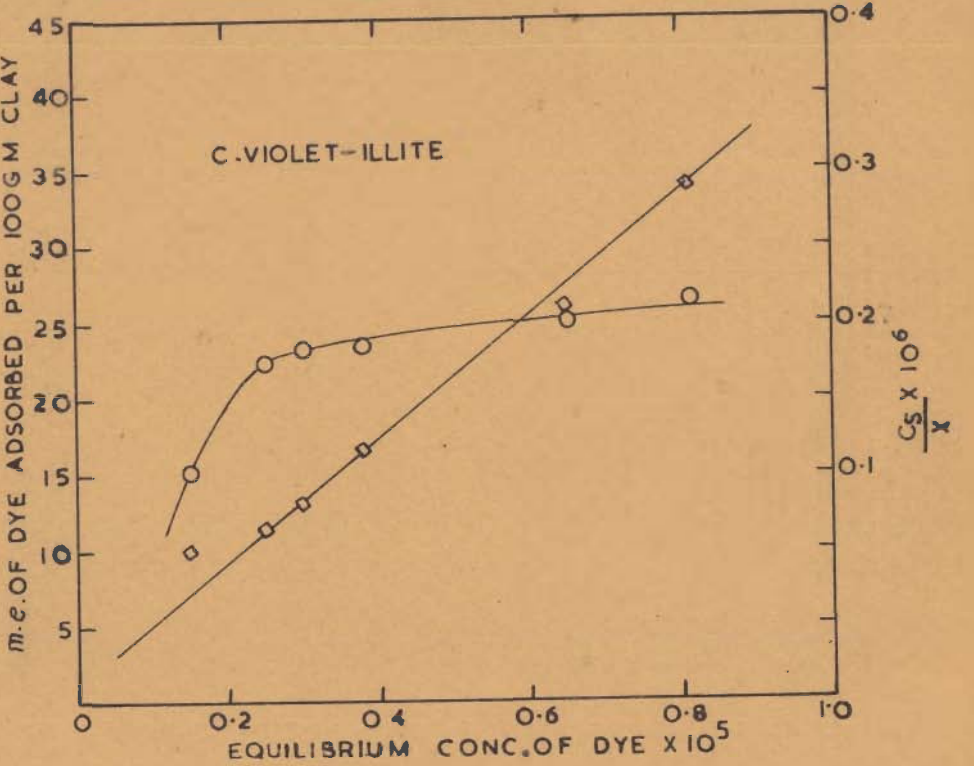

C.VIOLET–ILLITE
m.e.OF DYE ADSORBED PER 100 GM CLAY
$\frac{C_S}{X} \times 10^6$
EQUILIBRIUM CONC.OF DYE X 10^5
0
0·2
0·4
0·6
0·8
1·0
5
10
15
20
25
30
35
40
45
0·1
0·2
0·3
0·4

FIG 6

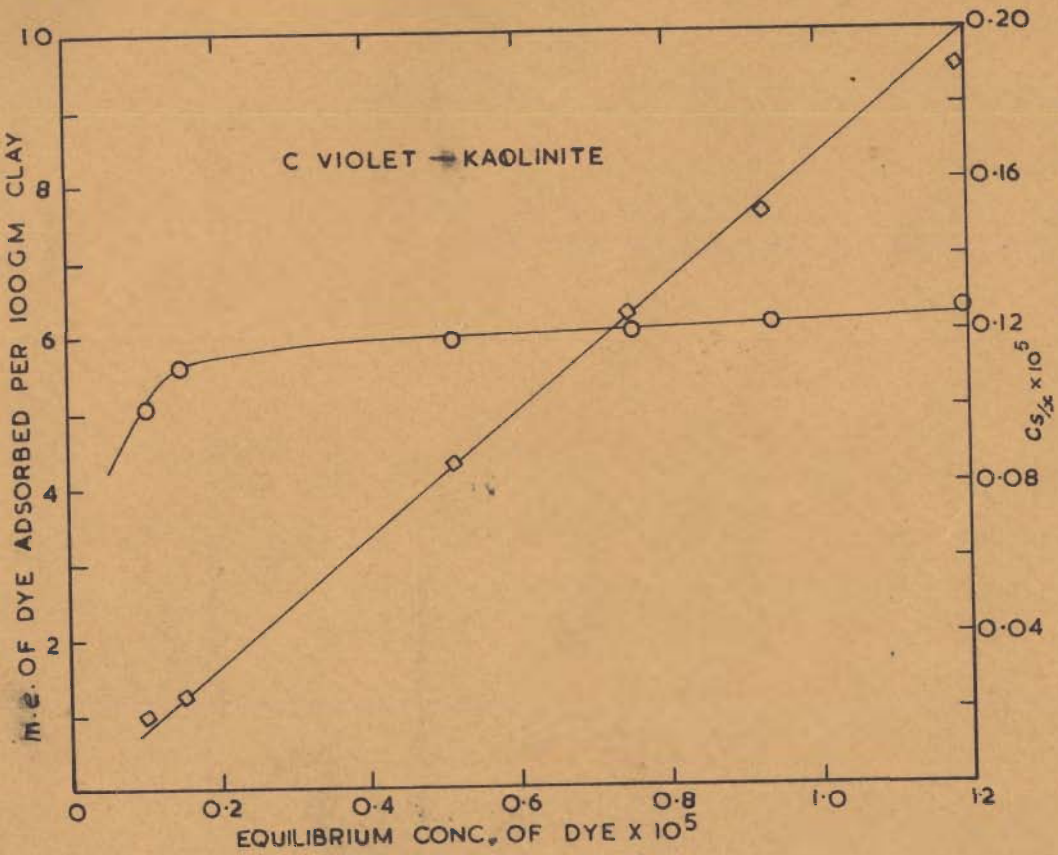

C VIOLET → KAOLINITE
m.e. OF DYE ADSORBED PER 100GM CLAY
EQUILIBRIUM CONC. OF DYE X 10^5
Cs/x x 10^5
0
0·2
0·4
0·6
0·8
1·0
1·2
2
4
6
8
10
0·04
0·08
0·12
0·16
0·20

FIG 7

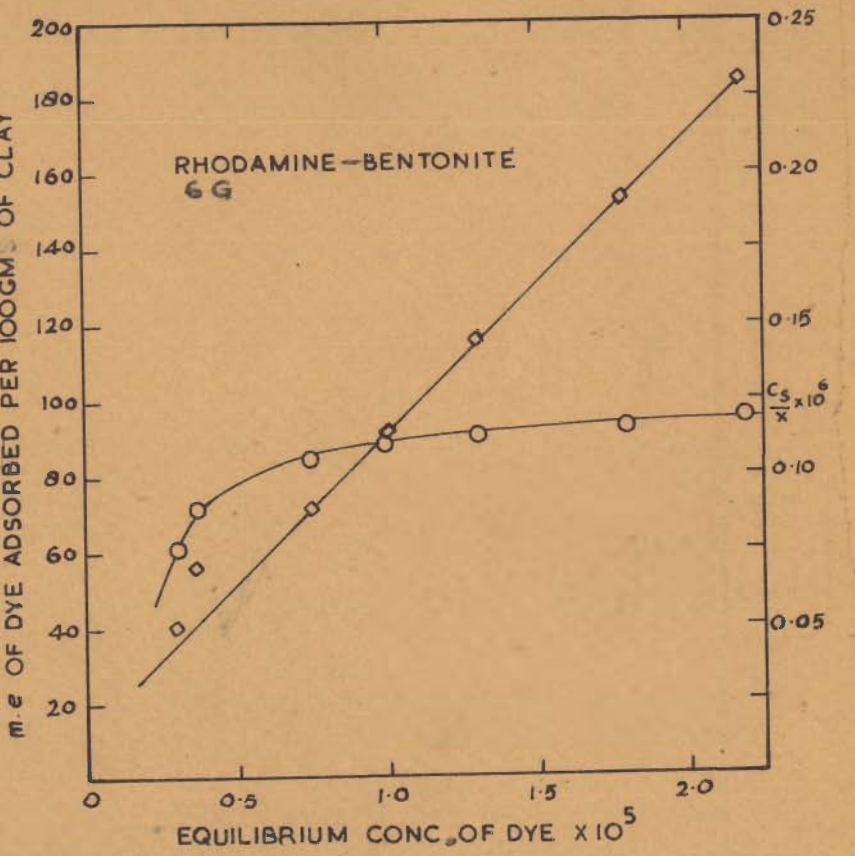

RHODAMINE–BENTONITE 6G
m.e OF DYE ADSORBED PER 100GM OF CLAY
EQUILIBRIUM CONC. OF DYE X 10^5
Cs/x x 10^6
0
0·5
1·0
1·5
2·0
20
40
60
80
100
120
140
160
180
200
0·05
0·10
0·15
0·20
0·25

FIG 8

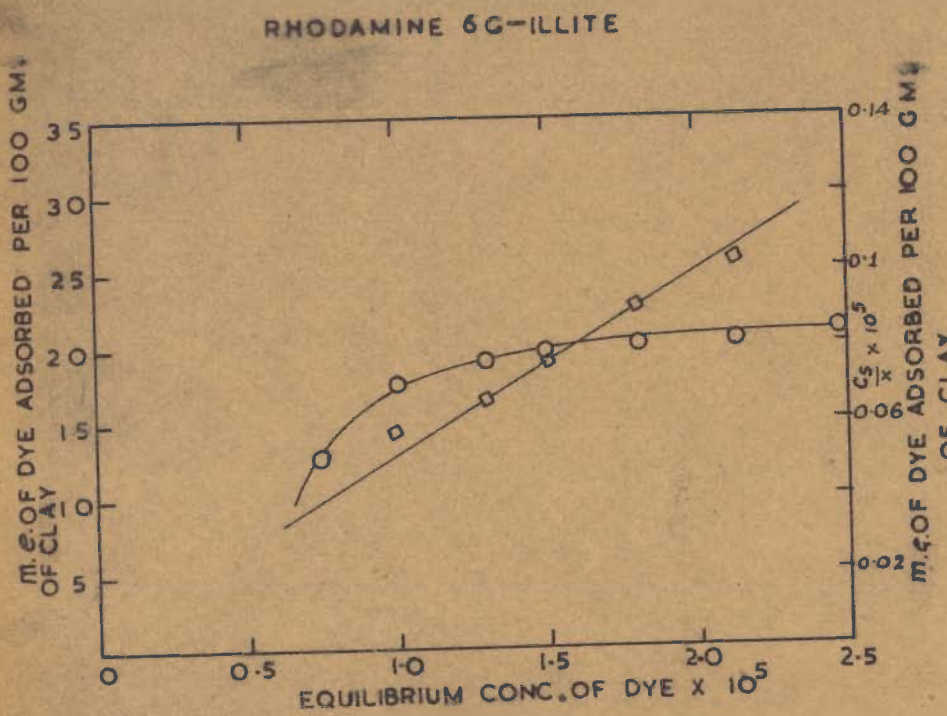

RHODAMINE 6G—ILLITE
m.e. OF DYE ADSORBED PER 100 GM. OF CLAY
m.e. OF DYE ADSORBED PER 100 GM. OF CLAY
$\frac{C_S}{x} \times 10^5$
EQUILIBRIUM CONC. OF DYE X 10^5
0
0·5
1·0
1·5
2·0
2·5
5
10
15
20
25
30
35
0·02
0·06
0·1
0·14

FIG. 9.

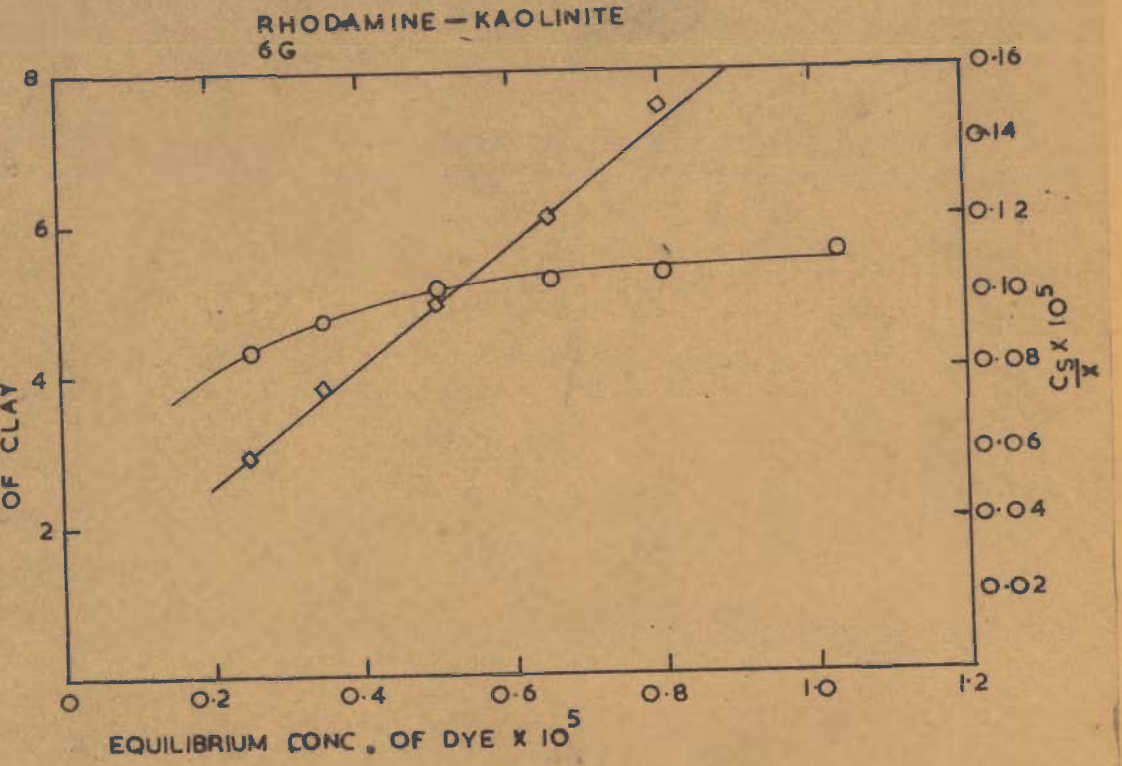

RHODAMINE—KAOLINITE 6G
m.e. OF DYE ADSORBED PER 100 GM. OF CLAY
$\frac{C_S}{x} \times 10^5$
EQUILIBRIUM CONC. OF DYE X 10^5
0
0·2
0·4
0·6
0·8
1·0
1·2
2
4
6
8
0·02
0·04
0·06
0·08
0·10
0·12
0·14
0·16

FIG. 10.

ADSORPTION OF METHYLENE BLUE BY BENTONITE AT DIFFERENT pH VALUES.

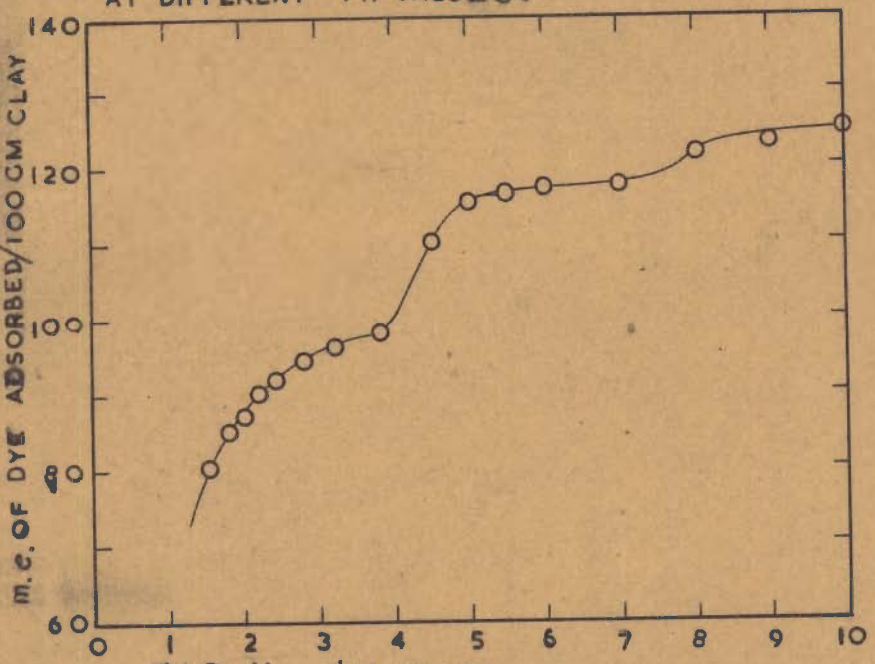


FIG. II.

ADSORPTION OF METHYLENE BLUE BY ILLITE AT DIFFERENT pH VALUES.

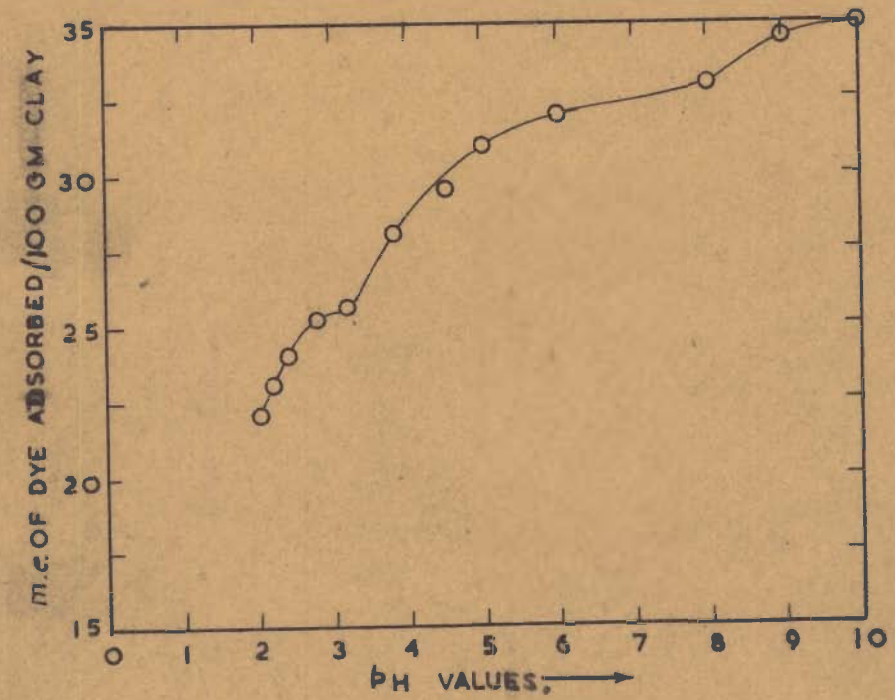


FIG 12

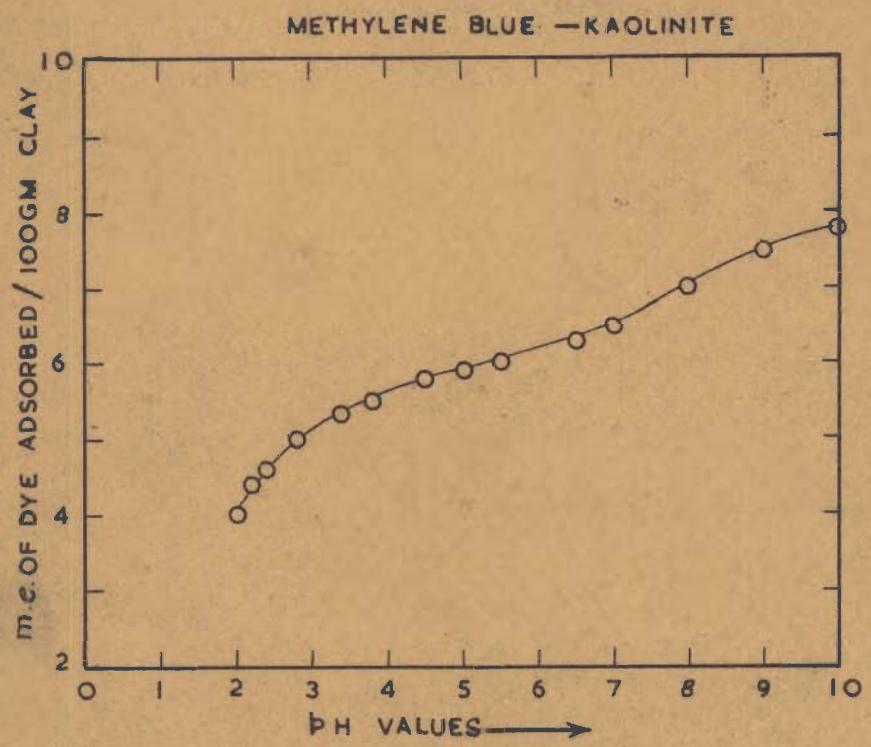

METHYLENE BLUE —KAOLINITE
m.e. OF DYE ADSORBED / 100GM CLAY
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
PH VALUES

FIG. 13.

RESULTS AND DISCUSSION

Nature of Isotherms.

Adsorption isotherms of various dyes with bentonite, kaolinite and illite are shown in Figs.2 to 10 (Vide Tables No.2 to 10). The plots have been drawn between the equilibrium concentration of dye and the milli equivalents of dye adsorbed per 100 gms.of clay. The isotherms indicate a positive adsorption. All the isotherms are regular and concave to the concentration axis. This suggests that a uni-molecular adsorption of the dye takes place. Further confirmation is obtained from the plot of Cs/x (equilibrium concentration/amount adsorbed) against the equilibrium concentration. The data are found to fit in well in the Langmuir adsorption equation showing that no multilayer formation of dye takes place.

Area of exchange sites.

The area associated with each dye molecule was calculated by dividing the surface area by the number of adsorbed dye molecules. The area of the exchange site was determined by computing the values of surface area and cation exchange capacity. Typical calculation is shown below,

Clay mineral — Bentonite
Adsorbate — Methylene blue

Area per exchange site.

Surface area = 750 m^2/gm = 750 x 10^4 cm^2/gm.

= 750 x 10^{20} A^{o2}/gm.

C.E.C. = 99 m.e./100 gm. = 99/10^5 equivalents per gm.

= 99 x 6.023 x $10^{23}/10^5$ cations per gm.

Area per exchange site

Surface area/No.of cations

$$= \frac{750 \times 10^{20} \times 10^5}{99 \times 6.023 \times 10^{23}}$$

= 125.8 A^{o2}

Area associated with each methylene blue molecule.

Methylene blue adsorbed = 0.3916 gm/gm of clay.

Actual number of methylene blue molecules.

$$= \frac{0.3916 \times 6.023 \times 10^{23}}{356}$$

Area associated with one methylene blue molecule.

$$= \frac{750 \times 10^{20} \times 356}{6.023 \times 10^{23} \times 0.3916}$$

= 113.2 A^{o2}

The results are tabulated in Table No.17.

Table No.17

Areas of exchange site and area associated per dye molecule

	Area per exchange site in A^{o2}.	Area associated per methylene blue molecule in A^{o2}.	Area associated per crystal violet molecule in A^{o2}.	Area associated per rhodamine 6G molecule in A^{o2}.
Bentonite	125.8	113.2	122.2	132.4
Illite	60.02	55.8	59.6	74.3
Kaolinite	47.4	56.7	47.1	53.7

The values of cation exchange capacity as determined from dye adsorption data are given below:

Table No.18

Cation exchange capacity as determined by

	Ammonium acetate method.	Methylene blue adsorption.	Crystal violet adsorption	Rhodamine 6G adsorption.
Bentonite	99m.e/100gm	110 m.e/100gm	102m.e/100gm	94m.e/100gm
Illite	26.0 ''	28.0 ''	26.3 ''	21.0 ''
Kaolinite	6.3 ''	4.3 ''	6.3 ''	4.3 ''

Kaolinite.

The c.e.c. values obtained from the dye adsorption method are 4.3 for methylene blue and rhodamine 6G and 6.2

in the case of crystal violet. The c.e.c.found from the conventional (ammonium acetate) method is 6.3. The discrepancy can be explained by comparing the data of the area per exchange site of the clay and the area covered by a particular dye. From the Table No.17 it can be seen that the area associated per dye molecule in the case of rhodamine 6G and methylene blue is greater than the area per exchange site . On the other hand the c.e.c.value determined by crystal violet adsorption coincides well with the standard c.e.c.of the mineral. The larger area associated per dye molecule in the case of rhodamine 6G and methylene blue as compared to area per exchange site naturally leads us to conclude that the coverage effect is more dominant in the case of these two dyes. In other words each dye molecule has an access to more than one exchange site. It is for this reason that the cation exchange capacity determined by these two dyes have lower values than the normal c.e.c.value.

Bentonite and illite.

In the case of bentonite and illite the area associated per methylene blue molecule is lesser than the area per exchange site which naturally gives a higher value of cation exchange capacity. The area associated per rhodamine 6G molecule is greater than the

area per exchange site and as a result this dye gives a lower value of c.e.c. than the standard value for both the minerals. Here again the most reasonable value is obtained from the adsorption data of crystal violet. The discrepancy in the case of these two minerals can again be explained in terms of coverage effect. Larger coverage than required on the basis of area per exchange site is,therefore dependant not only on the type of the dye bound but also on the clay mineral used. In the case of bentonite and illite the amount of methylene blue adsorbed exceeds the cation exchange capacity of the two minerals. The additional dye adsorption on both clays can be due to purely physical(van der Waal) adsorption. Assuming that the existence of the two kinds of adsorption sites on the mineral surface would not interfere with each other ,then the total dye adsorption capacity can be regarded as,

$$MB = MB^{C} + MB^{H}$$

where MB = Total dye adsorption capacity.

MB^{C} = Cation exchange capacity in m.e/gm.

MB^{H} = Physical adsorption in m.e/gm.

From this equation we can calculate the value of MB^{H},i.e., we can have an estimate of the surface concentration of the physical adsorption sites,

MB^{H} per cm^2 = MB - MB^{C}/Surface area in cm^2/gm.

The values obtained for the surface concentration of physical adsorption sites in case of bentonite and illite is,

MB^H m.e/cm^2 x 10^8 = 5.0 for bentonite and 5.9 for illite.

which agrees well with the value of MB^H per sq.cm. for silica (5.2)[22] where the dye adsorption can be considered solely due to physical adsorption.

Variation of adsorption with pH.

Tables No.11 to 13 and the corresponding curves (Figs.11 to 13) show the variations in adsorption due to change in pH. It can be seen that the adsorption of the dye increases with increase in pH in the case of all the three minerals. To explain this behaviour one has to take into account the possible existence of an electrical double layer on the edge surface also. This layer as pointed out by Van Olphen[24] would be entirely different from the layer existing at the basal pinacoids of clays which get a negative charge as a result of isomorphous substitution of Si^{4+} by Al^{3+} or other elements of lower valence. This negative charge persists despite changes in pH.

At the edges the tetrahedral silica sheets and the octahedral alumina sheets are disrupted and the primary bonds are broken. The picture may be depicted

by assuming -M-O-M-bond (M=Si or Al) at the edges breaking to give MO^- and M^+. A slightly acidic medium would result in the formation of a very weak,undissociable acid M-OH and thus a neutral surface is created. Addition of more acid(decreasing pH) would result in the adsorption of H^+ ions leading to the formation of a positive double layer. On the other hand with increasing pH the weak acid,M-OH would be converted into highly dissociable M-OR (R represents the alkali metal ion) resulting in the formation of negative electrical double layer. The edge would thus represent an electrical double layer of changing polarity depending on the pH of the medium.

The variations in adsorption due to change in pH can be interpreted in the light of the above discussion. Since at low pH the edges are positively charged therefore the dye cations are adsorbed only at clay surface and not at the edges. However,with increase of pH the edges become negatively charged,the dye cations get adsorbed at them also with the result that the adsorption of dye as a whole is increased.

It is further interesting to note that the amount of methylene blue adsorbed increases by 21 to 22 percent as we go from acidic to basic side in case of kaolinite and illite. This percentage is presumably similar to the percentage of the total surface area of the crystal by

the edge faces as found by Hendricks and Alexander[18].

In the case of bentonite and illite two distinct stages of adsorption can be recognised (from pH 2 to 10.0) one between 98 to 115 m.e.(difference 17 m.e.) and 115 to 124 m.e.(difference 9 m.e.) for bentonite and 24 to 31 m.e.(difference 7 m.e.) and 31 to 34 m.e. (difference 3 m.e.) for illite. The first stage (corresponding to a difference of 17 m.e.in case of bentonite and 7 m.e. in case of illite) would represent the adsorption at edges caused by Si-O sites and the second stage (corresponding to a difference of 9 m.e.in the case of bentonite and 3 m.e. in the case of illite) would represent the adsorption at the lateral surface constituted of Al-O adsorption sites. Since the silanol group (Si-OH) in silica sol readily dissociates to give SiO^- in slightly alkaline medium,the first stage represents adsorption of dye on the Si-O sites. On the other hand alumina due to its amphoteric character would get a negative charge at much higher pH and therefore the adsorption indicated by the second stage will be at Al-O sites.

Variation of dye adsorption with clay mineral content.

Table No.14

Variation of dye adsorption with C.E.C.of bentonite.

Percentage of silica.	Clay mineral percentage.	C.E.C.in m.e.per 100 gm. clay.	Amount of methylene blue adsorbed in gm. per gm.of clay.	Amount of rhodamine 6G adsorbed in gms. per gm.of clay.	Amount of crystal violet adsorbed in gm.per gm.of clay.
0.0	100	99.0	0.3916	0.4239	0.4523
20.0	80	78.12	0.3250	0.3412	0.3632
40.0	60	59.60	0.2358	0.2601	0.2760
50.0	50	48.20	0.2050	0.2123	0.2251
60.0	40	39.60	0.1568	0.1710	0.1850
80.0	20	18.40	0.0785	0.0847	0.0915

Fig.14.

Table No.15

Variation of dye adsorption with C.E.C. of illite.

Percentage of silica.	Clay mineral percentage.	C.E.C. in m.e. per 100 gm. clay.	Amount of methylene blue adsorbed in gm. per gm. of clay	Amount of rhodamine 6G adsorbed in gm. per gm. of clay.	Amount of crystal violet adsorbed in gm. per gm. of clay.
0.0	100	26.00	0.0996	0.0947	0.1167
20.0	80	21.00	0.0810	0.0760	0.0940
40.0	60	15.40	0.0598	0.0572	0.0710
50.0	50	13.70	0.0511	0.0480	0.0610
60.0	40	11.10	0.0399	0.0380	0.0466
80.0	20	5.20	0.0199	0.0189	0.0234

Fig.15

Table No.16

Variation of dye adsorption with C.E.C. of kaolinite.

Percentage of silica.	Clay mineral percentage.	C.E.C. in m.e. per 100 gm. clay.	Amount of methylene blue adsorbed in gm. per gm. of clay.	Amount of rhodamine 6G adsorbed in gm. per gm. of clay.	Amount of crystal violet adsorbed in gm. per gm. of clay.
0.0	100	6.30	0.0188	0.0252	0.0279
20.0	80	5.20	0.0151	0.0205	0.0229
40.0	60	3.80	0.0110	0.0152	0.0168
50.0	50	3.12	0.0089	0.0127	0.0135
60.0	40	2.80	0.0075	0.0105	0.0119
80.0	20	1.25	0.0034	0.0050	0.0055

Fig.16.

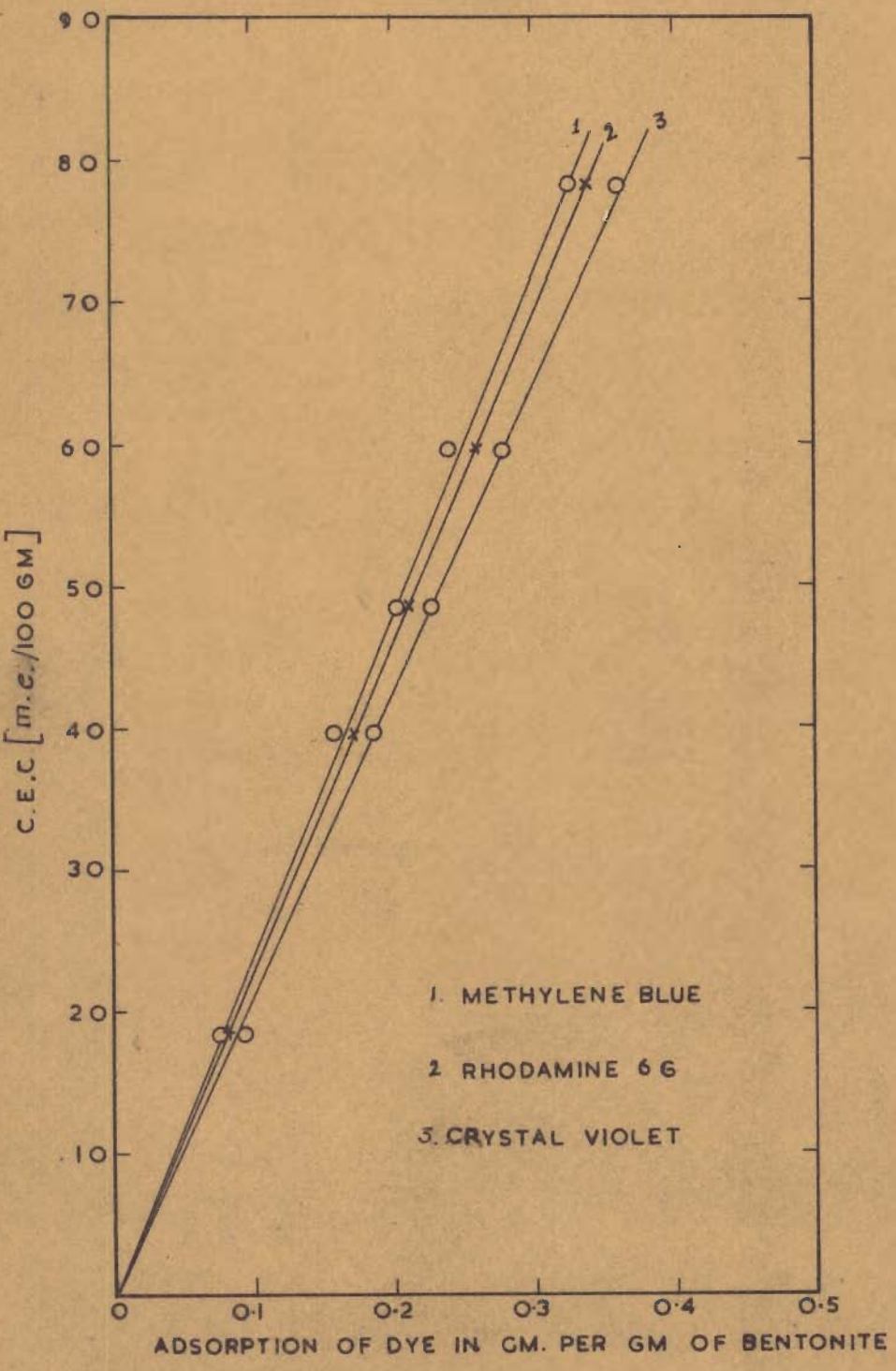

C.E.C [m.e./100 GM]
90
80
70
60
50
40
30
20
10
0
0·1
0·2
0·3
0·4
0·5
1
2
3
1. METHYLENE BLUE
2 RHODAMINE 6 G
3. CRYSTAL VIOLET
ADSORPTION OF DYE IN GM. PER GM OF BENTONITE

FIG. 14.

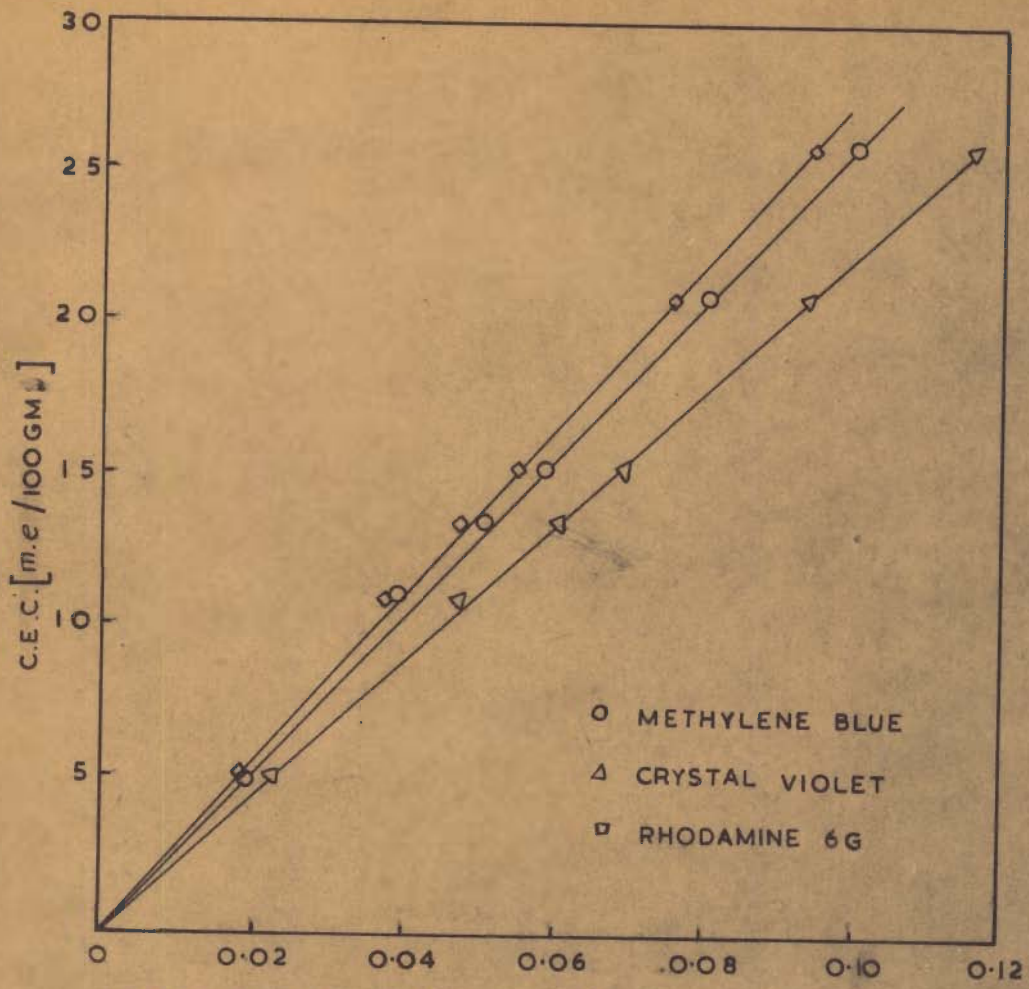

30
25
20
15
10
5
C.E.C. [m.e/100 GMS]
METHYLENE BLUE
CRYSTAL VIOLET
RHODAMINE 6G
0
0·02
0·04
0·06
0·08
0·10
0·12
ADSORPTION OF DYE IN GM. PER GM OF ILLITE.

FIG.15.

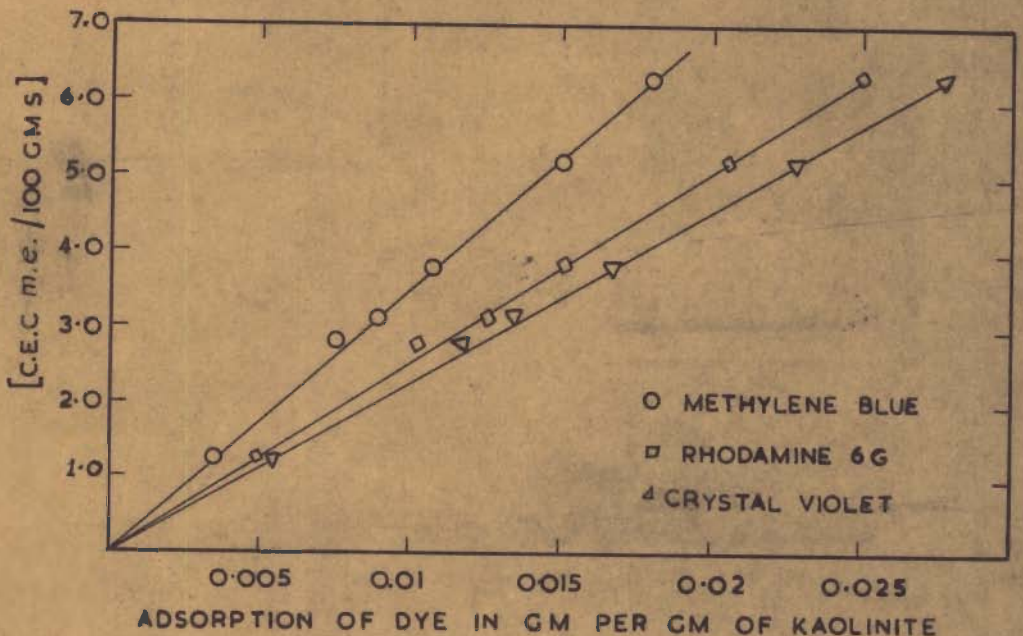

7·0
6·0
5·0
4·0
3·0
2·0
1·0
[C.E.C m.e./100 GMS]
METHYLENE BLUE
RHODAMINE 6G
CRYSTAL VIOLET
0·005
0.01
0·015
0·02
0·025
ADSORPTION OF DYE IN GM PER GM OF KAOLINITE

FIG.16.

Variation of dye adsorption with clay mineral content.

Each of the clay mineral was mixed with different proportions of silica passing through 100 mesh sieve. The cation exchange capacity of each sample was determined by the standard ammonium acetate method and the adsorption of dye was also determined by the method mentioned above. Tables 14,15 and 16 give the amount of dye adsorbed at different percentage of clay content. The amounts of these dyes adsorbed on silica used in these studies are 0.0015 gm./gm. for crystal violet and 0.0031 gms/gm. of methylene blue and 0.0022 gms/gm. of rhodamine 6G and hence in the calculation of dye adsorbed by samples of clay with different mineralogical percentage this amount was not considered.

Since the area associated per adsorbed molecule of dye depends on surface area and so the type of clay mineral, the dye adsorption should show a linear relationship with the clay mineral content or c.e.c. From the curves given in Figs.14,15 and 16 it can be seen that this relationship holds true in all the cases.

—

R E F E R E N C E S

1. Dyal,R.S. and Hendricks,S.B., Soil Sci.,69,421,1950.
2. Grim,R.E., Amer.Mineral., 45,259,1960.
3. Wintercorn,H.F., Colloid.Chem., 6,459,1946.
4. Bradley,W.F., J.Amer.Chem.Soc.,67,975,1945.
5. Hendricks,S.B., J.Phys.Colloid.Chem.,45,65,1941.
6. Allaway,W.H.,Cuthbert,F.L. and Grim,R.E., J.Amer.Cer.Soc., 30,137,1947.
7. Brindley,G.W. and Rustom,M., Amer.Min.,43,627,1958.
8. Brindley,G.W. and Hoffmann,R.W.,Bull.Geol.Soc.Amer., 70,1618,1959.
9. Badder,R.G. and Smith,J.B., Bull.Geol.Soc.Amer.,70, 1564,1959.
10. Doehler,R.W. and Young,W.A., Proc.of the ninth national Conf.on clay minerals. 468,1960.
11. Mackenzie,R.C., Trans.Farad.Soc.,44,368,1948.
12. Faust,G.T., U.S.Bureau of Mines,Rept.Invest.3522,1940.
13. Hendricks,S.B. and Alexander,L.T., J.Amer.Soc.Agron., 32,455,1940.
14. Weil-Malherbe,H. and Weiss,J., J.Chem.Soc.,2164,1948.
15. Shukervich,M.M., C.R.Acad.Sci. U.S.S.R. 94,327,1954.
16. Bossazza,V.L., Amer.Mineral.29,235,1944.
17. White,D. and Cowen,C.T., Trans.Brit.Cer.Soc.,57,210,1958.
18. Kipling,J.J. and Wilson,R.B., J.Appl.Chem.,10,109,1960.
19. Worrell,W., Trans.Brit.Cer.Soc., 57,210,1958.
20. Frysinger,G. and Thomas,H.C., 3rd Natl.Conf.Clays and Clay Minerals,1954.
21. Martland,M.M. and Mellor,J.L., Soil.Sci.Soc.Amer.Proc. 18,363,1964.

22. Piper C.S., Soil and Plant Analysis.154-190.

23. Robertson,R.H.S. and Ward,R.M., J.Pharmacy Pharmacol,3,27,1951.

24. Vanolphen,H., An Introduction to Clay Colloid Chemistry Inter-science Publishers, New York.

—

CHAPTER II.

Spectrophotometric studies of the aqueous suspensions of clay-dye complexes.

INTRODUCTION

The importance of dye clay reaction in identifying[1-3] clay minerals is well known. The reaction has also found use in determining the surface area[4] of clays. Quantitative studies on such reactions have, however, not been undertaken so far although the problem can be very well investigated from two angles:

(i) by carrying out adsorption studies using clays as adsorbents;

(ii) by studying the reaction entirely in the liquid phase using dilute clay suspensions.

The results on the first aspect have been thoroughly discussed in Chapter I. This chapter deals with the investigation on the second aspect of the problem.

Most of the dyes behave as ordinary electrolytes in aqueous solutions although many tend to exist as colloid. In the latter case dye aggregates are formed and sometimes this phenomenon is exhibited even in fairly dilute solutions. This fact should be kept in view when planning studies on dye-clay reactions.

Reaction of dyes with colloidal suspensions other than clays were recently initiated in these laboratories. The binding of a number of dyes to hydrous oxide sols[5] and surfactants[6] was investigated using the spectrophotometric method. On the basis of these studies the mode and extent

of binding of various dyes with hydrous oxide sols and the range of their micelle size has been estimated.

The results on the binding of methylene blue, crystal violet and rhodamine 6G to clays are described in the following pages. Dye solutions below the aggregation point were used and Klotz's equation[7] was employed to calculate the binding.

—

EXPERIMENTAL

Reagents.

Methylene blue, crystal violet, and rhodamine 6G used in these investigations were B.D.H. products. Standard clay minerals bentonite, kaolinite and illite were obtained from Ward's Natural Science Establishment New York. H-clays were prepared as described in Chapter I.

Apparatus.

A Bausch and Lomb 'Spectronic 20' was used for absorption measurements. The molar extinction coefficients were calculated from the relationship,

$$\epsilon = \left(\frac{1}{C.D}\right) \log I_o/I$$ where ϵ is the molar extinction coefficient; I_o is the intensity of the incident light falling on medium of thickness d. I is the intensity of transmitted light and c is concentration.

pH measurements were made with a Cambridge Bench Type pH-meter.

Formula used

The binding of dye was calculated using Klotz's equation which was originally employed to study the reaction of proteins with organic anions. The validity of this equation was extended to very dilute clay-dye suspensions. The equation is,

$$\alpha = \frac{\epsilon_{\text{apparent}} - \epsilon_{\text{bound}}}{\epsilon_{\text{free}} - \epsilon_{\text{bound}}}$$

where ϵ apparent is the apparent molar extinction

coefficient, ϵ bound is the molar extinction coefficient of bound dye and ϵ free is the molar extinction coefficient of free dye. α is the fraction of free dye which has been left behind unreacted.

Procedure.

Requisite amounts of dyes were taken in tubes to get the effective concentration as 1 $x10^{-5}$M, 5 x 10^{-6}M and 2 x 10^{-6}M. The solution was made up to 25 c.c. by double distilled water. The absorption of these tubes were noted to calculate the molar extinction coefficient of free dye.

A second set containing the same amount of dyes as mentioned above and varying amounts of clay suspensions (Tables 1-19) was arranged. The total was made up to 25 c.c. by double distilled water. A time of about 5 hours was allowed to lapse before making absorption measurements. The data were used to know the apparent molar extinction coefficient. The molar extinction coefficient of the bound dye was found as follows:

The curve between the clay concentration and the absorption values of clay-dye suspension was first plotted (Fig. 1-19). Since there was no change in the absorption values after a certain clay concentration, a flat portion was obtained in the curve. This flat portion was extrapolated to zero clay concentration in order to evaluate the exact absorbance of the bound dye. Dividing this absorbance

value by the dye concentration the value of molar extinction coefficient of bound dye was determined.

—

Table No.1

Absorption spectra of $1x10^{-5}$M methylene blue in presence of different quantities of H-bentonite at pH 3.5.

A B S O R B A N C E

Wave length in mμ.	1 0.016%	2 0.020%	3 0.024%	4 0.028%	5 0.032%	6 0.036%	7 0.04%
655	0.59	0.61	0.65	0.72	0.73	0.79	0.85
660	0.61	0.64	0.68	0.75	0.74	0.80	0.88
665	0.64	0.66	0.69	0.76	0.77	0.82	0.92
670	0.65	0.66	0.71	0.76	0.78	0.82	0.93
675	0.61	0.63	0.64	0.70	0.75	0.79	0.90

Total volume of solution in each case=25 c.c.

Fig.1.
Curve 1.

Table No.2

Absorption spectra of $1x10^{-5}$M methylene blue in presence of different quantities of H-bentonite at pH 9.0.

A B S O R B A N C E

Wave length in mμ.	1 0.012%	2 0.016%	3 0.020%	4 0.024%	5 0.028%	6 0.032%	7 0.036%
655	0.42	0.42	0.44	0.45	0.48	0.53	0.56
660	0.43	0.44	0.44	0.49	0.50	0.56	0.60
665	0.44	0.44	0.46	0.50	0.53	0.58	0.61
670	0.44	0.45	0.46	0.52	0.55	0.59	0.63
675	0.42	0.44	0.42	0.50	0.53	0.53	0.61

Total volume of solution in each case = 25 c.c.

Fig.1.
Curve 2.

Percentage of clay added in each case is given at the top of each table.

Table No.3

Absorption spectra of 5×10^{-6}M methylene blue in presence of varying amounts of H-bentonite at pH 3.5.

ABSORBANCE

Wave length in mμ.	1 0.012%	2 0.014%	3 0.016%	4 0.018%	5 0.02%	6 0.022%
655	0.200	0.236	0.284	0.309	0.337	0.387
660	0.207	0.244	0.301	0.318	0.346	0.408
665	0.214	0.251	0.301	0.332	0.356	0.414
670	0.214	0.259	0.309	0.337	0.366	0.414
675	0.207	0.251	0.302	0.318	0.361	0.403

Total volume of solution in each case = 25 c.c.

Fig.2.
Curve 1.

Table No.4

Absorption spectra of 5×10^{-6}M methylene blue in presence of varying amounts of H-bentonite at pH 9.0.

ABSORBANCE

Wave length in mμ.	1 0.01%	2 0.012%	3 0.014%	4 0.016%	5 0.018%	6 0.02%
655	0.301	0.332	0.366	0.387	0.475	0.522
660	0.314	0.342	0.376	0.408	0.494	0.537
665	0.327	0.356	0.397	0.420	0.508	0.552
670	0.332	0.361	0.397	0.431	0.515	0.560
675	0.327	0.351	0.395	0.420	0.507	0.552

Total volume of solution in each case = 25 c.c.

Fig.2.
Curve 2.

Percentage of clay added in each case is given at the top of each table.

Table No.5

Absorption spectra of $1x10^{-5}$M methylene blue in presence of different quantities of H-illite at pH 4.0.

ABSORBANCE

Wave length inmμ.	1 0.038%	2 0.04%	3 0.06%	4 0.08%	5 0.10%	6 0.12%	7 0.14%
565	0.314	0.426	0.494	0.530	0.628	0.721	0.920
570	0.318	0.434	0.501	0.537	0.633	0.725	0.939
575	0.327	0.437	0.508	0.545	0.642	0.732	0.958
580	0.337	0.443	0.511	0.560	0.647	0.769	0.978
585	0.327	0.431	0.508	0.552	0.638	0.757	0.958

Total volume of solution in each case =25 c.c.

Fig.3.
Curve 1.

Table No.6

Absorption spectra of $1x10^{-5}$M methylene blue in presence of different amount of H-illite at pH 9.0.

ABSORBANCE

Wave length in mμ.	1 0.036%	2 0.038%	3 0.04%	4 0.06%	5 0.08%	6 0.10%	7 0.12%
565	0.301	0.327	0.408	0.443	0.552	0.657	0.353
570	0.309	0.356	0.420	0.445	0.568	0.677	0.886
575	0.356	0.361	0.426	0.462	0.576	0.681	0.920
580	0.366	0.371	0.437	0.468	0.585	0.688	0.939
585	0.351	0.366	0.431	0.455	0.568	0.677	0.920

Total volume of solution in each case = 25 c.c.

Fig.3.
Curve 2.

Percentage of clay added in each case is given at the top of each table.

Table No.7

Absorption spectra of 5×10^{-6}M methylene blue in presence of different quantities of H-illite at pH 4.0.

ABSORBANCE

Wave length in mμ.	1 0.024%	2 0.028%	3 0.032%	4 0.036%	5 0.04%	6 0.048%	7 0.068%
565	0.193	0.251	0.301	0.314	0.371	0.507	0.585
570	0.197	0.255	0.305	0.318	0.376	0.508	0.593
575	0.200	0.259	0.309	0.327	0.381	0.511	0.598
580	0.207	0.267	0.314	0.337	0.381	0.515	0.602
585	0.200	0.259	0.309	0.323	0.375	0.508	0.593

Total volume in each case = 25 c.c.

Fig.4
Curve 1.

Table No.8.

Absorption spectra of 5×10^{-6}M methylene blue in presence of different quantities of H-illite at pH 9.0.

ABSORBANCE

Wave length in mμ	1 0.022%	2 0.024%	3 0.028%	4 0.032%	5 0.036%	6 0.040%	7 0.060%
565	0.180	0.221	0.267	0.301	0.337	0.443	0.494
570	0.183	0.225	0.271	0.305	0.342	0.449	0.508
575	0.190	0.229	0.275	0.309	0.346	0.455	0.522
580	0.197	0.232	0.279	0.318	0.351	0.468	0.537
585	0.193	0.225	0.275	0.314	0.346	0.462	0.530

Total volume of solution in each case = 25 c.c.

Fig.4
Curve 2.

Percentage of clay added in each case is given at the top of each table.

Table No.9

Adsorption spectra of 5×10^{-6}M methylene blue in presence of different quantities of H-kaolinite at pH 4.0.

ABSORBANCE

Wave length in mμ.	1 0.06%	2 0.1%	3 0.14%	4 0.18%	5 0.26%	6 0.28%
560	0.327	0.443	0.593	0.836	1.15	1.18
565	0.337	0.449	0.610	0.892	1.16	1.22
570	0.342	0.455	0.619	0.903	1.18	1.26
575	0.346	0.462	0.619	0.920	1.26	1.30
580	0.337	0.455	0.585	0.879	1.22	1.22

Total volume of solution in each case = 25 c.c.

Fig.5
Curve 1.

Table No.10

Absorption spectra of 2×10^{-6}M methylene blue in presence of different amounts of H- kaolinite at pH 4.0.

ABSORBANCE

Wave length in mμ.	1 0.04%	2 0.06%	3 0.08%	4 0.10%	5 0.12%	6 0.15%
560	0.175	0.292	0.376	0.488	0.522	0.537
565	0.177	0.296	0.381	0.494	0.537	0.552
570	0.180	0.301	0.387	0.501	0.552	0.585
575	0.183	0.305	0.397	0.508	0.576	0.593
580	0.180	0.301	0.392	0.501	0.571	0.585

Total volume of solution in each case= 25 c.c.

Fig.5.
Curve 2.

Percentage of clay added in each case is given at the top of each table.

Table No.11

Absorption spectra of $1x10^{-5}$M crystal violet in presence of different amounts of H-bentonite at pH 4.0.

A B S O R B A N C E

Wave length in mμ.	1 0.016%	2 0.020%	3 0.024%	4 0.028%	5 0.032%	6 0.036%	7 0.038%
640	0.481	0.537	0.638	0.721	0.769	0.853	0.958
645	0.508	0.560	0.667	0.744	0.809	0.886	1.000
650	0.522	0.568	0.688	0.769	0.823	0.920	1.040
655	0.530	0.585	0.699	0.782	0.838	0.939	1.040
665	0.481	0.552	0.638	0.721	0.740	0.869	1.000

Total volume of solution in each case = 25 c.c.

Fig.6.
Curve 1.

Table No.12

Absorption spectra of $5x10^{-6}$M crystal violet in presence of varying amounts of H-bentonite at pH 4.0.

A B S O R B A N C E

Wave length in mμ.	1 0.012%	2 0.014%	3 0.015%	4 0.016%	5 0.018%	6 0.019%	7 0.02%
635	0.337	0.463	0.508	0.552	0.619	0.677	0.699
645	0.366	0.501	0.576	0.610	0.667	0.721	0.744
650	0.387	0.530	0.602	0.628	0.688	0.732	0.769
655	0.397	0.537	0.610	0.638	0.699	0.744	0.769
660	0.371	0.501	0.568	0.585	0.647	0.699	0.721

Total volume taken in each case = 25 c.c.

Fig.6.
Curve 2.

Percentage of clay added in each case is given at the top of each table.

Table No.13

Absorption spectra of 6×10^{-6}M crystal violet in presence of varying amounts of H-illite at pH 4.0.

ABSORBANCE

Wave length in mμ.	1 0.02%	2 0.032%	3 0.044%	4 0.056%	5 0.068%	6 0.072%
540	0.397	0.494	0.522	0.657	0.744	0.769
545	0.408	0.501	0.537	0.667	0.769	0.795
550	0.417	0.508	0.545	0.688	0.782	0.795
555	0.408	0.501	0.537	0.677	0.769	0.782
560	0.395	0.491	0.522	0.657	0.744	0.769

Total volume of solution in each case =25 c.c.

Fig.7.

Table No.14

Absorption spectra of 2.5×10^{-6}M crystal violet in presence of different amounts of H-illite at pH 4.0.

ABSORBANCE

Wave length in mμ.	1 0.010%	2 0.012%	3 0.024%	4 0.028%	5 0.036%	6 0.037%
540	0.197	0.229	0.267	0.397	0.494	0.522
545	0.200	0.232	0.271	0.408	0.508	0.530
550	0.204	0.236	0.280	0.420	0.522	0.530
555	0.200	0.229	0.275	0.408	0.515	0.522
560	0.193	0.221	0.267	0.397	0.508	0.508

Total volume of solution in each case = 25 c.c.

Fig.8.

Percentage of clay added in each case is given at the top of each table.

Table No.15

Absorption spectra of $1x10^{-5}$M rhodamine 6G in presence of varying amounts of H-bentonite at pH 4.00.

A B S O R B A N C E

Wave length in mμ.	1 0.012%	2 0.016%	3 0.02%	4 0.024%	5 0.032%	6 0.04%	7 0.042%
515	0.552	0.628	0.699	0.782	0.939	1.08	1.15
520	0.602	0.699	0.744	0.823	1.000	1.12	1.18
525	0.657	0.744	0.795	0.836	1.040	1.15	1.22
530	0.677	0.769	0.823	0.903	1.070	1.22	1.26
535	0.653	0.744	0.809	0.886	1.040	1.20	1.26

Total volume of solution taken in each case =25 c.c.

Fig.9.
Curve 1.

Table No.16.

Absorption spectra of $5x10^{-6}$M rhodamine 6G in presence of varying amounts of H-bentonite at pH 4.0.

A B S O R B A N C E

Wave length in mμ.	1 0.008%	2 0.012%	3 0.014%	4 0.016%	5 0.018%	6 0.02%
515	0.449	0.545	0.694	0.809	0.853	0.886
520	0.475	0.568	0.721	0.823	0.869	0.903
525	0.494	0.585	0.732	0.853	0.886	0.920
530	0.508	0.602	0.744	0.853	0.892	0.939
535	0.505	0.593	0.744	0.842	0.886	0.928

Total volume of solution in each case = 25 c.c.

Fig.9.
Curve 2.

Percentage of clay added in each case is given at the top of each table.

Table No.17.

Absorption spectra of 1×10^{-5}M rhodamine 6G in presence of different amounts of H-illite at pH 4.0.

A B S O R B A N C E

Wave length in mμ.	1 0.056%	2 0.064%	3 0.072%	4 0.08%	5 0.12%	6 0.16%
525	0.481	0.530	0.593	0.667	0.920	1.04
530	0.537	0.568	0.638	0.699	0.958	1.07
535	0.552	0.619	0.677	0.744	0.978	1.09
540	0.568	0.638	0.699	0.757	1.000	1.12
545	0.522	0.619	0.677	0.744	0.958	1.09

Total volume taken in each case = 25 c.c.

Fig.10.

Table No.18

Absorption spectra of 5×10^{-6}M rhodamine 6G in presence of different amounts of H-illite at pH 4.0.

A B S O R B A N C E

Wave length in mμ.	1 0.024%	2 0.032%	3 0.036%	4 0.060%	5 0.068%	6 0.074%
525	0.361	0.420	0.481	0.657	0.757	0.769
530	0.376	0.426	0.488	0.667	0.769	0.782
535	0.387	0.443	0.508	0.677	0.774	0.795
540	0.395	0.449	0.511	0.688	0.782	0.809
545	0.387	0.443	0.501	0.667	0.769	0.795

Total volume taken in each case = 25 c.c.

Fig.11.

Percentage of clay added in each case is given at the top of each table.

Table No.19

Absorption spectra of 5×10^{-6}M rhodamine 6G in presence of varying amount of H-kaolinite at pH 4.0.

ABSORBANCE

Wave length in mμ.	1 0.14%	2 0.16%	3 0.20%	4 0.24%	5 0.26%	6 0.28%	7 0.30%
525	0.392	0.462	0.515	0.593	0.647	0.732	0.744
530	0.408	0.468	0.522	0.602	0.657	0.744	0.749
535	0.420	0.481	0.525	0.610	0.661	0.757	0.757
540	0.431	0.488	0.530	0.619	0.667	0.757	0.769
545	0.414	0.468	0.520	0.593	0.638	0.744	0.744

Total volume taken in each case = 25 c.c.

Fig.12.

Percentage of clay added in each case is given at the top of each table.

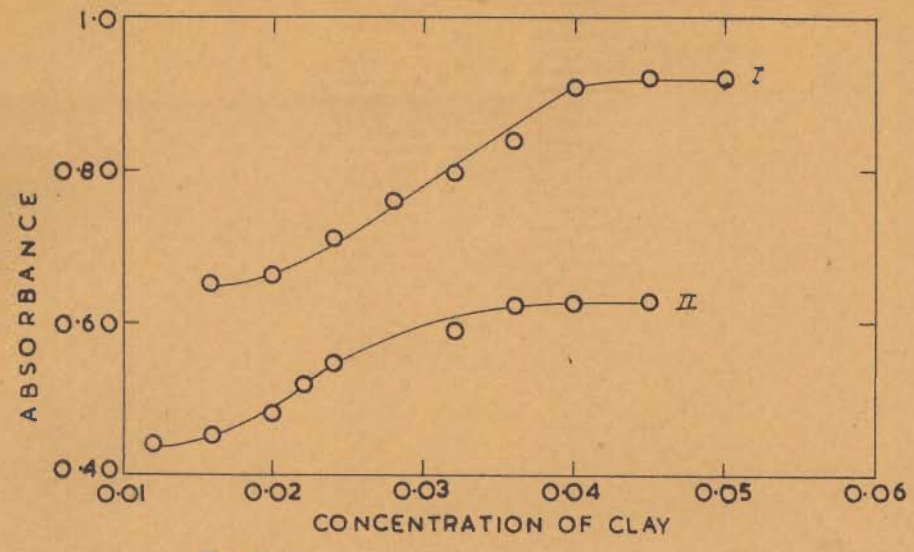


FIG.1.

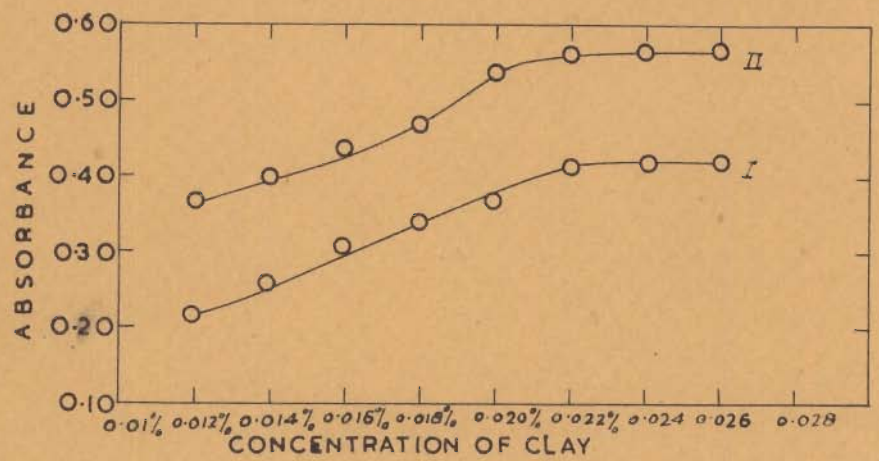


FIG.2.

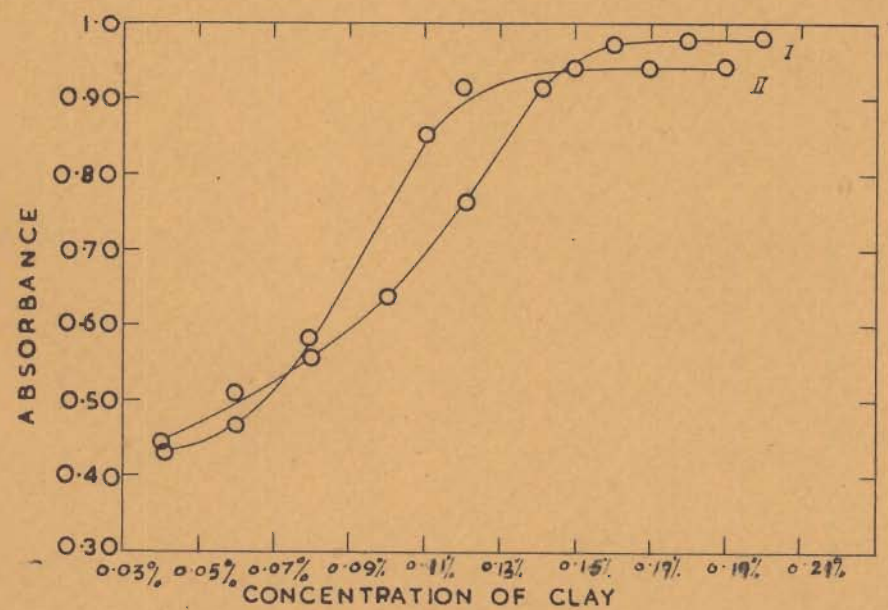


FIG.3.

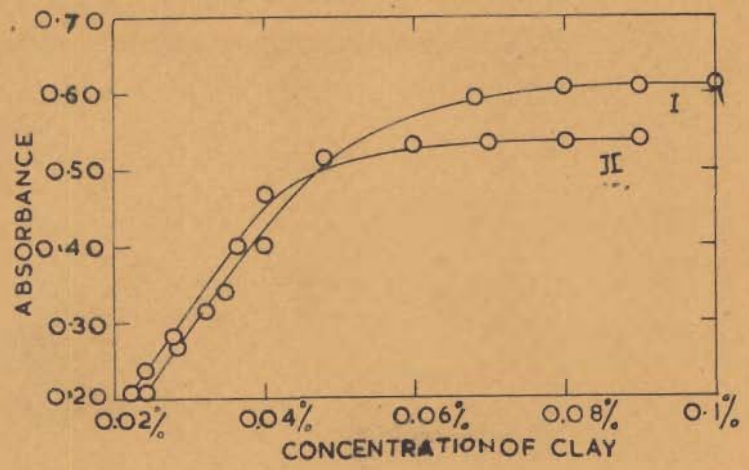


FIG.4.

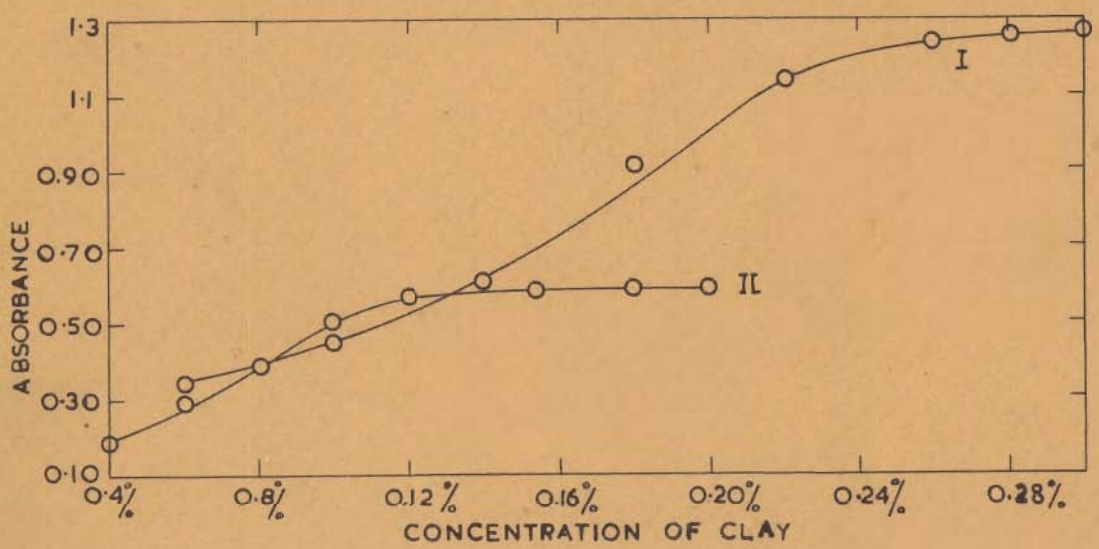


FIG. 5.

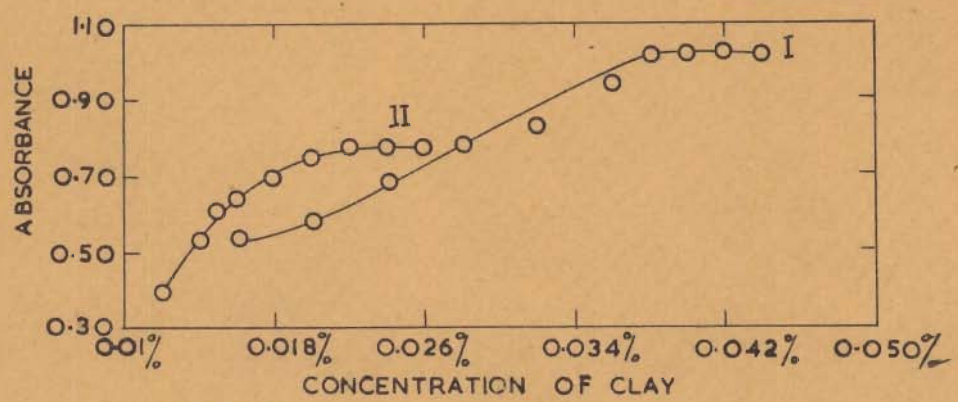


FIG. 6.

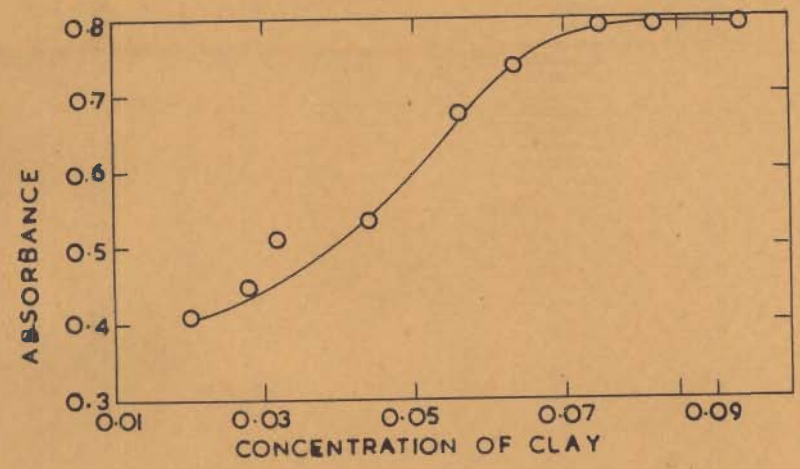


FIG.7.

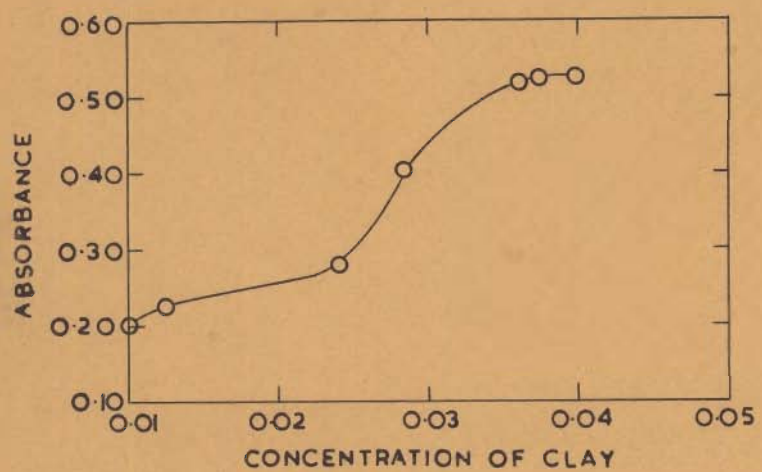


FIG.8.

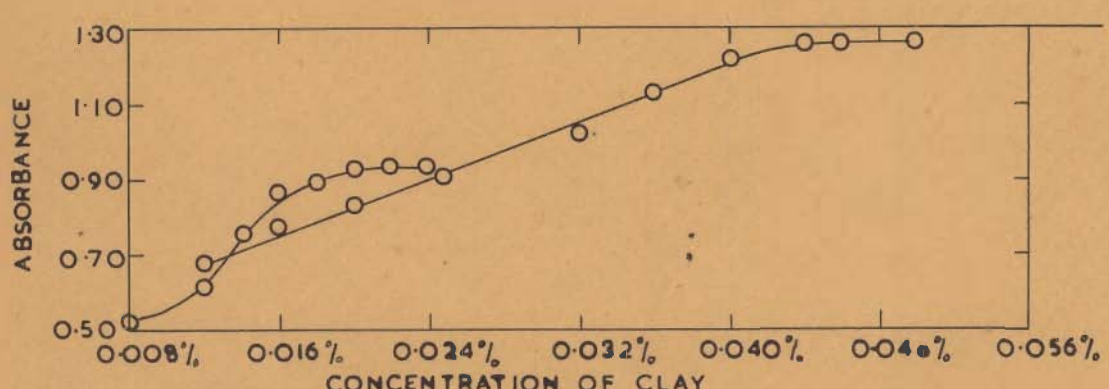


FIG.9.

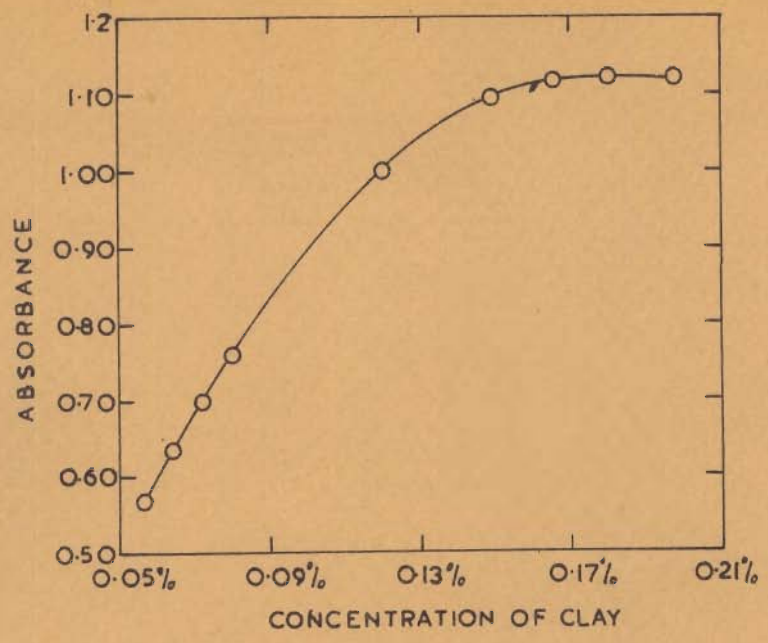


FIG 10

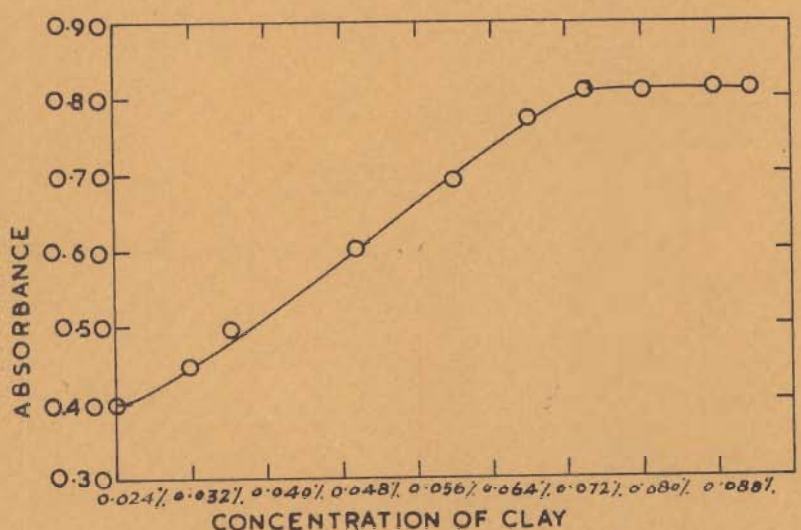


FIG 11

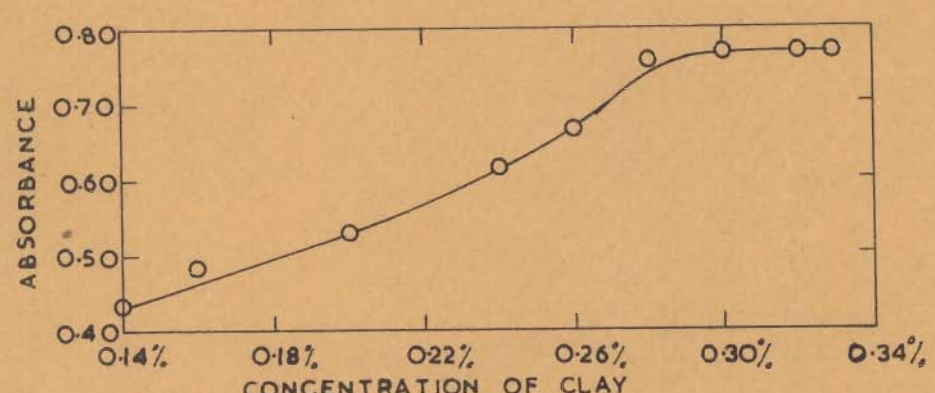


FIG 12

RESULTS AND DISCUSSION

From the experimental results summarized in Tables (1-19) it can be seen that by the addition of even a very small amount of clay to the dye a marked shift in maxima is observed. The shifts observed for the different cases are:

Dye	Absorption maxima without clay. mμ.	Absorption maxima with clay. mμ.	Clay
Methylene blue.	655	670	Bentonite
"	"	590	Illite
"	"	575	Kaolinite
Crystal violet.	580	655	Bentonite
"	"	555	Illite
Rhodamine 6G.	520	530	Bentonite
"	"	540	Illite
"	"	540	Kaolinite

The above observations provide sufficient proof of the fact that clay dye interaction takes place and this should be possible through hydrogen bonding with the -SiOH and -AlOH groups existing at the clay surface. The possibility of shifts in maxima due to structural changes are completely ruled out since no possibility of dye-dye interaction exists in the concentration range studied. The pH of the clay suspension also does not bring about any change in maxima.

The cation exchange capacity values calculated from the data of the amount of dye bound (Tables 20-38) give values which are very close to C.E.C. values obtained by ammonium acetate method.

Clays	C.E.C. values obtained from Methylene blue in m.e./100 gm.	Crystal violet in m.e./100gm.	Rhodamine 6G in m.e./100gm.	Ammonium acetate in m.e./100 gm.
Bentonite	100.0	100.0	98.0	99.0
Kaolinite	6.5	-	6.6	6.3
Illite	29.0	27.0	25.0	26.0

Table No.20

Binding of methylene blue with H-bentonite at pH 3.5.
Dye concentration 1×10^{-5}M.

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye ($\times 10^{-5}$M.)	Conc.of bound dye. ($\times 10^{-5}$M.)
4.0	0.6576	0.520	0.520	0.480
5.0	0.6676	0.500	0.500	0.500
6.0	0.7100	0.423	0.420	0.577
7.0	0.7696	0.313	0.313	0.687
8.0	0.7825	0.290	0.290	0.710
9.0	0.8239	0.203	0.203	0.797
10.0	0.9393	0.000	0.000	1.000

ϵ bound=0.9393 $\times 10^5$ ϵ free=0.3979 $\times 10^5$

Table No.21

Binding of methylene blue with H-bentonite at pH 9.0.
Dye concentration 1 $\times 10^{-5}$M.

Conc.of clay(mg.)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-5}$M)	Conc.of bound dye. ($\times 10^{-5}$M)
3.0	0.4437	0.52	0.52	0.48
4.0	0.4559	0.49	0.49	0.51
5.0	0.4685	0.45	0.45	0.55
6.0	0.5229	0.31	0.31	0.69
7.0	0.5528	0.23	0.23	0.77
8.0	0.5935	0.12	0.12	0.88
9.0	0.6386	0.00	0.00	1.00

ϵ bound = 0.6386$\times 10^5$ ϵ free= 0.2676 $\times 10^5$

Table No. 22

Binding of methylene blue with H-bentonite at pH 3.5.
Dye concentration 5 x 10^{-6}M.

Conc. of clay (mg)	ϵ apparent (x10^{5})	α	Conc. of free dye. (x10^{-6}M)	Conc. of bound dye. (x10^{-6}M)
3.0	0.429	0.684	3.420	1.580
3.5	0.519	0.529	2.645	2.355
4.0	0.619	0.359	1.800	3.200
4.5	0.674	0.264	1.350	3.650
5.0	0.733	0.166	0.830	4.170
5.5	0.829	0.000	0.000	5.000

ϵ bound = 0.829 x10^{5} ϵ free = 0.244 x10^{5}

Table No. 23.

Binding of methylene blue with H-bentonite at pH 9.0.
Dye concentration 5 x10^{-6}M.

Conc. of clay (mg)	ϵ apparent (x10^{5})	α	Conc. of free dye. (x10^{-6}M)	Conc. of bound dye. (x10^{-6}M)
2.5	0.66	0.560	2.80	2.20
3.0	0.72	0.480	2.40	2.60
3.5	0.79	0.400	2.00	3.00
4.0	0.86	0.310	1.55	3.45
4.5	1.03	0.100	0.50	4.50
5.0	1.12	0.000	0.00	5.00

ϵ bound = 1.12 x10^{5} ϵ free = 0.297 x10^{5}

Table No.24

Binding of methylene blue with H-illite at pH 4.0.
Dye concentration 1×10^{-5}M.

Conc.of clay(mg.)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye ($\times 10^{-5}$M)	Conc.of bound dye. ($\times 10^{-5}$M)
9.5	0.337	0.756	0.756	0.244
10.0	0.443	0.631	0.631	0.369
15.0	0.511	0.551	0.551	0.449
20.0	0.560	0.493	0.493	0.507
25.0	0.647	0.390	0.390	0.610
30.0	0.769	0.246	0.246	0.754
35.0	0.978	0.000	0.000	1.000

ϵ bound = 0.978×10^5 ϵ free = 0.130×10^5

Table No.25

Binding of methylene blue with H-illite at pH 9.0.
Dye concentration 1×10^{-5}M.

Conc.of clay(mg.)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-5}$M)	Conc.of bound dye. ($\times 10^{-5}$M)
9.0	0.366	0.708	0.708	0.292
9.5	0.371	0.702	0.702	0.298
10.0	0.437	0.620	0.620	0.380
15.0	0.468	0.582	0.582	0.418
20.0	0.585	0.438	0.438	0.562
25.0	0.683	0.311	0.311	0.689
30.0	0.939	0.000	0.000	1.000

ϵ bound = 0.939×10^5 ϵ free = 0.130×10^5

Table No.26

Binding of methylene blue with H-illite at pH 4.0.

Dye concentration 5×10^{-6}M.

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M)
6.0	0.414	0.722	3.610	1.39
7.0	0.534	0.612	3.060	1.94
8.0	0.628	0.527	2.630	2.360
9.0	0.674	0.485	2.420	2.57
10.0	0.763	0.404	2.020	2.98
12.0	1.030	0.387	1.930	3.06
17.0	1.204	0.000	0.000	5.00

ϵ bound = 1.204×10^5 ϵ free = 0.11×10^5

Table No.27

Binding of methylene blue with H-illite at pH 9.0.

Dye concentration 5×10^{-6}M

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M.)
5.5	0.394	0.704	3.520	1.480
6.0	0.464	0.632	3.160	1.840
7.0	0.558	0.533	2.665	2.335
8.0	0.636	0.452	2.260	2.740
9.0	0.702	0.383	1.910	3.080
10.0	0.936	0.139	0.690	4.300
15.0	1.070	0.000	0.000	5.000

ϵ bound = 1.07 $\times 10^5$ ϵ free = 0.11 $\times 10^5$

Table No.28

Binding of methylene blue with H-kaolinite at pH 4.0.
Dye concentration 5×10^{-6}M.

Conc.of clay(mg.)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M.)
15.0	0.692	0.775	3.87	1.13
25.0	0.924	0.684	3.42	1.58
35.0	1.230	0.556	2.78	2.22
45.0	1.840	0.310	1.55	3.45
65.0	2.520	0.033	0.16	4.83
70.0	2.600	0.000	0.00	5.00

ϵ bound = 2.6×10^5 ϵ free = 0.15×10^5

Table No.29

Binding of methylene blue with H-kaolinite at pH 9.0.
Dye concentration 2×10^{-6}M.

Conc.of clay(mg.)	ϵ apparent $\times 10^5$	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M.)
10.0	0.915	0.744	1.480	0.51
15.0	1.520	0.524	1.040	0.95
20.0	1.980	0.357	0.714	1.28
25.0	2.540	0.152	0.300	1.70
30.0	2.880	0.029	0.058	1.94
35.0	2.960	0.000	0.000	2.00

ϵ bound = 2.96×10^5 ϵ free 0.215×10^5

Table No.30

Binding of crystal violet with H-bentonite at pH 4.0.
Dye concentration 1×10^{-5}M.

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-5}$M)	Conc.of bound dye. ($\times 10^{-5}$M)
4.0	0.530	0.503	0.503	0.497
5.0	0.585	0.453	0.453	0.547
6.0	0.699	0.339	0.339	0.661
7.0	0.782	0.257	0.257	0.743
8.0	0.838	0.202	0.202	0.798
9.0	0.939	0.104	0.104	0.896
9.5	1.046	0.000	0.000	1.000

ϵ bound = 1.046×10^5 ϵ free = 0.022×10^5

Table No.31

Binding of crystal violet with H-bentonite at pH 4.0.

Dye concentration 5×10^{-6}M.

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M)
3.0	0.795	0.485	2.42	2.58
3.5	1.075	0.303	1.41	3.49
3.8	1.220	0.204	1.02	3.98
4.0	1.270	0.171	0.85	4.14
4.5	1.398	0.087	0.43	4.56
4.8	1.480	0.033	0.16	4.84
5.0	1.53	0.000	0.00	5.0

ϵ bound = 1.53×10^5 ϵ free = 0.017×10^5

Table No.32

Binding of crystal violet with H-illite at pH 4.0.
Dye concentration 5×10^{-6}M.

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M)
5.0	0.835	0.738	3.690	1.310
8.0	1.017	0.561	2.800	2.200
11.0	1.090	0.489	2.440	2.550
14.0	1.376	0.219	1.095	3.905
17.0	1.565	0.026	0.130	4.870
18.0	1.591	0.000	0.000	5.000

ϵ bound = 1.591×10^5 ϵ free = 0.568×10^5

Table No.33

Binding of crystal violet with H-illite at pH 4.0.
Dye concentration 2.5×10^{-6}M.

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M)
2.5	0.816	0.7520	1.880	0.620
3.0	0.946	0.6770	1.690	0.807
6.0	1.130	0.5680	1.420	1.080
7.0	1.680	0.2530	0.632	1.867
9.0	2.091	0.0167	0.041	2.450
9.2	2.120	0.0000	0.000	2.500

ϵ bound = 2.120×10^5 ϵ free = 0.388×10^5

Table No.34

Binding of rhodamine 6G with H-bentonite at pH 4.0.
Dye concentration 1×10^{-5}M.

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-5}$M)	Conc.of bound dye. ($\times 10^{-5}$M)
3.0	0.677	0.703	0.703	0.297
4.0	0.769	0.595	0.595	0.405
5.0	0.823	0.516	0.516	0.484
6.0	0.903	0.430	0.430	0.570
8.0	1.071	0.220	0.220	0.780
10.0	1.220	0.088	0.088	0.985
10.4	1.260	0.015	0.015	1.000

ϵ bound = 1.260×10^5 ϵ free = 0.4318×10^5

Table No. 35.

Binding of rhodamine 6G with H-bentonite at pH 4.0.
Dye concentration 5×10^{-6}M.

Conc.of clay(mg)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M)
2.0	1.016	0.610	3.050	1.95
3.0	1.204	0.384	1.920	3.08
3.5	1.480	0.275	1.370	3.62
4.0	1.707	0.120	0.600	4.40
4.5	1.785	0.065	0.325	4.67
5.0	1.878	0.000	0.000	5.00

ϵ bound = 1.878×10^5 ϵ free = 0.464×10^5

Table No.36

Binding of rhodamine 6G with H-illite at pH 4.0.
Dye concentration 1×10^{-5}M.

Conc.of clay(mg.)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-5}$M)	Conc.of bound dye. ($\times 10^{-5}$M)
14.0	0.568	0.698	0.698	0.302
16.0	0.638	0.611	0.611	0.389
18.0	0.699	0.534	0.534	0.466
20.0	0.757	0.463	0.463	0.537
30.0	1.000	0.157	0.157	0.843
40.0	1.120	0.000	0.000	1.000

ϵ bound = 1.12×10^5 ϵ free = 0.3279×10^5

Table No.37

Binding of rhodamine 6G with H-illite at pH 4.0.
Dye concentration 5×10^{-6}M.

Conc.of clay(mg.)	ϵ apparent ($\times 10^5$)	α	Conc.of free dye. ($\times 10^{-6}$M)	Conc.of bound dye. ($\times 10^{-6}$M.)
6.0	0.789	0.663	3.310	1.680
8.0	0.899	0.574	2.870	2.130
9.0	1.022	0.478	2.390	2.610
15.0	1.370	0.194	0.970	4.030
17.0	1.560	0.044	0.223	4.777
18.5	1.619	0.000	0.000	5.000

ϵ bound = 1.619×10^5 ϵ free = 0.367×10^5

Table No.38

Binding of rhodamine 6G with H-kaolinite at pH 4.0.

Dye concentration 5 $x10^{-6}$M.

Conc.of clay(mg.)	ϵ apparent ($x10^5$)	α	Conc.of free dye. ($x10^{-6}$M.)	Conc.of bound dye. ($x10^{-6}$M.)
35.0	0.363	0.529	2.640	2.350
40.0	0.976	0.440	2.200	2.800
50.0	1.060	0.375	1.875	3.120
60.0	1.239	0.234	1.170	3.830
65.0	1.335	0.159	0.795	4.205
70.0	1.514	0.028	0.140	4.360
75.0	1.539	0.000	0.000	5.000

ϵ bound = 1.539 $x10^5$ ϵ free = 0.026 $x10^5$

Number of bentonite units bound per dye mole.

Analytical data give the following composition of bentonite.

SiO_2	....	60.96%
Al_2O_3	....	18.27%
Fe_2O_3	....	2.83%
FeO	....	0.14%
MgO	....	2.96%
CaO	....	0.10%
Na_2O	....	1.44%
K_2O	....	0.31%
H_2O	....	12.00%
TiO_2	....	0.08%

The general formula for the members of montmorillonite group is,

$$(Si_{4-y}\ Al_y)(Al_{2-y}\ Fe^3_b\ Fe^2_c\ Mg_d\ Cr^3_e\ Mn^2_g\ Mn^3_f\ Li_h)O_{10}\cdot(OH,F)_2$$

$$\downarrow$$

$$Xx(\text{Exchangeable cations})$$

By making the following assumptions the formula of the clay can be calculated from its chemical composition.

1. There are twenty oxygen atoms and four -OH groups per unit cell.
2. All the silica present is assigned to tetrahedral sheet. The remainder of the tetrahedral positions are filled exclusively by Al. Any other Al is assigned to octahedral sheet.

The analytical values for the percentage of the oxides of the lattice elements and the exchangeable ions must be reduced to atomic proportions. The percentage of the oxide of lattice elements is divided by the molecular weight of the oxides and multiplied by the number of atoms of the positive element in the oxide. The percentage of oxides of the exchangeable ions are divided by the equivalent weight of the oxides. The atomic proportions thus obtained are represented by the same letters as those given in the general formula but the letters are capitalized. these proportions must be multiplied by a factor K to obtain actual amounts in the formula. K x D = d. The atomic proportions of silica are represented by Z, therefore K x Z = 4 -y. Since the total number of negative charges should be equal to the total number of positive charges, $K(4Z+3Y+3A-3Y+3B+2C+2D+3E+3F+2G+H+X) = 22$.

From these equations K and Y can be calculated and the formula for this clay is obtained as,

$$(Si_{3.91}\ Al_{0.09})\ (Al_{1.32}\ Fe_{0.13}\ Mg_{0.29})\ O_{10}\ (OH)_2$$

$$\downarrow$$

$$(Na_{0.20}\ Ca_{0.02}\ K_{0.03})$$

The formula for H-bentonite would be,

$$(Si_{3.91}\ Al_{0.09})\ (Al_{1.32}\ Fe_{0.13}\ Mg_{0.29})\ O_{10}\ (OH)_2$$

$$\downarrow$$

$$(H_{0.25})$$

with a weight = 356.46. Considering this to be the weight

of bentonite unit, the number of such clay units bound per mole of dye has been calculated in the following table,

Dye	Concentration of bound dye (x 10^{-5}M)	Concentration of clay in gm.	Number of clay units bound to 1 mole of dye.
Methylene blue.	1.000	0.010	110
	0.577	0.006	112
	0.500	0.005	111
Crystal violet.	1.000	0.0095	106
	0.661	0.006	102
	0.547	0.005	100
Rhodamine 6G.	1.000	0.0104	116
	0.570	0.006	111
	0.434	0.005	113

From these observations the following conclusions may be drawn:

1. A fraction of the clay unit offers site for reaction with the dye.
2. Since Klotz's equation which is applicable to macromolecular solutions has been applied, it can be said that 100 to 110 clay units form the colloidal micelle which would interact with 1 mole of the dye.

REFERENCES

1. Faust, G.T., U.S. Bureau of Mines, Rept. Invest. 3522, 1940.
2. Hendricks, S.B. and Alexander, L.T., J. Amer. Soc. Agron., 32, 455, 1940.
3. Weil-Malherbe, H. and Weiss, J., J. Chem. Soc. 2164, 1948.
4. Dyal, R.S. and Hendricks, S.B., Soil Sci., 69, 421, 1950.
5. Khan, I.A., Ph.D. Thesis Roorkee University.
6. Malik, W.U. and Verma, S.P., J. Phys. Chem., 70, 26, 1966.
7. Klotz, L.M., J. Amer. Chem. Soc. 68, 2299 (1946).

CHAPTER III.

Polarographic reduction of methylene blue in presence of clay minerals.

I N T R O D U C T I O N

Of the various electrometric techniques employed in investigating physico-chemical phenomenon, the polarographic method occupies a unique position as far as its universal applicability in different branches of chemistry is concerned. It has not only helped in solving problems relating to inorganic cations but is also being successfully used in reactions involving organic compounds and products of biological importance.

Most of the polarographic studies, which have been taken up, concern with the reduction or oxidation of substances at the dropping mercury electrode in the form of true solutions[1,2]. Very few references are available on the polarographic behaviour of colloidal solutions.

Mica[3] while working with suspensions of active charcoal has shown that the solid materials may also be determined by polarographic technique. He has further shown that various insoluble substances[4-6], in the form of suspension, undergo direct reduction at dropping mercury electrode. He has further observed that in a suspension of heavy metal oxides[4], which is kept stirred by means of a stream of nitrogen bubbled through a polarographic cell, a relatively great reduction current flows through the cell, while in a quiet suspension no current is observed.

It will not be out of place to mention that the function of stirring is not only to keep the particles suspended and to renew the surfaces near the dropping mercury electrode,but also to eliminate the diffusion current as far as possible.

In a more recent work Mica[7] has shown that the current flowing during the production of the maxima, corresponds to the electrochemical reduction of particles in suspension. In case of carbon black the current is either anodic or cathodic,depending upon the chemical treatment of the material.

Zagorski[8] has also carried out work with solid suspensions,connecting the dissolution rate of the suspension with its surface area.

Kalvoda[9] has reported certain interesting observations,regarding the polarographic reduction of suspensions, (a) colour of the substance has a certain significance, (b) larger the radius of the cation the better the reducibility of the insoluble substance,(c) size of the insoluble particles also plays an important part.

Besides this attempts have also been made to apply polarographic technique to problems of mineralogy though not with great success. Puri and Hoon[10] in a bid to determine cation exchange capacity took some current

voltage curves of soil suspensions saturated with various cations and compared these polarograms with those obtained from the chlorides of the respective cations, drawing the close analogy between the two, but did not elaborate their findings.

Malik and Gupta[11] did polarography of clay suspensions without the addition of any supporting electrolyte with a view to distinguish various clay minerals. They observed a well defined wave with $E_{1/2}$ = - 1.6 volt and few kinks or maxima in all clay suspensions except bentonite. These data were used in distinction of clay minerals.

Certain dyes like methylene blue, are known to give well defined waves in buffered media. The polarographic reduction of dyes has been utilised to study their aggregation as well as for the estimation of surfactant concentration[12]. The effect of clay minerals or other suspensions on the polarographic reduction of dyes has however not been investigated so far. Investigations were therefore planned in this direction in order to know whether it will be possible to differentiate between various clay minerals with the help of polarographic technique. The results of these studies are incorporated in this chapter.

—

EXPERIMENTAL

Reagents.

Bentonite, kaolinite and illite used in these investigations were obtained from Ward's Natural Science Establishment New York.

Methylene blue used in these studies was obtained from B.D.H.

Stock suspension of these minerals were prepared and converted into H-clay by the procedure mentioned in Chapter I.

Apparatus.

Heyrovsky polarograph (Model LP 55A) was operated manually in conjunction with Pye Scalamp Galvanometer (Model 7903/5). Iriple distilled mercury was used for the dropping mercury electrode. pH adjustments were made with a Cambridge Bench Type pH meter.

Procedure.

The following dye-clay mixtures were prepared:

1. Requisite amount of dye (effective concentration 1×10^{-3}M and 8×10^{-5}M) was taken in tubes along with different amounts of H-bentonite suspension. The volume was made up to 20 c.c. and the pH was maintained at 2.9, 4.9 and 9.2.
2. 8×10^{-5}M dye solution was mixed with different amounts of kaolinite and illite suspension and the pH

was maintained at 2.9. The mixtures were kept for few hours till equilibrium was attained.

Before taking the polarograms the mixtures were deaerated by passing pure hydrogen for about 20 to 30 minutes in the polarographic cell. The sensitivity of the instrument was kept 1:7 throughout. All measurements were carried out at 25° ± 0.1°C in water thermostat.

Figs.(1 to 5) show the polarograms of methylene blue (at different concentrations) in presence of varying amounts of bentonite at different pH.

Figs.(6 and 7) show the polarograms of methylene blue (concentration 8×10^{-5}M) in presence of different amounts of kaolinite and illite.

—

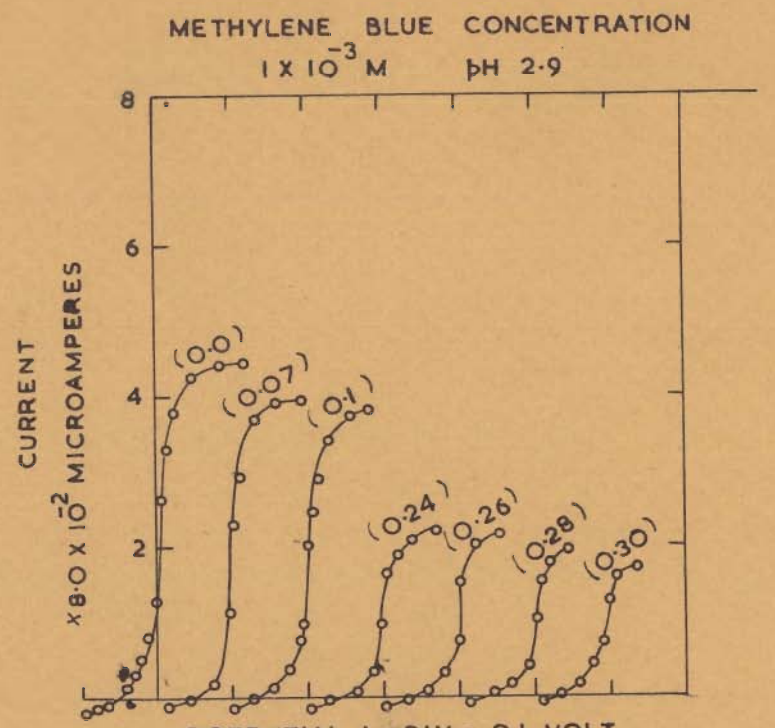

METHYLENE BLUE CONCENTRATION
1 X 10⁻³ M pH 2·9
CURRENT
X 8·0 X 10⁻² MICROAMPERES
8
6
4
2
(0·0)
(0·07)
(0·1)
(0·24)
(0·26)
(0·28)
(0·30)
POTENTIAL 1 DIV = 0·1 VOLT
EACH CURVE STARTS AT + 0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS
THE AMOUNT OF BENTONITE IN GM.

FIG. 1.

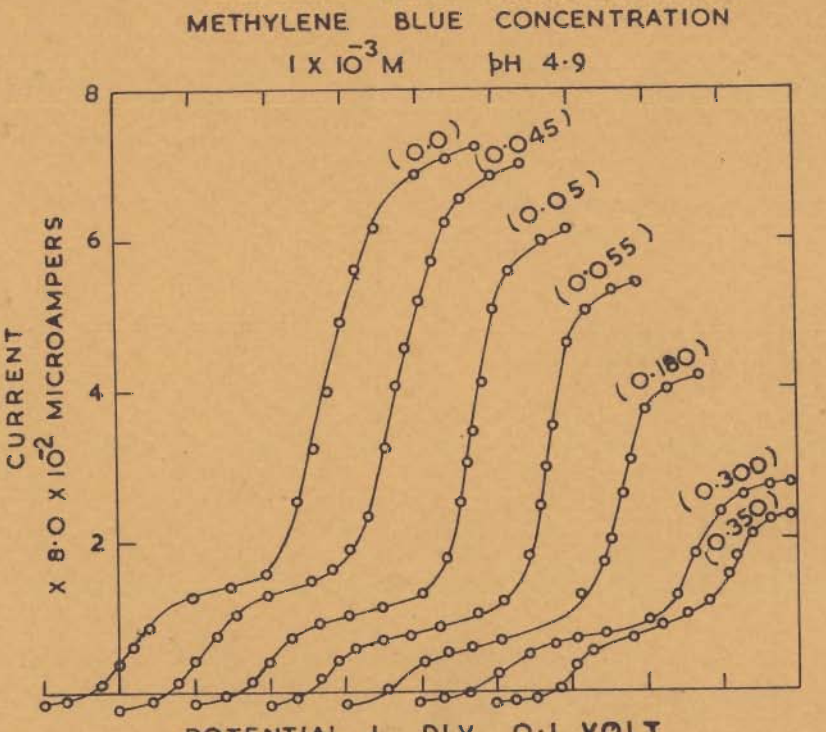
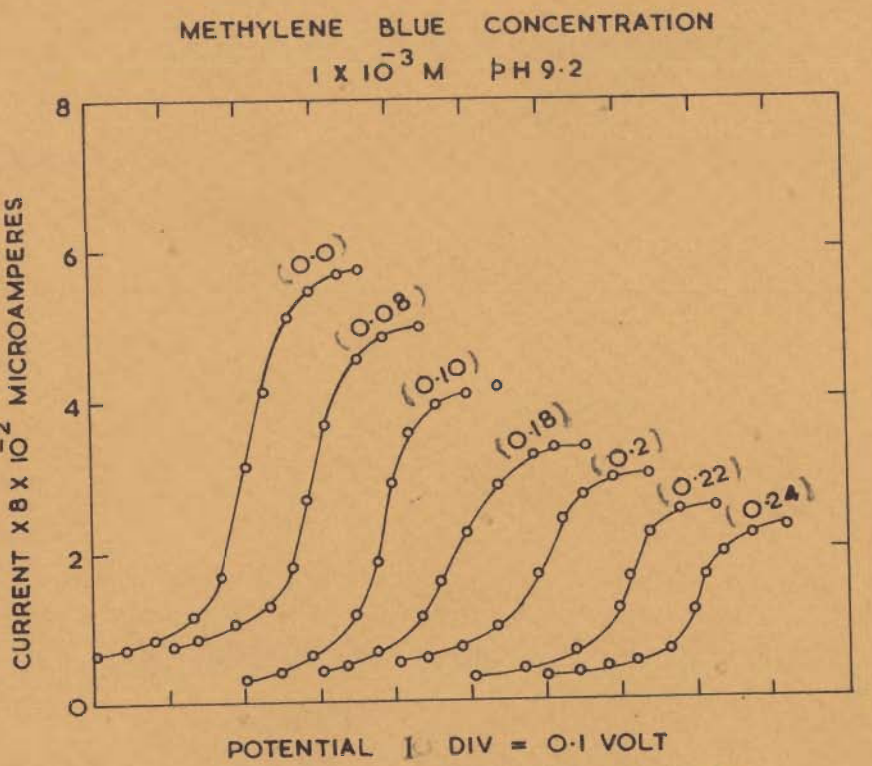
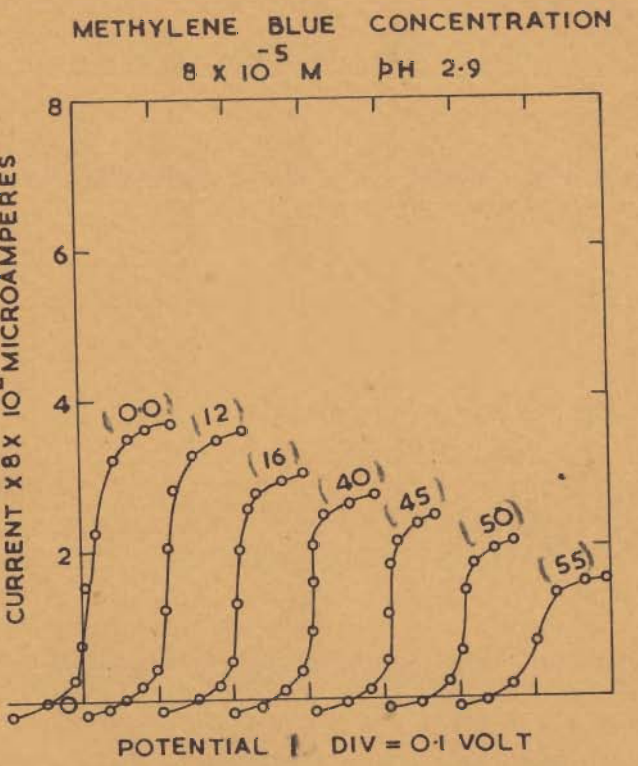

METHYLENE BLUE CONCENTRATION
1 X 10⁻³ M pH 4·9
CURRENT
X 8·0 X 10⁻² MICROAMPERS
8
6
4
2
(0·0)
(0·045)
(0·05)
(0·055)
(0·180)
(0·300)
(0·350)
POTENTIAL 1 DIV 0·1 VOLT
EACH CURVE STARTS AT + 0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS
THE AMOUNT OF BENTONITE IN GM.

FIG. 2.



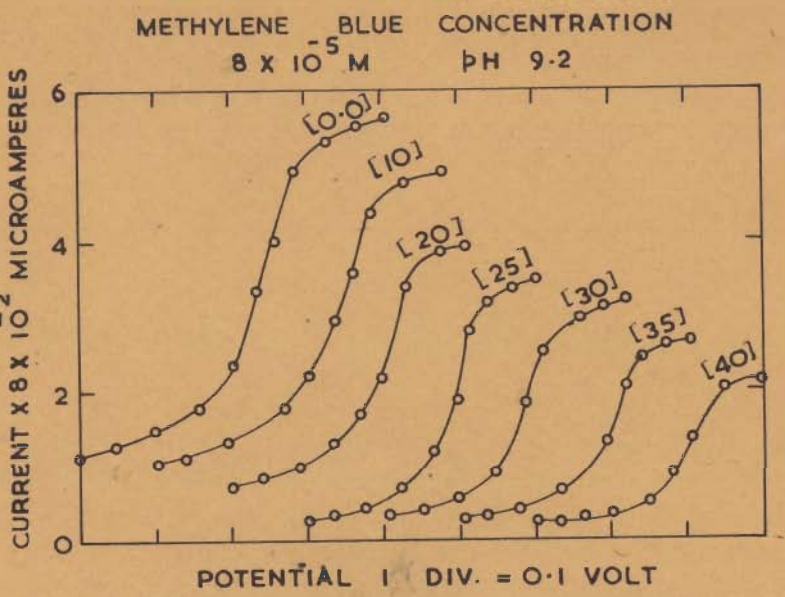
EACH CURVE STARTS AT − 0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS THE
AMOUNT OF BENTONITE IN GM.

FIG 3



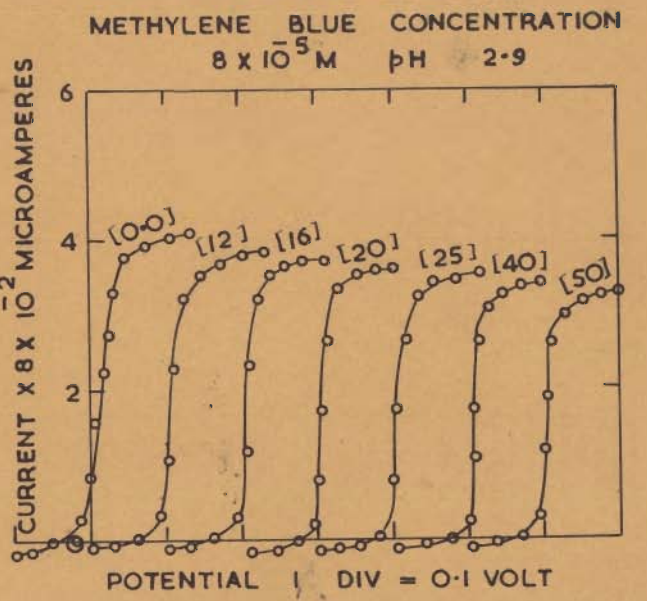
EACH CURVE STARTS AT + 0·1 VOLT
VALUE IN PARENTHESIS REPRSENTS
THE AMOUNT OF BENTONITE IN mg.

FIG 4



EACH CURVE STARTS AT −0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS
AMOUNT OF BENTONITE IN MG

FIG 5



EACH CURVE STARTS AT+0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS
THE AMOUNT OF KAOLINITE IN MG

FIG 6

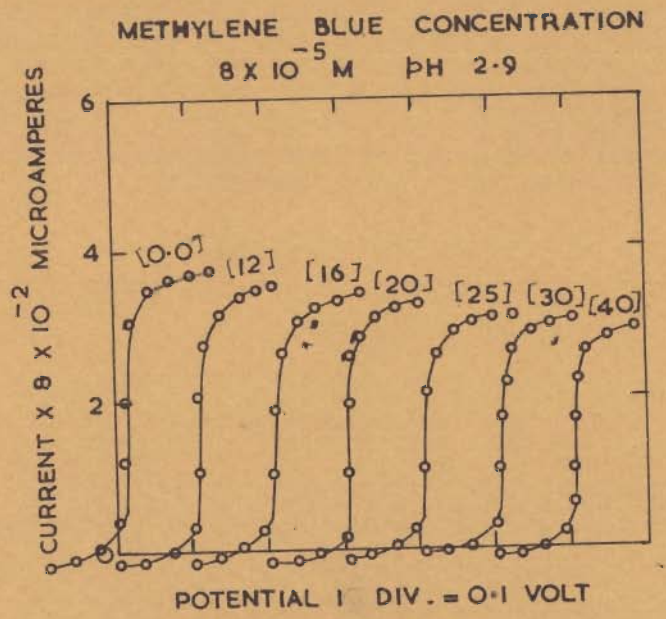

METHYLENE BLUE CONCENTRATION
8 X 10⁻⁵ M pH 2·9
CURRENT X 8 X 10⁻² MICROAMPERES
6
4
2
[0·0]
[12]
[16]
[20]
[25]
[30]
[40]
POTENTIAL 1 DIV. = 0·1 VOLT
EACH CURVE STARTS AT + 0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS
THE AMOUNT OF ILLITE IN MG.

FIG 7

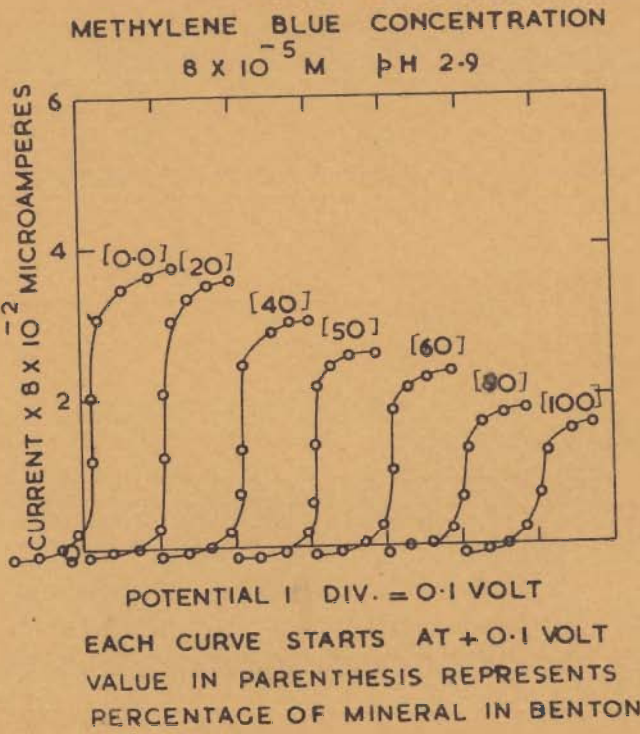

METHYLENE BLUE CONCENTRATION
8 X 10⁻⁵ M pH 2·9
CURRENT X 8 X 10⁻² MICROAMPERES
6
4
2
[0·0]
[20]
[40]
[50]
[60]
[80]
[100]
POTENTIAL 1 DIV. = 0·1 VOLT
EACH CURVE STARTS AT + 0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS
PERCENTAGE OF MINERAL IN BENTONITE

FIG 8

Table No.1

Binding of methylene blue with bentonite at pH 2.9.
Dye concentration 1 x 10^{-3}M.
$(i_d)_o$ = 42 x 8 x 10^{-2} microamperes.

Amount of clay added (gm.)	(i_d) ($\times 8 \times 10^{-2}$) microamperes.	$(i_d)_o - (i_d)$ ($\times 8 \times 10^{-2}$) microamperes.	$\frac{(i_d)}{(i_d)_o}$	Concentration of free dye ($\times 10^{-3}$M)	Concentration of bound dye ($\times 10^{-3}$M)
0.07	35	7	0.83	0.83	0.17
0.10	33	9	0.78	0.78	0.22
0.24	20	22	0.47	0.47	0.53
0.26	17	25	0.40	0.40	0.60
0.28	13	29	0.30	0.30	0.70
0.30	12	30	0.28	0.28	0.72

Fig.11.

Table No.2

Binding of methylene blue with bentonite at pH 4.9.
Dye concentration 1 x 10^{-3}M.
$(i_d)_o$ = 46 x 8 x 10^{-2} microamperes.

Amount of clay added(gm.)	(i_d) (x8x10^{-2}) microamperes.	$(i_d)_o - (i_d)$ (x8x10^{-2}) microamperes.	$\frac{(i_d)}{(i_d)_o}$	Concentration of free dye (x10^{-3}M)	Concentration of bound dye (x10^{-3}M)
0.045	44	2	0.95	0.95	0.05
0.05	42	4	0.91	0.91	0.09
0.055	39	7	0.84	0.84	0.16
0.18	24	22	0.52	0.52	0.48
0.30	12	34	0.26	0.26	0.74
0.35	9	37	0.19	0.19	0.81

Fig.11.

Table No.3

Binding of methylene blue with bentonite at pH 9.2.

Dye concentration 1×10^{-3}M.

$(i_d)_o = 42 \times 8 \times 10^{-2}$ microamperes.

Amount of clay added. (gm.)	(i_d) $(x8x10^{-2})$ microamperes.	$(i_d)_o - (i_d)$ $(\times 8 \times 10^{-2})$ microamperes.	$\frac{(i_d)}{(i_d)_o}$	Concentration of free dye. $(\times 10^{-3}M)$	Concentration of bound dye. $(\times 10^{-3} M)$
0.08	30	12	0.71	0.71	0.29
0.10	28	14	0.66	0.66	0.34
0.18	20	22	0.47	0.47	0.53
0.20	19	23	0.45	0.45	0.55
0.22	16	26	0.38	0.38	0.62
0.24	14	28	0.33	0.33	0.33

Fig.12.

Table No.4.

Binding of methylene blue with bentonite at pH 2.9.
Dye concentration 8 x 10^{-5}M.

$(i_d)_o$ = 33 x 8 x 10^{-2} microamperes.

Amount of clay added. (mg.)	(i_d) ($x8x10^{-2}$) microamperes.	$(i_d)_o - (i_d)$ (x 8 x 10^{-2}) microamperes.	$\frac{(i_d)}{(i_d)_o}$	Concentration of free dye ($x10^{-5}$M)	Concentration of bound dye. ($x10^{-5}$M)	m.e.of dye bound per 100 gm.clay.
12	28.0	5.0	0.85	6.80	1.20	101
16	26.0	7.0	0.78	6.24	1.76	100
40	16.0	17.0	0.48	3.84	4.16	102
45	14.0	19.0	0.42	3.36	4.64	103
50	12.0	21.0	0.36	2.88	5.12	102
55	10.0	23.0	0.30	2.40	5.60	101

Fig.13.

Table No.5

Binding of methylene blue with bentonite at pH 9.2.
Dye concentration 8×10^{-5}M.
$(i_d)_o = 32 \times 8 \times 10^{-2}$ microamperes.

Amount of clay added(mg.)	(i_d) $(x8x10^{-2})$ microamperes.	$(i_d)_o - (i_d)$ $(x8 \times 10^{-2})$ microamperes.	$\frac{(i_d)}{(i_d)_o}$	Concentration of free dye ($x10^{-5}$M)	Concentration of bound dye ($x10^{-5}$M)	m.e.of dye bound per 100 gm.clay.
10	27.0	5.0	0.84	6.72	1.28	128
20	21.0	11.0	0.65	5.20	2.80	140
25	19.0	13.0	0.59	4.72	3.28	135
30	17.0	15.0	0.53	4.24	3.76	125
35	15.0	17.0	0.46	3.68	4.32	123
40	12.0	20.0	0.37	2.96	5.04	126

Fig.13.

Table No.6

Binding of methylene blue with kaolinite at pH 2.9.
Dye concentration 8×10^{-5}M.
$(i_d)_o = 33 \times 8 \times 10^{-2}$ microamperes.

Amount of clay added. (mg.)	(i_d) ($\times 8 \times 10^{-2}$) microamperes.	$(i_d)_o - (i_d)$ ($\times 8 \times 10^{-2}$) microamperes.	$\frac{(i_d)}{(i_d)_o}$	Concentration of free dye. ($\times 10^{-5}$M)	Concentration of bound dye. ($\times 10^{-5}$M)	m.e.of dye bound per 100 gm.of clay.
12	32.6	0.4	0.990	7.92	0.07	5.8
16	32.4	0.6	0.985	7.88	0.11	6.8
20	32.2	0.8	0.982	7.86	0.13	7.0
25	32.0	1.0	0.980	7.83	0.165	6.6
40	31.6	1.4	0.965	7.72	0.28	6.8
50	31.5	1.5	0.958	7.67	0.33	6.3

Fig.14.

Table No.7

Binding of methylene blue with illite at pH 2.9.

Dye concentration 8 x 10^{-5}M.

$(i_d)_o$ = 33 x 8 x 10^{-2} microamperes.

Amount of clay added. (mg.)	(i_d) ($x8x10^{-2}$) microamperes.	$(i_d)_o - (i_d)$ ($x8x10^{-2}$) microamperes.	$\frac{(i_d)}{(i_d)_o}$	Concentration of free dye. ($x10^{-5}$M)	Concentration of bound dye. ($x10^{-5}$M)	m.e.of dye bound per 100 gm.of clay.
12	31.6	1.4	0.957	7.66	0.330	27.0
16	31.2	1.8	0.944	7.55	0.44	28.0
20	30.5	2.5	0.927	7.42	0.58	29.0
25	29.5	3.5	0.90	7.27	0.72	28.0
30	29.0	4.0	0.89	7.13	0.87	29.0
40	28.0	5.0	0.86	6.88	1.12	28.0

Fig.15.

Table No.8

Variation of i_d of methylene blue(8×10^{-5}M) at pH 2.9 by adding bentonite of varying mineral percentage.

$(i_d)_o = 33 \times 8 \times 10^{-2}$ microamperes.

Percentage of silica.	Clay mineral percentage.	C.E.C. of the sample in m.e. per 100 gm. of clay.	(i_d) ($\times 8 \times 10^{-2}$) microamperes.	$(i_d)_o - (i_d)$ ($\times 8 \times 10^{-2}$) microamperes.
0.0	100.0	99.0	12.0	21.0
20.0	80.0	78.12	16.0	17.0
40.0	60.0	59.60	21.0	12.0
50.0	50.0	48.20	23.0	10.0
60.0	40.0	39.60	26.0	7.0
80.0	20.0	18.40	30.0	3.0

Fig.16.

Table No.9

Variation of (i_d) of methylene blue (8×10^{-5}M) at pH 2.9 by adding kaolinite of varying mineral percentage.

$(i_d)_o = 33 \times 8 \times 10^{-2}$ microamperes.

Percentage of silica	Clay mineral percentage.	C.E.C. of the sample in m.e. per 100gm. clay.	(i_d) ($\times 8 \times 10^{-2}$) microamperes.	$(i_d)_o - (i_d)$ ($\times 8 \times 10^{-2}$) microamperes.
0.0	100.0	6.3	31.5	1.5
20.0	80.0	5.2	31.8	1.2
40.0	60.0	3.30	32.2	0.8
50.0	50.0	3.12	32.5	0.5
60.0	40.0	2.80	32.7	0.3
80.0	20.0	1.25	-	-

Fig.17

Table No.10

Variation of (i_d) of methylene blue ($8 \times 10^{-5}M$) at pH 2.9 by adding illite of varying mineral percentage.
$(i_d)_o = 33 \times 8 \times 10^{-2}$ microamperes.

Percentage of silica.	Clay mineral percentage.	C.E.C. of the same in m.e. per 100 gm. clay.	(i_d) $(\times 8 \times 10^{-2})$ microamperes.	$(i_d)_o - (i_d)$ $(\times 8 \times 10^{-2})$ microamperes.
0.0	100.0	26.00	28.0	5.0
20.0	80.0	21.00	29.0	4.0
40.0	60.0	15.40	30.0	3.0
50.0	50.0	13.70	30.5	2.5
60.0	40.0	11.10	31.0	2.0
80.0	20.0	5.20	32.0	1.0

Fig. 17.

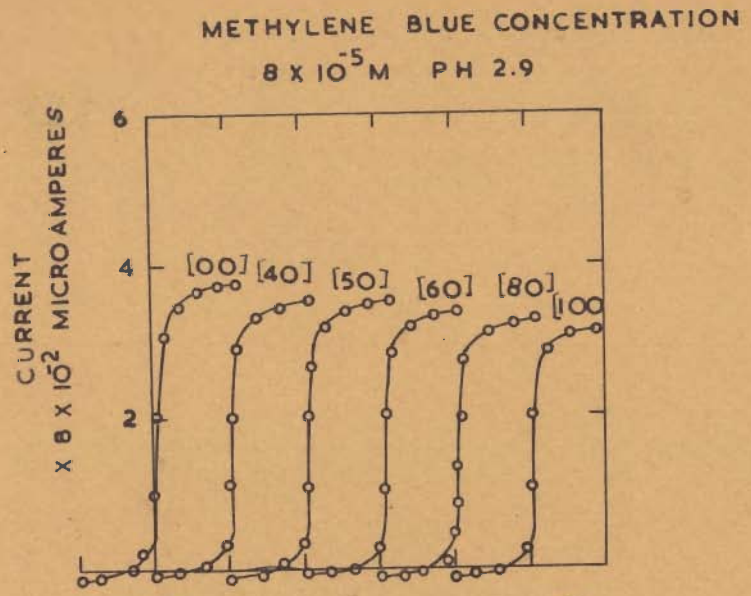

METHYLENE BLUE CONCENTRATION
8 X 10⁻⁵M PH 2.9
CURRENT
X 8 X 10⁻² MICROAMPERES
6
4
2
[00]
[40]
[50]
[60]
[80]
[100]
POTENTIAL 1 DIV = 0·1 VOLT
EACH CURVE STARTS AT +0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS
PERCENTAGE OF MINERAL IN
KAOLINITE

FIG 9

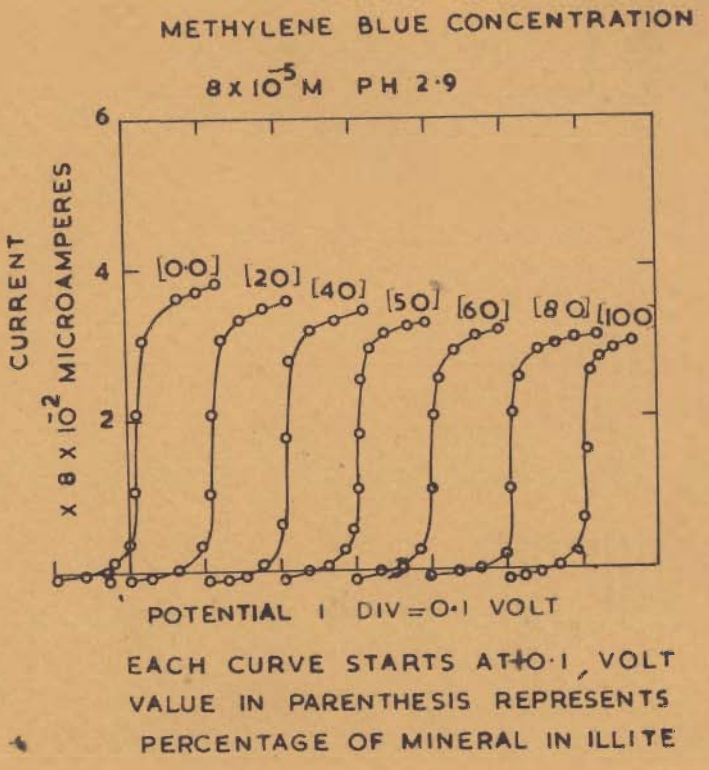

METHYLENE BLUE CONCENTRATION
8 X 10⁻⁵M PH 2·9
CURRENT
X 8 X 10⁻² MICROAMPERES
6
4
2
[0·0]
[20]
[40]
[50]
[60]
[80]
[100]
POTENTIAL 1 DIV = 0·1 VOLT
EACH CURVE STARTS AT +0·1 VOLT
VALUE IN PARENTHESIS REPRESENTS
PERCENTAGE OF MINERAL IN ILLITE

FIG 10

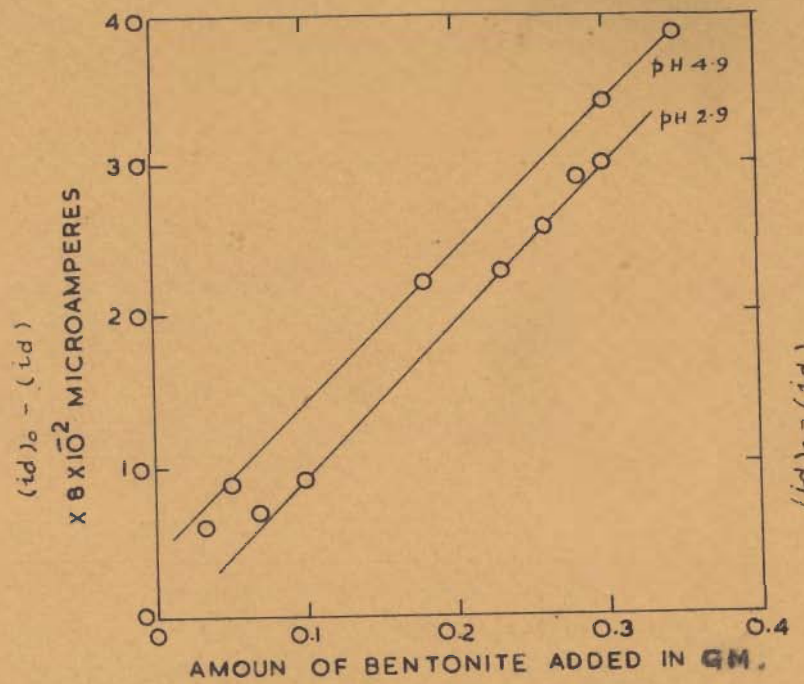


FIG 11

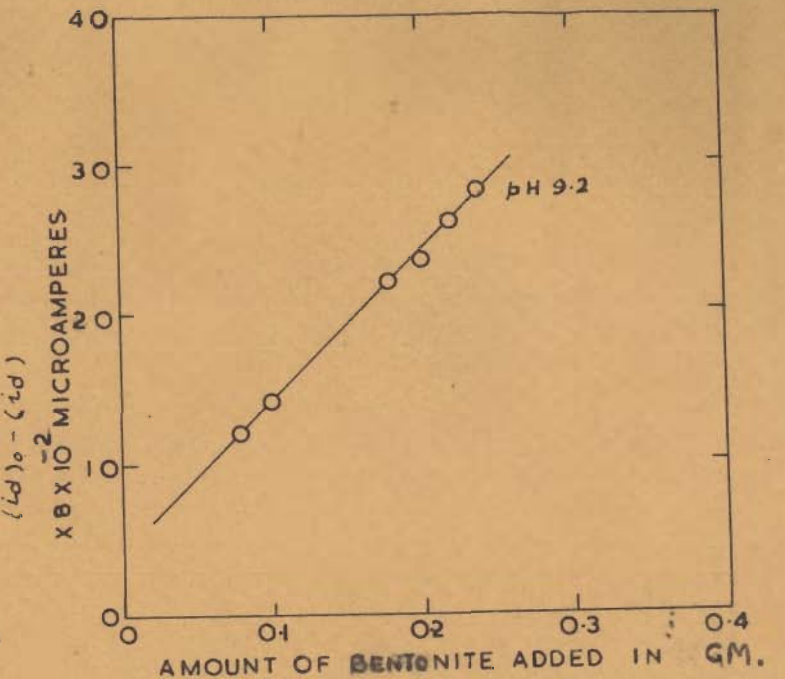


FIG 12

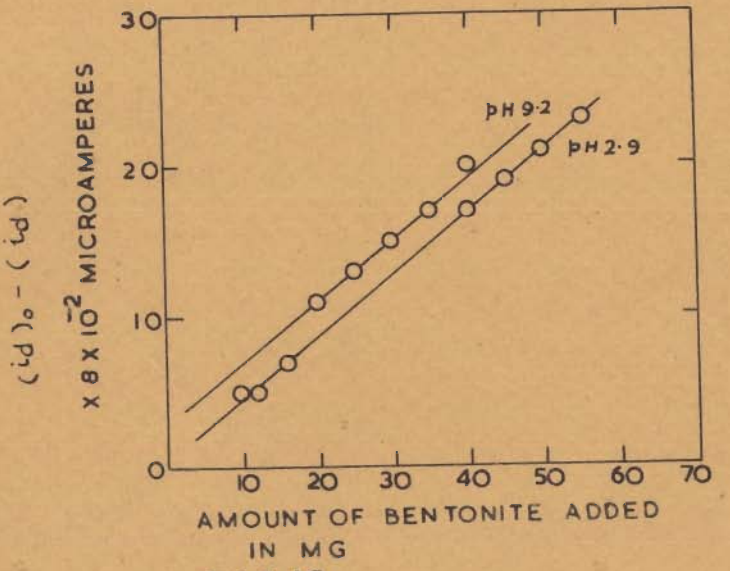


FIG 13

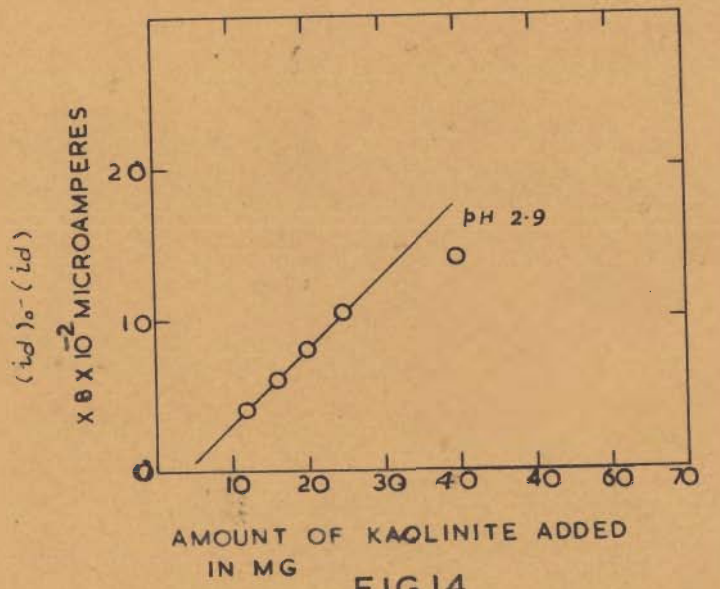


FIG 14

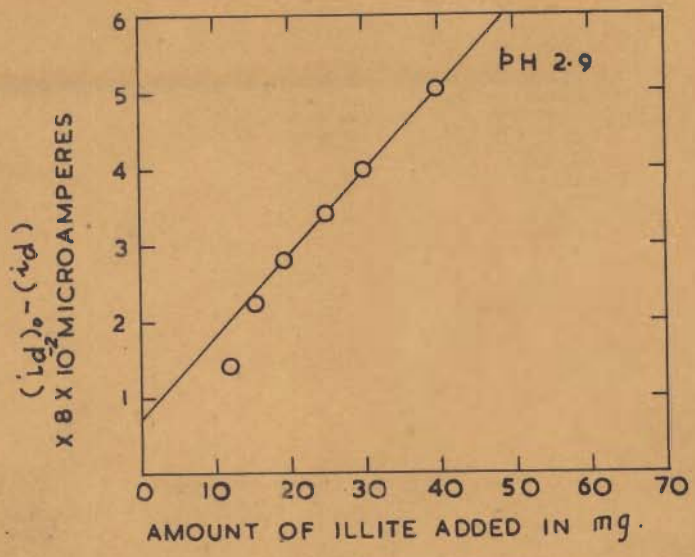


FIG.15.

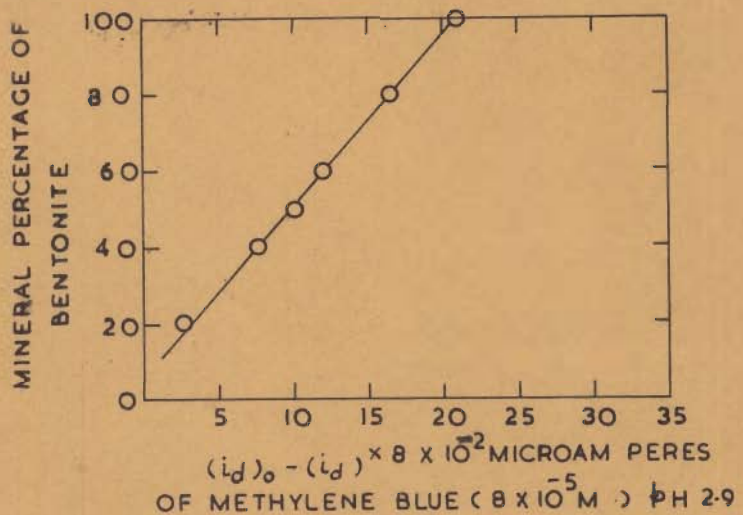


FIG.16.

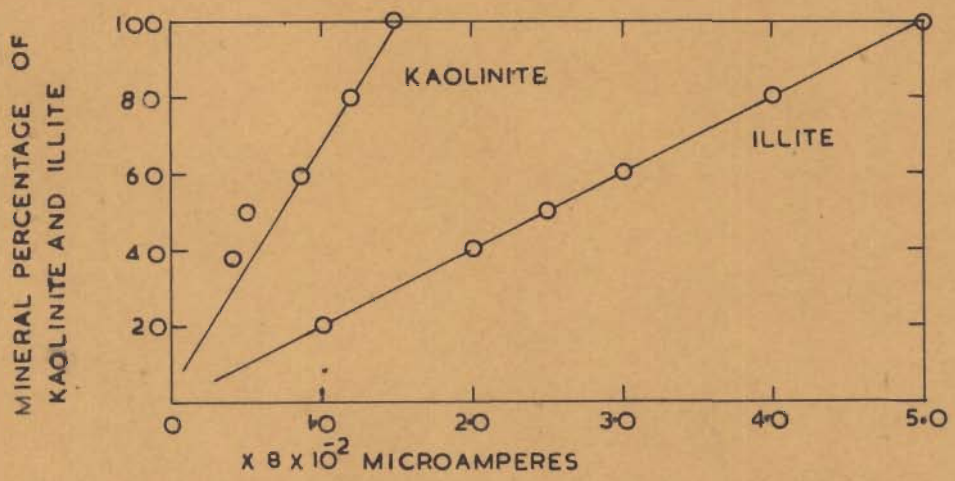


FIG.17.

RESULTS AND DISCUSSION

Clark[13] observed a well defined wave of methylene blue and found that the half wave potential of the normal wave corresponds closely,but not exactly,to the oxidation potential of the thermodynamically reversible methylene blue system and that it changes with pH. In a study of the reduction of organic compounds in acid medium Brdicka[14] reported the existence of a prewave just before the normal reduction wave. The compounds studied included substances like riboflavin,methylene blue etc..

In these studies the reduction of methylene blue at the dropping mercury electrode was observed at pH 2.9, 4.9 and 9.2. The half wave potential of this dye was found to be -0.02 volts at pH 2.9,-0.28 volts at pH 4.9 and -0.32 volts at pH 9.2. A prewave with $E_{1/2}$ =-0.01 volts was realised with solution of pH 4.9. The normal reduction wave disappeared below the concentration 6×10^{-5}M and therefore a concentration higher than this was chosen for subsequent studies.

From the polarograms of methylene blue (Figs.1 to 5) in presence of varying amounts of bentonite at different pH it can be seen that the diffusion current of the dye solute is considerably reduced. The $E_{1/2}$,however,does not change. A similar decrease in diffusion current of the dye,

(without any change in $E_{1/2}$) although to a lesser extent is also observed in the cases of kaolinite and illite. The results in the case of these two clays are shown in Figs.6 and 7.

The effect of clay minerals on the diffusion current of methylene blue is most pronounced in the case of bentonite(Table No.4). The diffusion current reduces from 33 x 8 x 10^{-2} microamperes to 16 x 8 x 10^{-2} microamperes in presence of 40 mg.of clay. The corresponding decrease in the case of illite and kaolinite for the same amount of clay mineral is very much less,being 28.8x8x10^{-2} microamperes and 31.6 x 8 x 10^{-2} microamperes respectively. The order for the decrease in diffusion current in the presence of the three clay minerals is bentonite > illite > kaolinite.

A linear relationship is found to exist in between the decrease in wave height $(i_d)_o - (i_d)$ and the amount of mineral present (Figs.11 to 15). It should thus be possible to estimate the clay fraction present in a soil sample by the polarographic method.

The data on the amount of dye bound to the three clay minerals at different pH are given in tables (1 to 7). The amount of dye bound to bentonite is abnormally high

with concentrated solution 1 x 10^{-3}M. This can be explained on the basis of aggregation of dye at such higher concentration and multiple adsorption. At the lower concentration i.e.,8 x 10^{-5}M the binding (Table 4 to 7) is small and is of the same order as required for exchange adsorption (Chapter I.).

Polarograms at pH 4.9 (Fig.11 Table 2) show that low amounts of bentonite added have no effect on prewave but decrease the i_d of the normal reduction wave. Higher amounts of this mineral,however,shift the half wave potential of prewave towards more positive side along with the decrease in the height of the normal reduction wave.

It is further observed that the amount of bound dye increases with increase in pH. This can again be explained by assuming that the edges acquire a negative charge at higher pH (through broken Si-O and Al-O bonds) with the result that more of the cationic dye is adsorbed. Similar data were obtained on the basis of adsorption studies described in Chapter I.

Variation of i_d with the percentage of the mineral content.

Powdered silica passing through a 100 mesh sieve was mixed in different amounts with bentonite,kaolinite, and illite in order to increase the amount of non clay material and prepare samples of varying cation exchange capacity. Polarograms of methylene blue of concentration

8×10^{-5}M were then taken at pH 2.9 in presence of samples of bentonite, kaolinite and illite having different mineral composition. The polarograms are shown in Figs.(8 to 10) and the i_d values are given in Tables (8 to 10). A linear relationship is found to exist between the mineral percentage of these samples (which is proportional to cation exchange capacity) and the decrease in diffusion current i.e. $(i_d)_o - (i_d)$ (vide figs.16 and 17). This goes to show that polarographic reduction of methylene blue in presence of any clay or soil sample can be a very quick method of determining its cation exchange capacity. It may, however, be emphasized that the above relationship holds for one particular clay sample and not between the cation exchange capacities of different clays and their decrease in the diffusion current.

—

R E F E R E N C E S

1. Meites,L.,Polarographic Techniques Inter-science Publishers New York.

2. Kolthoff,I.M. and Lingane,J.J.,Polarography.Inter-science Publishers New York.

3. Mica,K., Chem.Listy.51,233,1957.

4. Mica,K.,Coll.Czechoslov.Chem.Communs.21,647,1956.

5. Mica,K.,Czeskoslav.farm.5,133,1956.

6. -idum-Coll.Czech.Chem.Communs.22,1400,1957.

7. Mica,K.,Advances in Polarography Vol.III,Pergamon Press.London.

8. Zagorski,Z.P.Proc.2nd Intern.Polarographic Conf. Cambridge 1959.

9. Kalvoda,R., -ibid-

10. Puri,A.N. and Hoon,R.C.,Soil Sci.44,399,1937.

11. Malik,W.U. and Gupta,G.C.,Talanta.15,39,1968.

12. Puran Chandra,Ph.D.Thesis,Roorkee University.

13. Clark,W.M.Cohen,B.and Gibbs,H.D.,Public Health Reports 40,1155,1925.

14. Brdicka,R.,Z.Electrochem., 48,278,1942.

—

CHAPTER IV.

Interaction of proteins with clay minerals.

I_N_T_R_O_D_U_C_T_I_O_N

For quite some time investigations[1-3] are being carried out to find the interrelationship between the clay mineral of the soil and its nitrogenous component. The possibility of the two existing in the form of a complex compound is an attractive hypothesis although very little has been said about the nature of such a complex.

The presence of various kinds of amino acids in soil hydrolysates suggest the possibility of the existence of proteins in the soil. Bremer[4] has reported that 35 to 40 % of the total nitrogen in most of the soils is in the form of proteins. It can therefore be assumed that any search for finding the interrelationship between the clay part of the soil and its nitrogenous component should envisage an interaction of proteins with clays. Thinking in this direction has revealed that protein-clay complexes can exist in the soil since the conditions necessary for the existence of such complexes are supposed to be present in the soil system. Ensminger[5,6] and Giesking have prepared bentonite lysozyme complexes and found that the adsorption of protein of low molecular dimension takes place in interlamellar spaces of the mineral also in addition to the amount bound at the surface. Waksman[7] has separated the humus complex into

two fractions, alpha humus and beta humus, and recognised the second as a chemical compound between protein and one of the clay constituents of the soil. The adsorption of enzymes on clay surface has also been studied by Maclaren[8]. The problem of the stability of these complexes as influenced by microbial degradation has also been a subject of interest with the soil chemist.

The investigations described in this chapter are concerned with a more fundamental approach to the problem of clay-protein reaction than hitherto taken up and deals with the following aspects of the problem:

1. Extent of adsorption of fibrillar (transfusion gelatin) and globular (egg albumin) on bentonite, illite and kaolinite including the role of iso-electric pH on adsorption.
2. Stability of clay-protein complexes with changing pH and in presence of potassium chloride.
3. Turbidimetric studies on clay-protein reaction carried out in dilute solutions of clay as well as protein.

—

E X P E R I M E N T A L

Reagents.

Clay minerals bentonite, kaolinite and illite used as adsorbents were obtained from Wards Natural Science Est. United States. Pure crystalline egg albumin used in these studies was a B.D.H. product. Transfusion gelatin was obtained from National Chemical Laboratory Poona.

Minerals were finely powdered, passed through a 100 mesh sieve, suspended in double distilled water and converted into hydrogen form. The concentration of the stock suspension was determined by drying it in air at 120°C for few hours.

Solution of egg albumin was prepared by dissolving it in double distilled water. The solution was centrifuged and its concentration determined by drying a known aliquot in an air oven at 105°C. Transfusion gelatin was originally supplied as a 6% solution.

Apparatus.

Absorbance measurements were carried out with Unicam S.P.500 spectrophotometer. Turbidity was measured by Bosch and Lomb 'Spectronic-20' at a wave length 3750 A°. pH measurements were made with a Cambridge Bench Type pH meter.

Procedure for adsorption measurements.

A number of tubes were set up having a clay

concentration of 0.05% and varying amounts of proteins (vide tables 2 to 7). The pH in these tubes was adjusted by adding buffars. The total volume was made up to 20 ml. in each case. The tubes were intermittently agitated in an electrical shaker for about six hours and then left over night to attain equilibrium. The suspension was then allowed to sediment by centrifugation and the concentration of protein determined by noting the absorbance at wave lengths of 2610 A° and 2810 A° for transfusion gelatin and egg albumin respectively.

Turbidity measurements.

Very dilute solutions of clay-protein mixtures (vide tables 11 to 14) were prepared in buffar solutions and a time of about ten hours was allowed for equilibration of these mixtures. Turbidimetric measurements of the suspensions were carried out at 3750 A°.

Tables 2 to 7 give the adsorption data of the two proteins at iso-electric pH. Adsorption isotherms at various pH values are given in Figs.5 to 10. Figures 11 to 13 depict the changes in adsorption due to pH.

The iso-electric point of the proteins and their ionisable groups.

Transfusion gelatin, a fibrillar protein is a modified form of gelatin and is suitable for intravenous injections as a substitute for plasma. The average molecular weight of this protein is 75000. Egg albumin is a globular protein with a mol.wt.of 45000.

Proteins have many ionizable groups. The extent of their ionization in solution depends on the hydrogen ion concentration. The iso-electric points of these proteins were checked by observing the cataphoretic velocity of proteins stained with Rhodamine B (these proteins do not react with this dye over the entire pH range). The results are depicted in Table I (Fig.I).

Table No.1

Amount of 0.01N HCl added in ml.	Egg albumin		Transfusion gelatin	
	pH	Velocity μ per sec.	pH	Velocity μ per sec.
0.4	9.6	-2.75	9.1	-1.38
0.8	9.2	-2.63	8.7	-1.01
1.6	6.8	-2.52	5.9	-0.67
2.0	-	-	5.0	±0.00
2.6	5.0	-0.38	4.7	+0.61
2.7	4.8	±0.00	4.5	+0.78
2.8	4.75	+0.20	-	-
3.0	4.7	+0.52	4.1	+1.21
3.6	4.4	+1.42	3.4	+1.32

From Fig.I it is observed that egg albumin is isoelectric at pH 4.8 while transfusion gelatin is isoelectric at

pH 5.0. A protein containing a high proportion of the diamino-mono carboxylic acids arginine and lysine might be expected to be more basic and less acid and to have a higher iso-electric pH than proteins having a low content of these constituents. The different ionizable groups of egg albumin and transfusion gelatin are given below:

Ionizable groups in transfusion gelatin

Ionizable groups	Reasonable analytical value.	Observed value.
∝ Carboxyl	-	(1)
β Carboxyl	83	83
Imidazole	3.4	3.8
∝ Amino	-	(1)
∈ Amino	72	22
Phenolic	1	1
Guanidino	39	48
Total cationic	65	70

Ionizable groups in egg albumin

Groups	By total residues weight.	By titration
Aspartic	32	-
Glutamic	52	-
Amide	39	-
Free carboxylic	45	51
Phosphoric	2	-
Arginine	15	14
Histidine	6	5
Lysine	20	23
Total basic	41	40-42

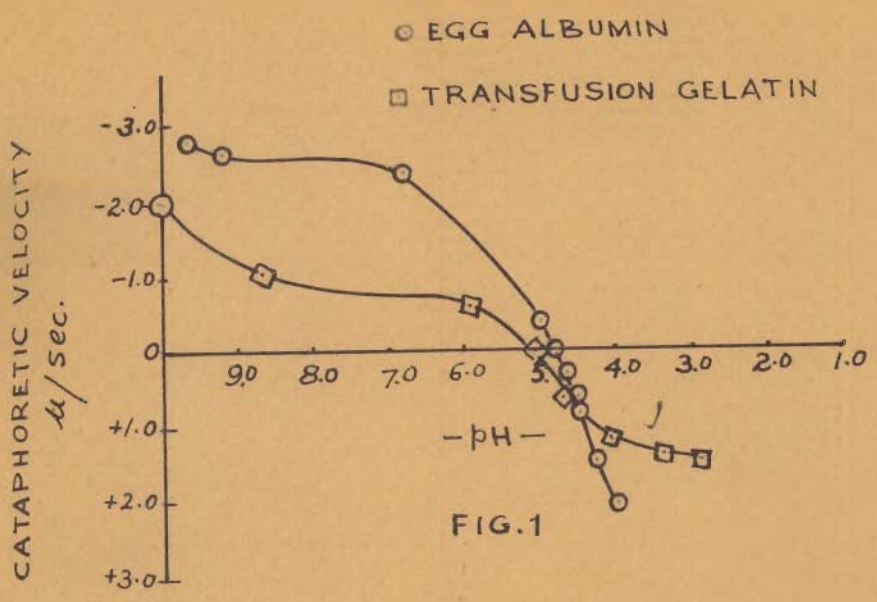


FIG.1

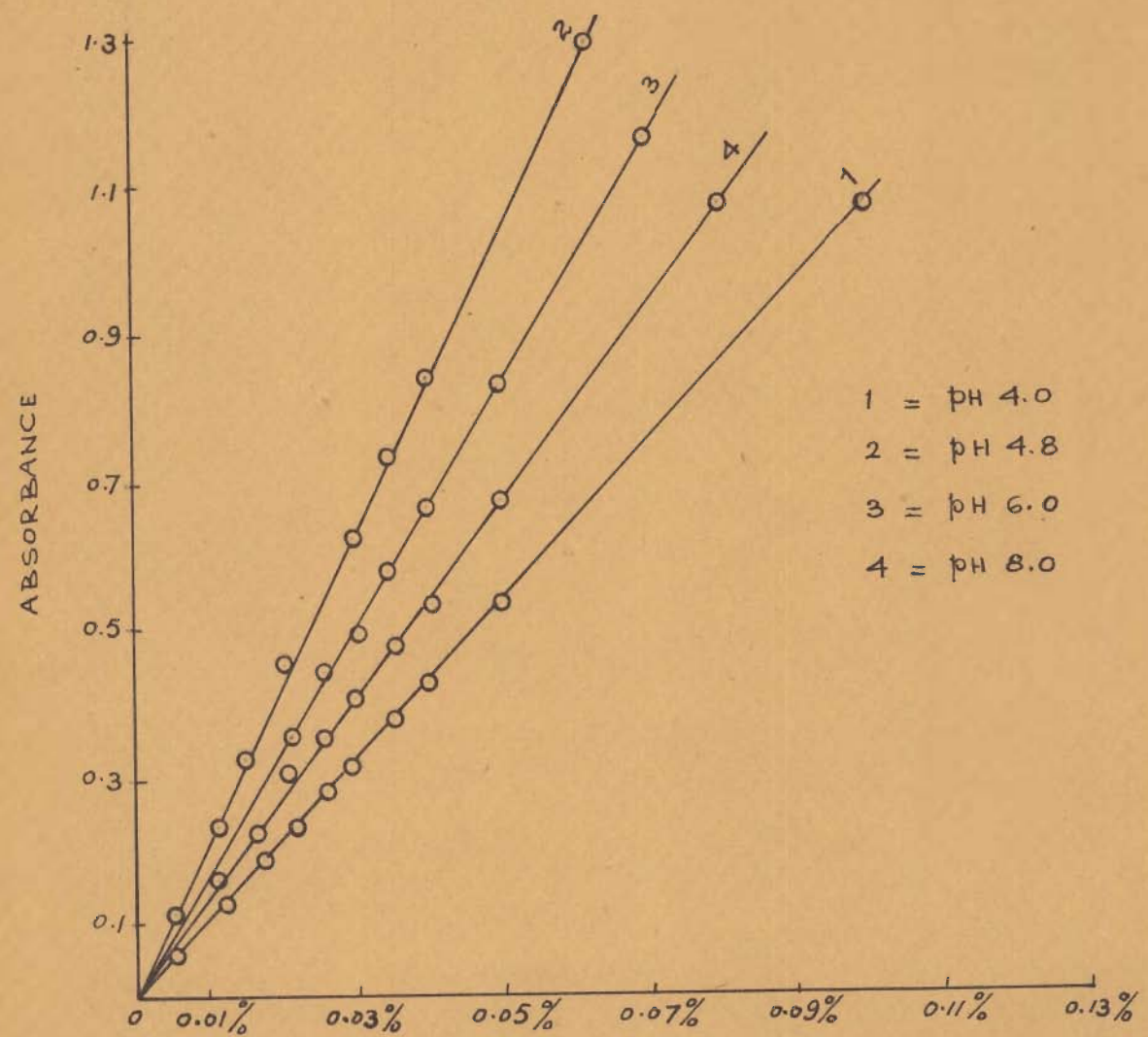


FIG.2 CONCENTRATION OF EGG ALBUMIN

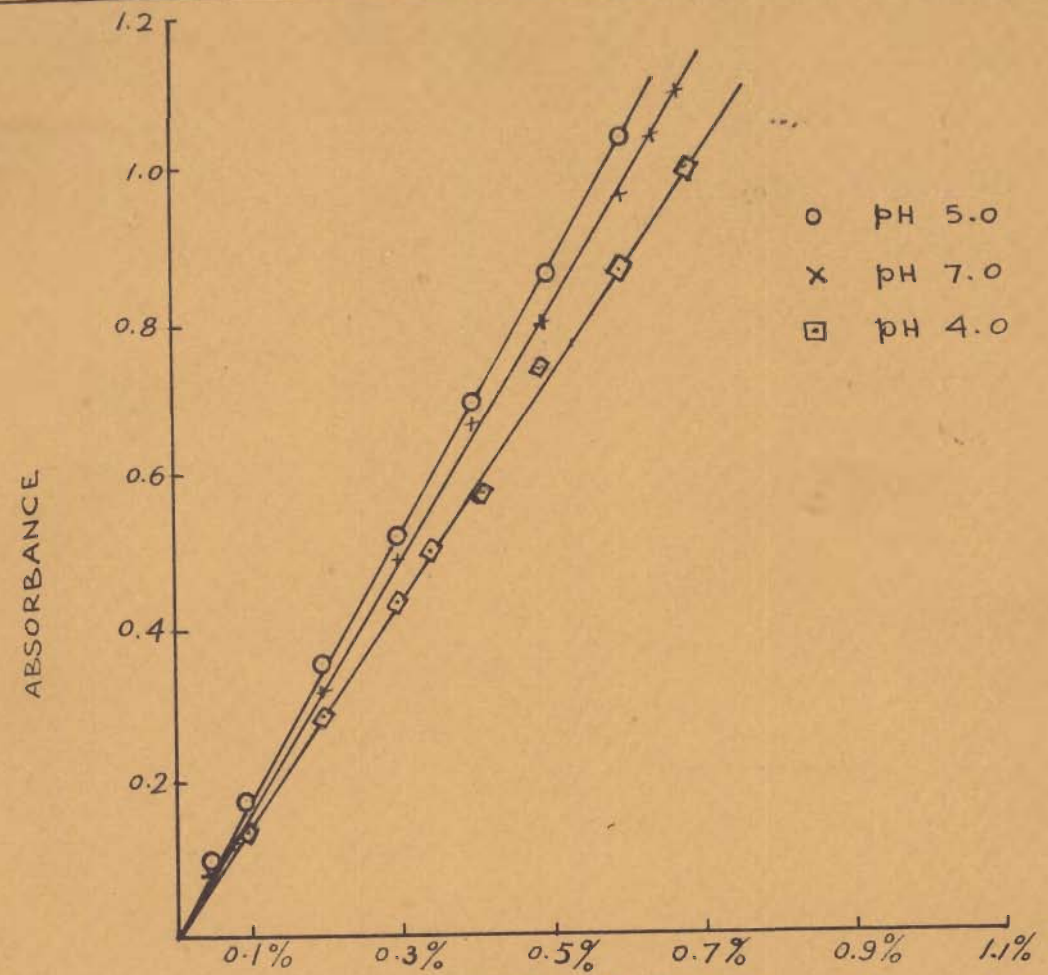


FIG. 3 CONCENTRATION OF TRANSFUSION GELATIN

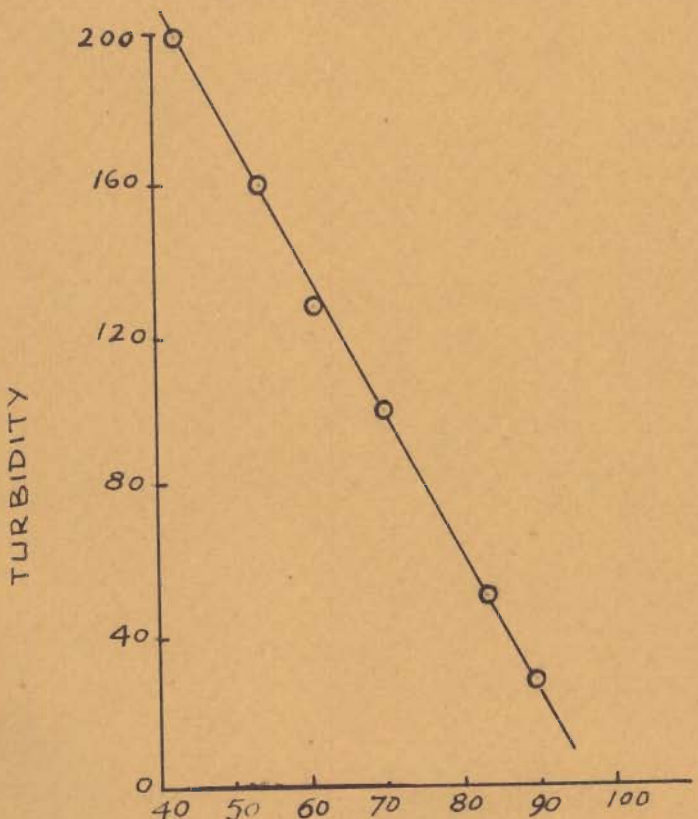


FIG. 4 TRANSMITTANCE

Calibration curves for the determination of protein concentration.

The ultra violet spectra of proteins can be divided into 3 regions (1) above 2500 A° (2) between 2100 to 2500 A° (3) below 2100 A°. The first region is simple while the second and third regions are complex.

Goldfarb[9] found that in several proteins the molar absorptivity per peptide bond falls in the range 2600 to 3100 A°. Following this lead several suggestions have appeared for determining the protein concentration from absorbance measurements.

Transfusion gelatin was found to give a sharp maxima at 2610 A° and egg albumin shows maximum absorbance at 2810 A°. The wave-length of maximum absorbance did not depend on pH in the case of these two proteins. Hence calibration curves (Figs.2 and 3) were obtained by plotting absorbance against concentration of protein solutions at different pH values. No change in absorbance was noted below 4 pH in both cases and above 7 pH in the case of transfusion gelatin and 8 pH in the case of egg albumin.

Calibration curve for turbidity measurements.

A calibration curve (Fig.4) was constructed to convert percentage transmittance into turbidity using a standard solution of silica (Hellige Standard Stock Suspension) equivalent to 200 parts per million of turbidity manufactured by Hellige Inc.,Garden city, New York.

-

Table No.2

Adsorption of egg albumin by H-bentonite at pH 4.8.

Initial conc. of protein solution.	O.D.of protein solution in equilibrium.	Equilibrium concentration of protein solution.		Protein solution adsorbed.	mg.of protein adsorbed per mg. of clay.
1.75%	0.05	0.002%	0.02 mg/ml.	1.748%	1.748
2.10%	0.11	0.005%	0.05 mg/ml.	2.09 %	2.09
2.42%	0.51	0.024%	0.24 mg/ml.	2.39 %	2.39
2.53%	0.84	0.04 %	0.40 mg/ml.	2.49 %	2.49
2.65%	1.34	0.064%	0.64 mg/ml.	2.536%	2.58
2.73%	1.75	0.082%	0.82 mg/ml.	2.648%	2.65

Concentration of clay suspension in each case = 0.05%

Total volume of solution in each case = 20 c.c.

Fig.5

Table No.3

Adsorption of egg albumin by H-illite at pH 4.8.

Initial concentration of protein solution.	O.D.of protein solution in equilibrium.	Equilibrium concentration of protein solution.		Protein solution adsorbed.	mg.of protein adsorbed per mg. of clay.
0.4 %	0.05	0.002%	0.02 mg/ml.	0.398%	0.398
0.7 %	0.21	0.01 %	0.1 mg/ml.	0.60 %	0.60
0.75%	0.5	0.024%	0.24 mg/ml.	0.726%	0.726
0.78%	0.92	0.044%	0.44 mg/ml.	0.736%	0.736
0.80%	1.27	0.061%	0.61 mg/ml.	0.739%	0.74
0.85%	1.7	0.082%	0.82 mg/ml.	0.768%	0.77

Concentration of clay suspension in each case = 0.05%

Total volume of solution in each case = 20 c.c.

Fig.6

Table No.4

Adsorption of egg albumin by H-kaolinite at pH 4.8.

Initial concentration of protein solution.	O.D.of protein solution in equilibrium.	Equilibrium concentration of protein solution.		Protein solution adsorbed.	mg.of protein adsorbed per gm.of clay.
0.02 %	0.05	0.002%	0.02 mg/ml.	0.018%	18.0
0.035%	0.21	0.010%	0.1 mg/ml.	0.025%	25.0
0.045%	0.38	0.018%	0.18 mg/ml.	0.027%	27.0
0.072%	0.92	0.044%	0.44 mg/ml.	0.028%	28.0
0.090%	1.3	0.062%	0.62 mg/ml.	0.028%	28.0
0.12 %	1.78	0.09 %	0.9 mg/ml.	0.030%	30.0

Concentration of clay suspension in each case = 0.05 %

Total volume of solution taken in each case = 20 c.c.

Fig.7.

Table No.5

Adsorption of transfusion gelatin by H-bentonite at pH 5.0.

Initial concentration of protein solution.	O.D. of protein solution in equilibrium.	Equilibrium concentration of protein solution.		Protein solution adsorbed.	mg. of protein adsorbed per mg. of clay.
1.35%	0.035	0.02%	0.2 mg/ml.	1.33%	1.33
1.80%	0.12	0.07%	0.7 mg/ml.	1.73%	1.73
2.00%	0.24	0.14%	1.4 mg/ml.	1.86%	1.86
2.20%	0.47	0.27%	2.7 mg/ml.	1.93%	1.93
2.40%	0.70	0.40%	4.0 mg/ml.	2.00%	2.00
2.60%	1.0	0.58%	5.8 mg/ml.	2.02%	2.02

Concentration of clay suspension in each case = 0.05 %

Total volume of solution in each case = 20 c.c.

Fig.8

Table No.6

Adsorption of transfusion gelatin by H-illite at pH 5.0.

Initial concentration of protein solution.	O.D. of protein solution in equilibrium.	Equilibrium concentration of protein solution.		Protein solution adsorbed.	mg. of protein adsorbed per mg. of clay.
0.45%	0.03	0.02%	0.2 mg/ml.	0.430%	0.43
0.60%	0.12	0.07%	0.7 mg/ml.	0.530%	0.53
0.68%	0.21	0.12%	1.2 mg/ml.	0.560%	0.56
0.78%	0.35	0.2 %	2.0 mg/ml.	0.580%	0.58
1.00%	0.70	0.4 %	4.0 mg/ml.	0.60 %	0.60
1.22%	1.05	0.61%	6.1 mg/ml.	0.61 %	0.61

Concentration of clay suspension in each case = 0.05 %

Total volume of solution in each case = 20 c.c.

Fig.9

Table No.7

Adsorption of transfusion gelatin by H-kaolinite at pH 5.0.

Initial concentration of protein solution.	O.D. of protein solution in equilibrium.	Equilibrium concentration of protein solution.		Protein solution adsorbed.	mg. of protein per gm. of clay.
0.035%	0.03	0.02%	0.2 mg/ml.	0.015%	15.0
0.077%	0.10	0.06%	0.6 mg/ml.	0.017%	17.0
0.130%	0.19	0.11%	1.1 mg/ml.	0.02%	20.0
0.490%	0.49	0.28%	2.8 mg/ml.	0.021%	21.0
0.530%	0.875	0.508%	5.08mg/ml.	0.022%	22.0
0.550%	0.905	0.527%	5.27mg/ml.	0.023%	23.0

Concentration of clay suspension in each case = 0.05%

Total volume of solution taken in each case 20.0 c.c.

Fig.10

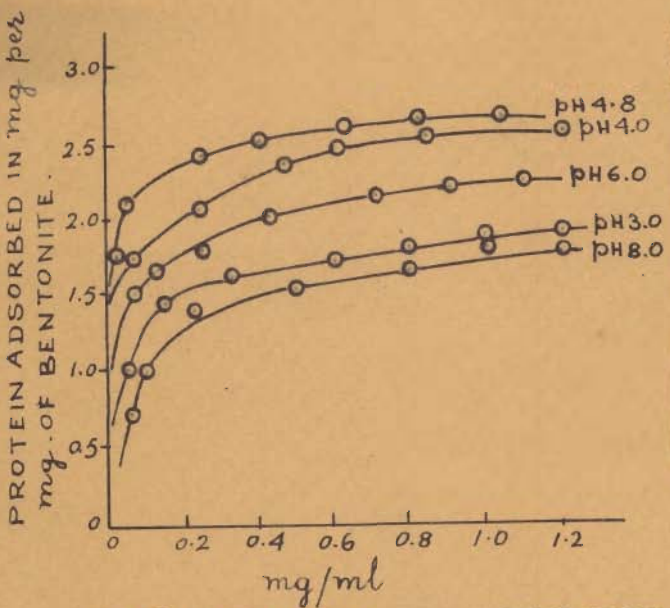


FIG. 5 EQUILIBRIUM CONCENTRATION OF EGG ALBUMIN.

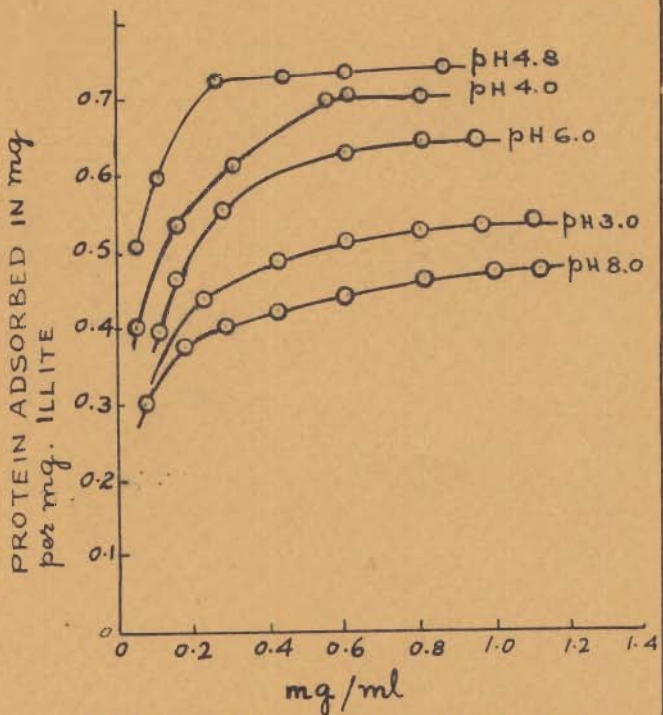


FIG. 6 EQUILIBRIUM CONCENTRATION OF EGG ALBUMIN.

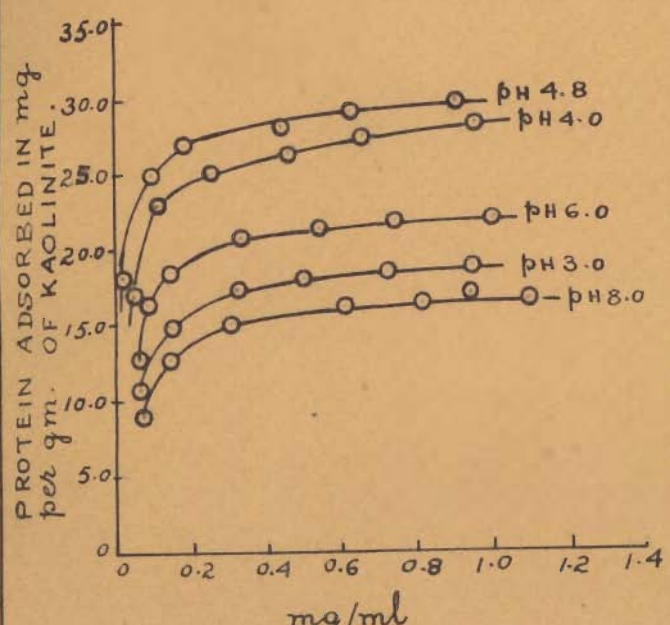


FIG. 7 EQUILIBRIUM CONCENTRATION OF EGG ALBUMIN

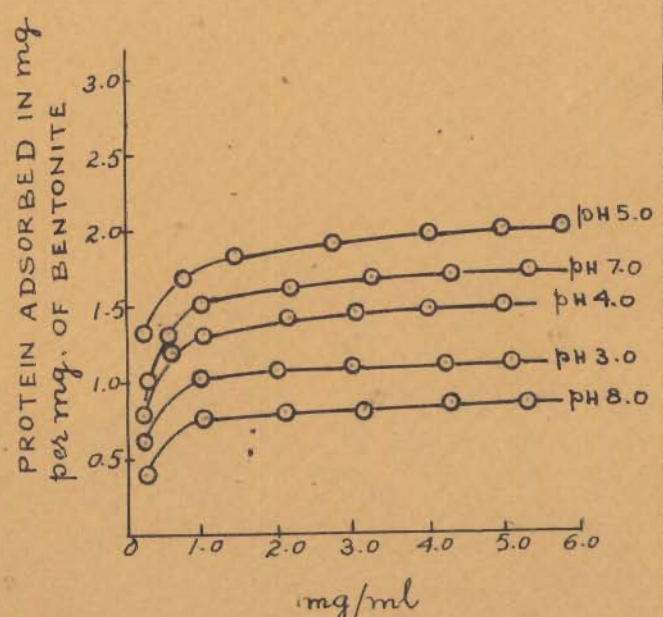


FIG. 8 EQUILIBRIUM CONCENTRATION OF TRANSFUSION GELATIN

Variation in the adsorption of proteins by clay minerals along with pH.

Table No.8

Clay mineral	Proteins	mg.of proteins adsorbed per mg.of clay				
				at pH		
		3.0	4.0	4.8	6.0	8.0
Bentonite	Egg albumin	1.75	2.45	2.60	2.10	1.60
				at pH		
		3.0	4.0	5.0	7.0	8.0
Bentonite	Transfusion gelatin	1.1	1.48	1.98	1.70	0.85

Fig.11

Table No.9

Clay mineral	Proteins	mg.of proteins adsorbed per mg.of clay				
				at pH		
		3.0	4.0	4.8	6.0	8.0
Illite	Egg albumin	0.52	0.71	0.74	0.63	0.44
				at pH		
		3.0	4.0	5.0	7.0	8.0
Illite	Transfusion gelatin	0.39	0.47	0.59	0.54	0.31

Fig.12

Table No.10

Clay mineral	Proteins	mg.of proteins adsorbed per mg.of clay				
				at pH		
		3.0	4.0	4.8	6.0	8.0
Kaolinite	Egg albumin	18.4	27.0	29.0	21.5	16.0
				at pH		
		3.0	4.0	5.0	7.0	8.0
Kaolinite	Transfusion gelatin	11.0	15.5	21.0	18.5	9.0

Fig.13.

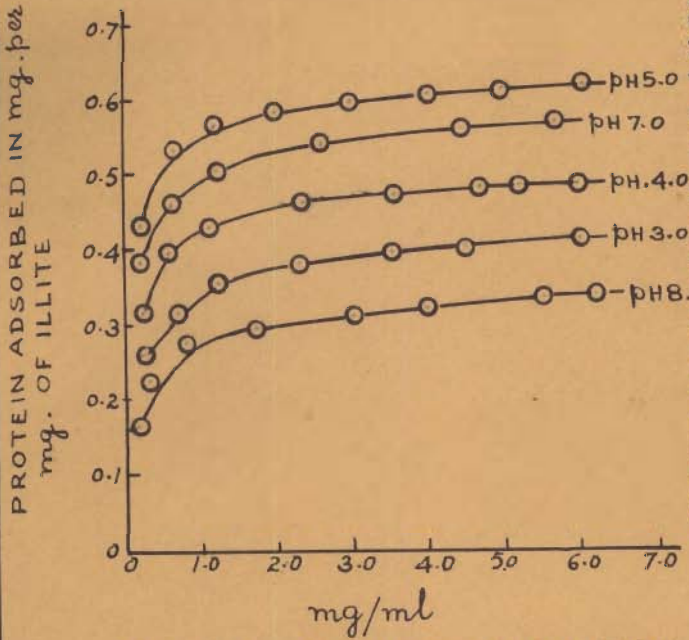


FIG.9 EQUILIBRIUM CONCENTRATION OF TRANSFUSION GELATIN

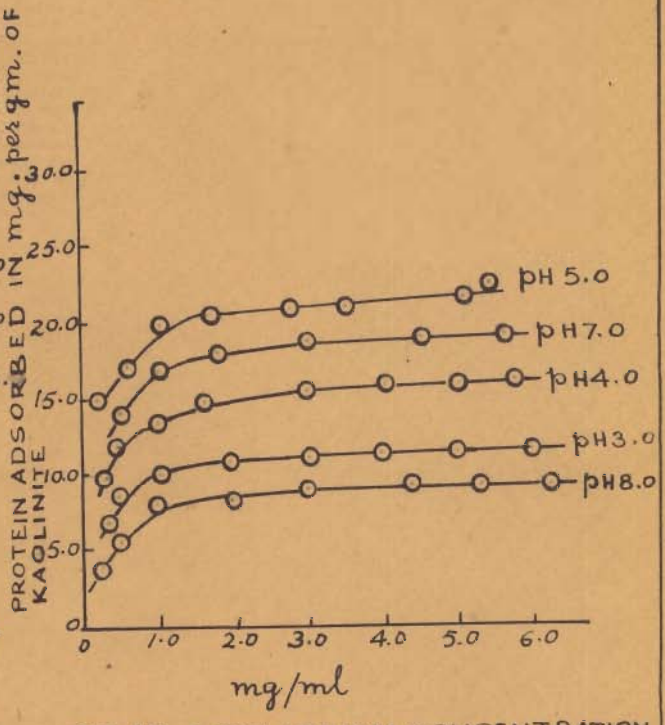


FIG.10 EQUILIBRIUM CONCENTRATION OF TRANSFUSION GELATIN

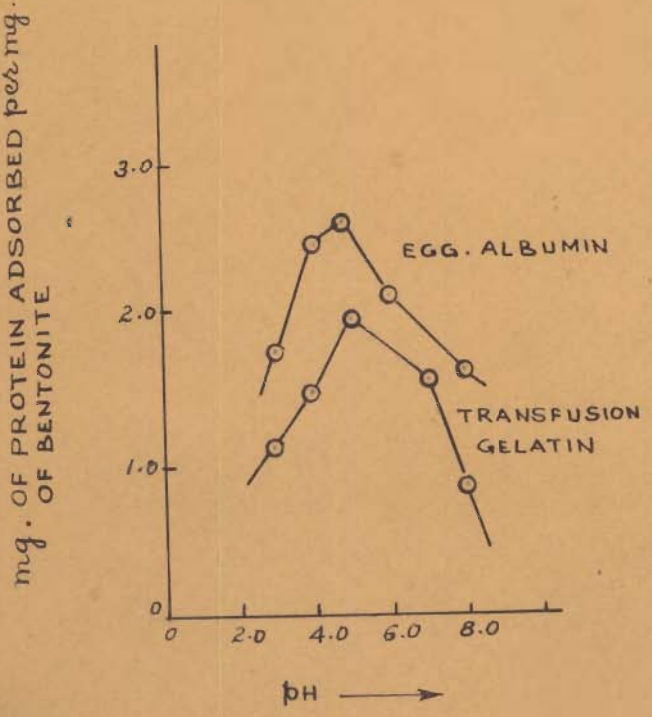


FIG. 11

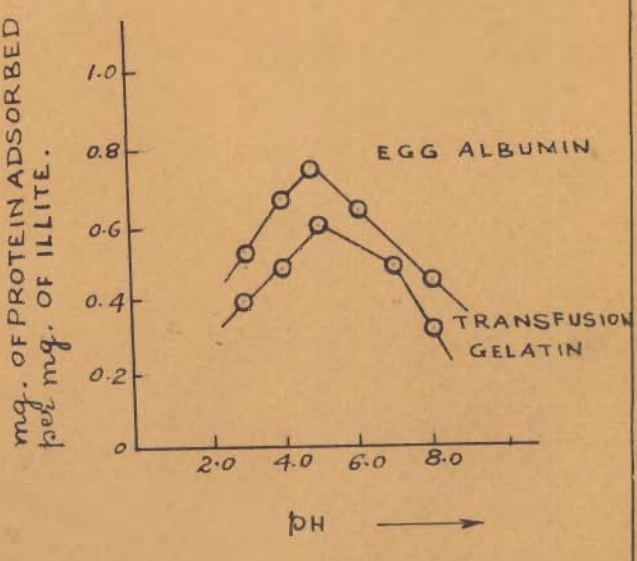


FIG. 12

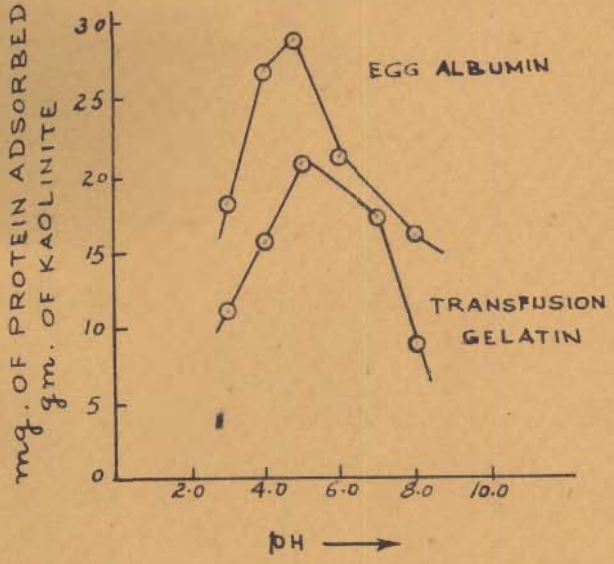


FIG. 13

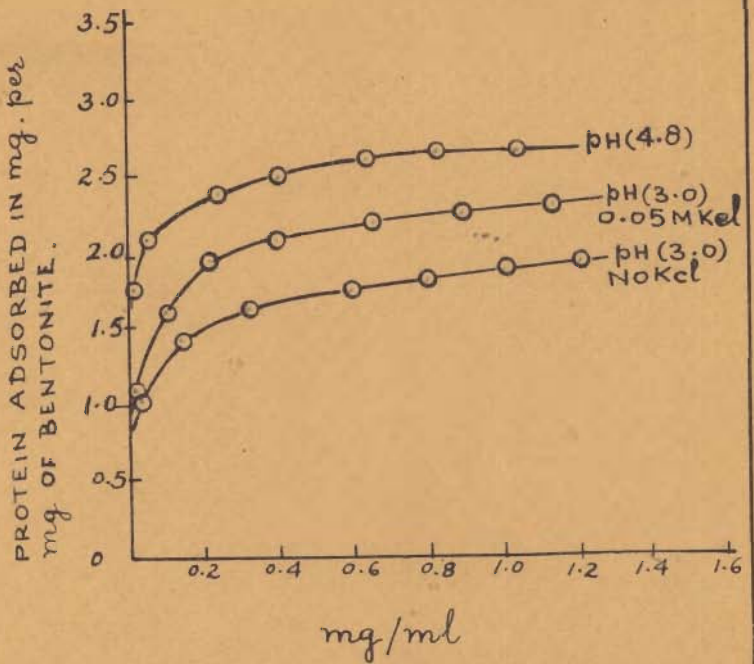


FIG. 14 EQUILIBRIUM CONCENTRATION OF EGG ALBUMIN

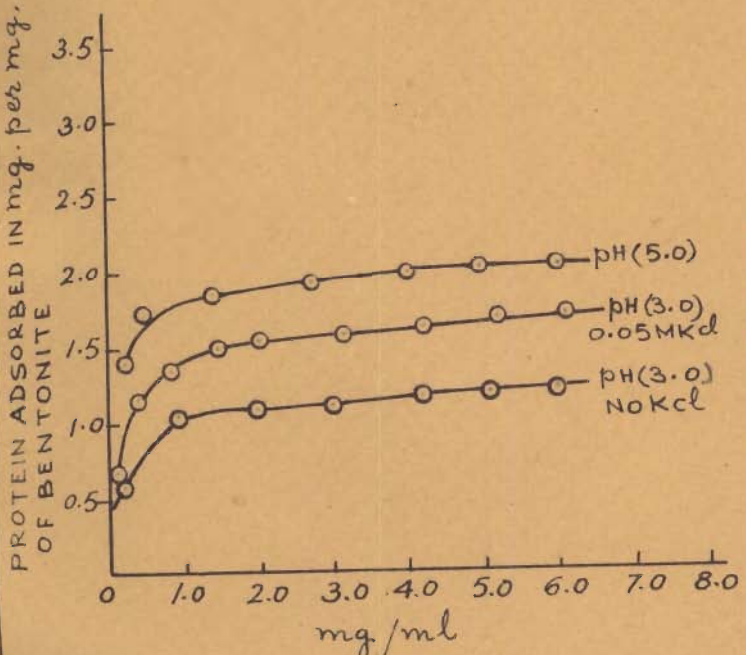


FIG. 15 EQUILIBRIUM CONCENTRATION OF TRANSFUSION GELATIN

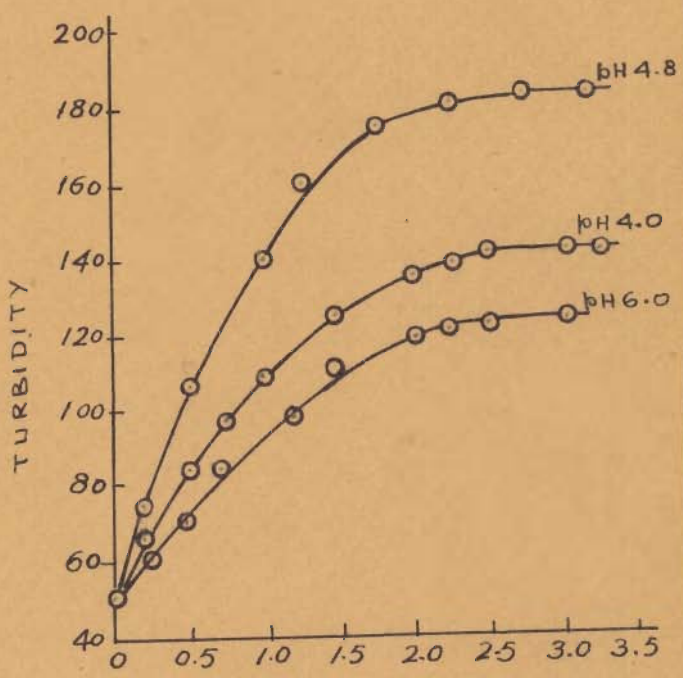


FIG. 16 mg. OF EGG ALBUMIN per mg. OF BENTONITE

Table No.11

Concentration of H-bentonite 0.02 %

Total volume in each tube = 25 ml.

Concentration of egg albumin.	mg/mg.clay.	Transmittance.	Turbidity
		pH 4.8	
-	-	83.0	50.0
0.004%	0.2	76.0	77.0
0.01 %	0.5	68.0	106.0
0.02 %	1.0	59.0	140.0
0.024%	1.2	53.5	160.0
0.036%	1.8	49.0	176.0
0.046%	2.3	47.5	182.0
0.054%	2.7	47.0	184.0
0.064%	3.2	47.0	184.0
		pH 4.0	
-	-	83.0	50.0
0.004%	0.2	79.0	66.0
0.01 %	0.5	74.0	84.0
0.015%	0.75	72.0	90.0
0.02 %	1.0	67.0	110.0
0.03 %	1.5	62.0	126.0
0.04 %	2.0	60.0	136.0
0.045%	2.25	59.0	140.0
0.05 %	2.5	59.0	142.0
		pH 6.0	
-	-	83.0	50.0
0.005%	0.25	80.5	60.0
0.01 %	0.5	77.5	70.0
0.015%	0.75	74.0	84.0
0.024%	1.2	70.0	98.0
0.03 %	1.5	66.8	110.0
0.04 %	2.0	64.0	120.0
0.045%	2.25	63.5	122.0
0.05 %	2.5	63.0	123.0

Fig.16.

Table No.12

Concentration of H-bentonite 0.02%

Total volume in each tube = 25 ml.

Concentration of transfusion gelatin.	mg/mg.clay	Transmittance.	Turbidity.
		pH 5.0	
-	-	83.0	50.0
0.005%	0.25	75.0	80.0
0.008%	0.4	68.5	104.0
0.014%	0.7	62.0	128.0
0.024%	1.2	58.0	144.0
0.034%	1.7	55.5	152.0
0.044%	2.2	54.0	158.0
0.052%	2.6	53.5	160.0
		pH 4.0	
-	-	83.0	50.0
0.005%	0.25	80.0	62.0
0.01 %	0.5	77.0	72.0
0.015%	0.75	74.5	82.0
0.02 %	1.0	72.0	90.0
0.03 %	1.5	70.0	100.0
0.04 %	2.0	68.5	104.0
0.05 %	2.5	68.5	104.0
		pH 7.0	
-	-	82.0	54.0
0.004%	0.2	78.2	68.0
0.01 %	0.5	74.0	84.0
0.015%	0.75	70.5	98.0
0.02 %	1.0	66.0	114.0
0.03 %	1.5	63.0	124.0
0.04 %	2.0	62.5	126.0
0.05 %	2.5	62.5	126.0

Fig.17

Table No.13

Concentration of H-illite sol = 0.02 %

Total volume in each tube = 25 ml.

Concentration of egg albumin.	mg/mg. clay.	Transmittance.	Turbidity.
		pH 4.8	
-	-	80.5	60.0
0.0006%	0.03	76.0	78.0
0.0016%	0.08	70.5	96.0
0.003 %	0.15	67.0	110.0
0.006 %	0.3	63.5	122.0
0.008 %	0.4	62.5	126.0
0.011 %	0.55	61.5	130.0
0.014 %	0.7	61.5	130.0
		pH 4.0	
-	-	80.5	60.0
0.0014%	0.07	77.5	71.0
0.0028%	0.14	72.5	90.0
0.004 %	0.2	70.0	100.0
0.006 %	0.3	68.0	106.0
0.008 %	0.4	67.0	110.0
0.011 %	0.55	65.5	114.0
0.014 %	0.7	65.5	114.0
		pH 6.0	
-	-	82.0	54.0
0.002%	0.1	77.0	73.0
0.004%	0.2	73.5	85.0
0.006%	0.3	72.0	92.0
0.008%	0.4	70.1	96.0
0.010%	0.5	70.0	98.0
0.012%	0.6	69.5	100.0
0.014%	0.7	69.5	100.0

Fig.18

Table No.14

Concentration of H-illite = 0.02 %

Total volume in each tube = 25 ml.

Concentration of transfusion gelatin.	mg/mg.clay.	Transmittance.	Turbidity.
		pH 5.0	
-	-	80.5	60.0
0.0006%	0.03	76.0	76.0
0.0014%	0.07	72.0	90.0
0.0024%	0.12	68.5	104.0
0.0042%	0.22	65.5	114.0
0.007 %	0.35	64.5	118.0
0.01 %	0.5	64.0	120.0
0.012 %	0.6	64.0	120.0
		pH 7.0	
-	-	80.5	60.0
0.0014%	0.07	75.5	77.0
0.003 %	0.15	74.0	84.0
0.0048%	0.24	70.08	96.0
0.007 %	0.35	69.6	100.0
0.009 %	0.45	68.5	104.0
0.011 %	0.55	68.5	104.0
0.013 %	0.65	68.5	104.0
		pH 4.0	
-	-	80.5	60.0
0.002 %	0.1	77.8	70.0
0.004 %	0.2	75.0	80.0
0.006 %	0.3	72.0	90.0
0.008 %	0.4	71.0	94.0
0.010 %	0.5	70.05	96.0
0.012 %	0.6	70.05	96.0

Fig.19.

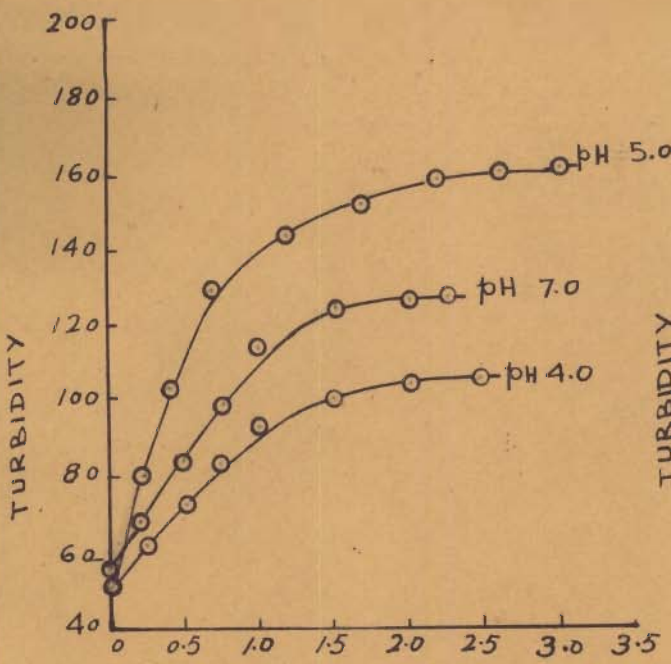


FIG. 17 mg. OF TRANSFUSION GELATIN per mg. OF BENTONITE

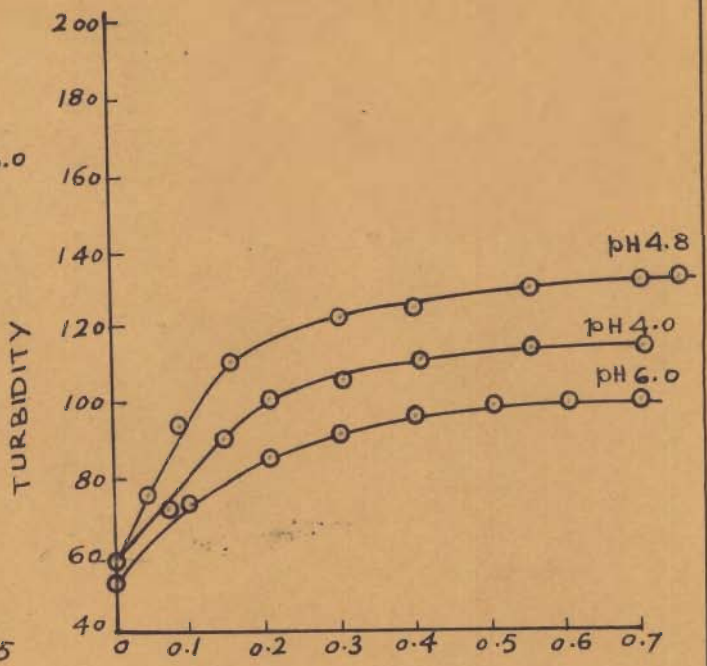


FIG. 18 mg. OF EGG ALBUMIN per mg. OF ILLITE.

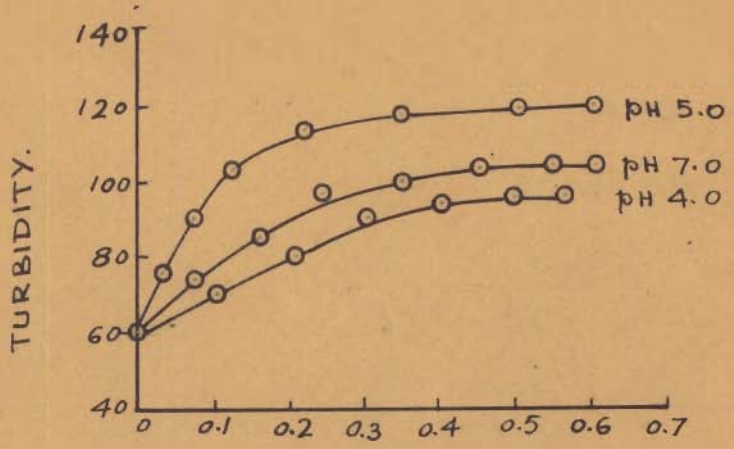


FIG. 19 mg. OF TRANSFUSION GELATIN per mg. OF ILLITE.

RESULTS AND DISCUSSION

The adsorption isotherms have been drawn by plotting the equilibrium protein concentration(in mg. per ml.) and amount of protein adsorbed. All the isotherms are regular and show a positive adsorption.The fact that the data neither fits in the Langmuir type nor in the Freundlich adsorption equation and that higher amounts of protein are adsorbed indicate that interlamellar spaces have also been covered by the adsorbate molecules.

From the shape of adsorption isotherm it would be observed that isotherms of clay and egg albumin rise more steeply than that of transfusion gelatin at lower protein concentrations. It may therefore be concluded that globular protein shows greater activity towards the adsorption sites as compared to fibrillar protein.

Apparently these isotherms have two distinct regions,one of the lower equilibrium concentration corresponding to initial rise in adsorption and the other is the region of higher equilibrium concentration. In the initial stages the adsorption is rapid and the up take of protein is more. This initial rise in adsorption reflects a binding to random sites on the clay surface. At higher values it is possible that protein-protein interaction and a necessity for such a translational movement of protein molecule is there which may

cover up the uncovered areas isolated by random adsorption,and too small to accommodate a protein molecule. These factors decrease the adsorption rate and prevent the isotherm from levelling off more sharply to parallel the concentration axis in the higher concentration region.

Normally the process of adsorption involves some ion-exchange as well as simple adsorption to the surface of the particles,still the binding can be most suitably accounted by the interaction between COOH and NH_3^+ groups of protein molecule and OH groups on a clay surface. Since a protein molecule has both anionic and cationic groups,both anionic and cationic displacement may play roles in the mechanism of protein binding to the clay surface.

Effect of pH on adsorption.

The amounts of protein adsorbed by the minerals, bentonite,kaolinite and illite is influenced by the pH of the system (vide Figs.11 to 13). It is found that in each case the maximum adsorption occurs close to iso-electric pH and falls low if the hydrogen ion concentration is changed on either side of iso-electric pH.

Because of amphoteric nature,the molecule of protein is cationic at pH below the iso-electric point and anionic above the iso-electric point. When protein is in cationic form its adsorption to clay mineral is

only due to the attraction of opposite charges on the adsorbate and the adsorbent.

Low values of the amount of protein adsorbed at pH less than iso-electric point, can be explained on the basis of increased charge density of protein molecule as acidity is increased. From the titration curves of Tanford and Wagner[10] it is known that the positive charge on protein molecule will increase with decrease in pH. The free amino groups are protonated at this pH and carry positive charges. The protonation of imidazole and carboxyl groups at lower pH would further increase the positive charge and thus the total number of positive charges carried per protein molecule is increased and less protein need be adsorbed to satisfy the negative charge on the clay surface. Thus the decreased adsorption with increasing acidity may be attributed to the increased charge density of protein molecule.

As the pH is increased above the iso-electric point a sharp decrease in adsorption of protein takes place. The protein molecule in this pH range is anionic and the decreased adsorption is a result of repulsion of the protein molecule by the clay particle. The basic medium also increases the negative charge on total

surface (plane + edges) of the clay particle and this further acts in reducing the extent of adsorption in this region. Still,however,there is some adsorption of protein on clay particles even above the iso-electric pH which can only be explained on the basis of following;

Mehta[11] has pointed out the importance of Al-bond mechanism and shows the existence of bonds joining the clay surface with an organic anion forming a bridging net work through a polyvalent inorganic cation like clay-Al-OOCR. Even in H-clay some Al exists at the surface and this along with the lattice Al at the broken edges of the clay platelets enter into the formation of such bonds. The aggregate stabilization in soil has also been explained on the basis of this theory. As such the binding of protein with clay when it is in the anionic form takes place by means of a bridging net work through a polyvalent inorganic cation(Al).

Another possibility is the presence of some localised sites on the protein molecule which may retain positive charges at pH values higher than iso-electric value. Any adsorption at this pH is due to attraction of these localised sites to the negatively charged clay surface. Any further increase in pH would reduce such localised sites which would cause a further reduction in

the amount adsorbed.

Effect of pH on clay protein complexes.

It would be of interest to see the effect of changing pH on protein-clay complexes.

These studies were carried out by preparing protein-bentonite and protein-illite complexes at the iso-electric pH. The isolated complexes were re-equilibrated in media below and above the iso-electric pH. The change in pH resulted in desorption of the protein from the original complex. The results are given below:

Bentonite	Egg albumin	Transfusion gelatin.
Amount of bentonite taken.	10.0 mg.	10.0 mg.
Amount of protein added.	2.73%	1.35 %
pH of the suspension.	4.8	5.0
Amount of protein adsorbed in mg./mg.of clay.	2.65	2.02
Readjusted pH.	3.2	8.2
Protein detected in solution in mg.	0.90	9.6
Protein released in mg/mg. clay.	0.09	0.96
Protein adsorbed in mg/mg. clay.	2.56	1.06
Protein released.	3.5%	42.5%

Percentage of protein released =

$$\frac{\text{Protein released per mg.of clay}}{\text{Protein adsorbed per mg.of clay}} \times 100$$

Illite	Egg albumin	Transfusion gelatin
Amount of illite taken.	10 mg.	10 mg.
Amount of protein added	0.85 %	1.22 %
pH of the suspension.	4.8	5.0
Amount of protein adsorbed in mg./mg.of clay.	0.77	0.61
Readjusted pH.	3.2	8.2
Protein detected in solution.	0.60 mg.	5.2 mg.
Protein released in mg/mg. of clay.	0.06	0.52
Protein adsorbed in mg/mg.of clay.	0.71	0.09
Protein released	7.7 %	85.0 %

It is observed from the above data that change in the pH of egg albumin below its iso-electric point resulted in the release of 3.5 % and 7.7 % of protein. Results in the pH range above the iso-electric point show that 42.5% and 85% of protein is released in the case of transfusion gelatin.

At pH values below the iso-electric point the adsorption as well as desorption of protein is small. On the other hand the protein desorbed at values higher than the iso-electric point is much higher. It goes to show that the protein from the interlayer spaces of clay minerals is removed at pH above the iso-electric point while it does not do so in the region below the iso-electric pH.

The ionization of carboxyl and amino groups would

depend on pH even when the protein is adsorbed on the mineral. At pH above the iso-electric point, the negative charge on the adsorbed protein will increase thus causing strong forces of repulsion between the protein molecules themselves and between protein molecules and clay surface. The forces of repulsion would be strong enough to remove the protein from the interlayer spaces of minerals.

On the other hand in the acidic side of iso-electric pH such forces of repulsion would exist only between the protein molecules and not between the protein molecules and clay surface with the result that very little protein would be removed from inter-layer spaces of the mineral.

Further more large amount of protein is desorbed from illite as compared to bentonite. This goes to show that lesser amount of protein goes in the inter-layer spaces of illite than that of bentonite.

Effect of electrolyte on adsorption.

The presence of electrolyte has a marked effect on the amount of protein adsorbed below the iso-electric pH. Figs.14 and 15 show the effect of 0.05 N KCl to buffered bentonite-egg albumin system at pH 3.0.

The amount of protein adsorbed increases in presence of electrolyte. It appears that the chloride ions form an ionic atmosphere around the positively charged protein molecule. This would result in a decrease in the charge density of the protein with the result that a larger amount of protein would be required to cover up the adsorption sites.

Turbidimetry.

Tables (11 to 14) give the turbidimetry data of clay protein interactions at various pH. Figs.(16 to 19) show the plot of turbidity developed in the solution against the amount of protein added. The flat portion of the curve depicts the point of maximum interaction beyond which no rise in turbidity value is noticed on further addition of protein.

Turbidity data also support the results reported in adsorption measurements. The extent of interaction which is denoted by the amount of turbidity developed depends on pH and being maximum at iso-electric-pH. Three regions of interaction may be considered:

1. Below the iso-electric pH the interaction takes place between the free amino, guanidino and imidazole groups which are protonated in this pH range and carry a positive charge.

2. At the iso-electric pH only the interaction of

carboxyl groups is possible, but since the number of COOH groups available for interaction would be much less in this region the binding of protein would be mostly due to physical forces.

3. Above the iso-electric point the imidazole groups, amino groups, etc. will be ionised imparting a negative charge to protein molecule. Under these conditions a small turbidity will be developed in the mixture.

—

R_E_F_E_R_E_N_C_E_S

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

2. Allison,F.E.,Doetsch,J.H., Soil Sci., 75,373,1953.

3. Pinck,L.A. and Allison,F.E.,Science 114,130,1951.

4. Bremner,J.M., J.Agri.Sci., 39,181,1949.

5. Ensminger,L.E. and Giesking,J.E.,Soil Sci.,48,467, 1937.

6. Ensminger,L.E. and Giesking,J.E., Soil Sci.,51,125, 1941.

7. Waksman,S.A., Soil Sci.,78,118,1954.

8. McLaren,A.D., Peterson,G.H. and Barshad,I.,Soil Sci. Soc.Am.Proc.,23,239,1958.

9. Goldfarb A.R., Science,114,177.

10. Tanford and Wagner,J.Amer.Chem.Soc.,76,3331-36.

11. Mehta,N.C., Ph.D.Thesis,Univ.of California.

—

CHAPTER V.

Flow properties of clay-protein mixtures.

INTRODUCTION

The viscosity measurements have proved to be of immense value in studying the colloid chemical behaviour of clays, and, although its theoretical aspects are less appreciated this technique finds great many industrial and technological applications. In fact, the flow properties (including the deformability) find so many diversified applications that a new field, known as, 'Rheology of clays' is fast developing.

The viscosity variations in clays are usually explained in terms of mutual interactions between clay particles, those between the suspended particles and dispersion medium or between molecules of dispersion medium itself. But other important factors to keep in view are the existence of clay colloidal micelles, their ionization and subsequent hydration for instance clay suspensions, inspite of their large dependence on charge and zeta potential for stability, unlike the hydrophobic colloids, show a fairly high degree of hydration. This unique behaviour has resulted in the endowment of properties like thixotropy, rheopexy, dilatancy etc. to clay minerals. Attention to these facts was drawn by Kruyt[1], Pauli[2] and Marshall[3] during the last few decades.

Equations with numerous modifications have been put forward from time to time to explain the variations in

viscosity with concentration. Einstein[4] gave the equation $\eta_s = \eta_m (1 + 2.5 \phi)$ for systems containing hydrated particles:where η_s is the viscosity of the suspension and η_m is the viscosity of the medium and ϕ is the ratio of the volume of dispersed phase to that of the whole system. His equation suffers from the draw back that it is only applicable to rigid and spherical particles,and that too,for the low concentration of the colloidal micelle. His equation is therefore not applicable for clay systems. Similarly the modified equation of Smoluchowski[5] which takes into account the electrical properties of the suspended particles cannot be applied to clay systems where factors other than charge and hydration play and also influence the viscosity.

In a dilute suspension during Newtonian flow the shape and orientation of the suspended particles affect the flow pattern and so the viscosity. If electrically charged,the orientation,interaction and hydrodynamics of the particles will be affected and in turn the viscosity. Many viscosity equations have been proposed to account for the particle-particle interaction and for the determination of the intrinsic viscosity. A modified form of Kraemer's[6] equation is the Schulz-Blaschke[7] equation:

$\eta_{sp/c} = (\eta)\ \eta_{sp} + (\eta)$

where η_{sp} is the specific viscosity, (η) is the intrinsic viscosity and k is the interaction index of the system and is valid at concentrations (c) such that $1/c > m$ where $m = k(\eta)$. Granquist[8] and Van der Watt[9] have tested the validity of Schulz-Blaschke equation over various systems and have emphasized its advantages over other equations mentioned in literature.

So far the reaction between the clays and the proteins has not been exploited to study the rheological data of clay minerals. It is felt that investigations in this direction can provide a better understanding of the problem and the role of many factors like electrical and hydrodynamic interaction, dissymmetry etc. hitherto ambiguous, can be clearly appreciated. With this aim in view the viscosity variations of bentonite in presence of transfusion gelatin and egg albumin were determined using Newtonian mixtures of the two. The results are reported in this chapter.

—

E_X_P_E_R_I_M_E_N_T_A_L

Reagents.

The clay mineral used in these investigations is bentonite obtained from Wards Natural Science Establishment United States. A suspension of H-bentonite of known concentration was prepared by the method mentioned in Chapter I.

Al and Ca clays were obtained by passing the hydrogen bentonite suspension repeatedly through Amberlite IR 120 column (originally labelled with aluminium and calcium cations) till the exchange was complete. Their concentrations were determined by the usual method.

Solution of egg albumin was prepared as mentioned in Chapter IV. The transfusion gelatin used was a 6% solution of the product.

Viscosity measurements.

The viscosity measurements were made at a constant temperature of 30°C. The method used was the one devised by Scarpa[10] as modified by Farrow[11] and latter by Joshi[12]. The apparatus used is shown in figure I. It consists of three parts (i) viscometer, (ii) apparatus for generating low constant pressure and (iii) thermostatic water bath(vide Fig.1).

Ostwald viscometer,B.S.S.Type number 2,was used

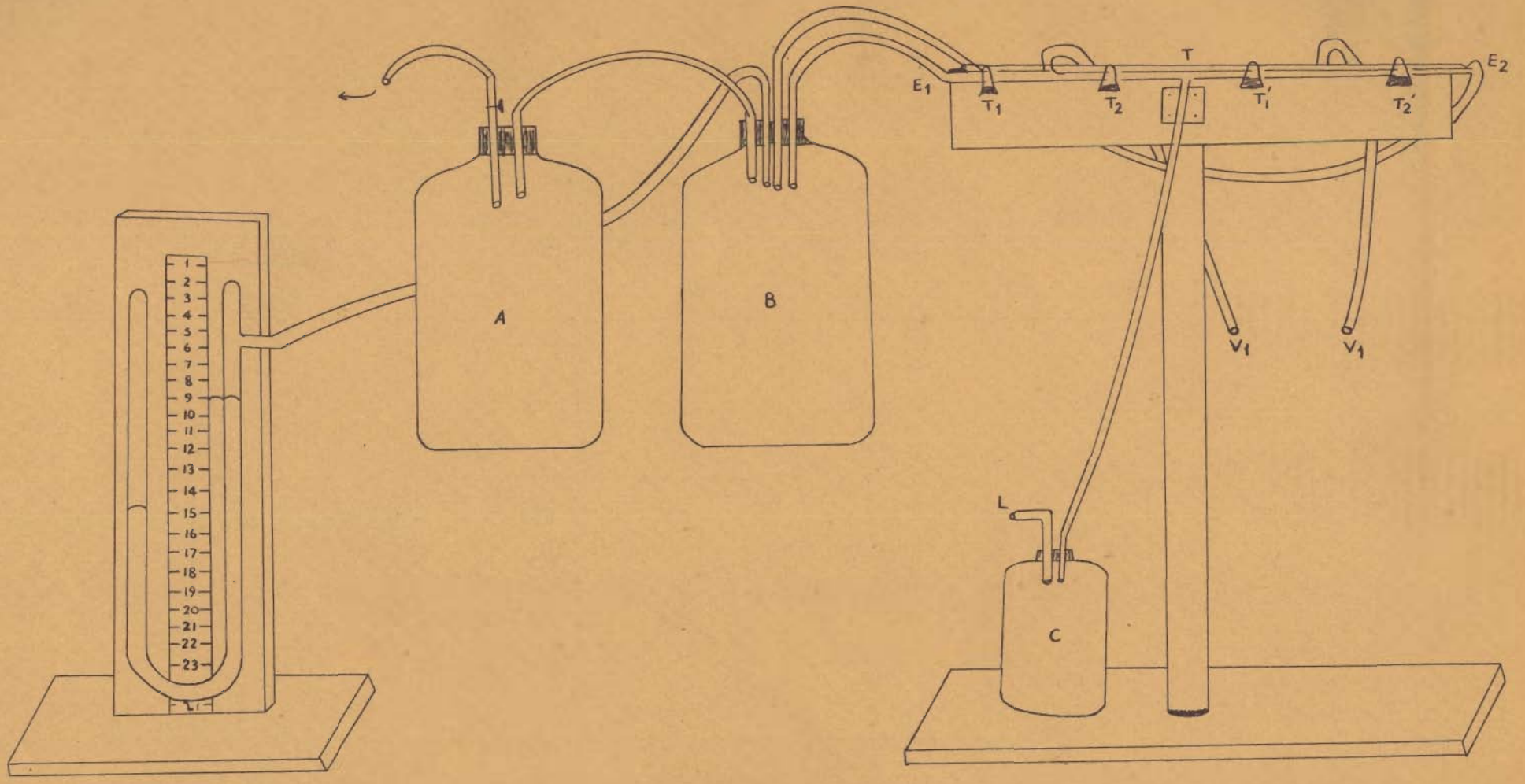

A
B
C
T
E1
E2
T1
T2
T1'
T2'
V1
V1
L
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23

FIG. 1.

for these estimations. A number of viscometers were used simultaneously to facilitate a larger number of determinations within a short time interval. The viscometers were connected through the rubber tubings to the source of reduced pressure. They were cleaned with chromic acid, washed with distilled water and dried by air blower before use in each case.

The second part of the apparatus consisted of two large aspirator bottles inter connected to each other with pressure rubber tubings so that any diminution of pressure in one caused a simultaneous and equivalent reduction in the other and could be read off by a manometer (Fig.1). The manometer consisted of a clean glass U tube of suitable dimensions fixed along a scale to read the pressure. Coloured water was used in the manometer. The reduction of pressure could be effected by means of a suction pump connected to the bottle A and was carefully regulated. The bottle B was connected to two glass tubes mounted over a stand each carrying a system of stoppers. A vacuum could be applied on the viscometer connected at V_1 or V_2 by opening the stoppers T_1 or T_2 respectively. This caused a rise in the liquid placed in the viscometer. Access to air was provided to the viscometers by opening suitable stoppers.

The temperature was controlled by immersing the viscometers in a thermostatic water bath, the temperature of which was regulated by means of a mercury regulator which worked through an electromagnetic relay to an accuracy of ± 0.01°C. Sufficient time was allowed for the viscometer to attain the temperature of the bath before actual measurements were carried out. The viscosity was determined by the formula:

$$\eta = k \frac{t_1 t_2}{t_1 + t_2}$$

where η is the viscosity of the liquid and t_1 and t_2 are the times for rise and fall of a given volume of liquid under constant pressure and temperature and k is the viscometer constant. The value of the constants of the viscometers used were obtained by determining t_1 and t_2 for standard solvents at a pressure of 22 cms. of water and a temperature of 30°C. Great care was taken in regulating the pressure so that it remains constant at the time of operation.

The procedure employed for determining the viscosity of unknown mixtures was as follows: A constant volume of mixture was taken in viscometer which was fitted to a clamp stand attached to the constant temperature bath.

The limb carrying the capillary of the viscometer was connected to aspirators for introducing low pressure. After a definite time the readings were taken by opening the stopper T_1 and keeping T closed. This caused the liquid in the viscometer to rise. The time of rise t_1 was noted. The stopper T_1 was then closed and T was opened. The liquid now started to fall and the time of fall t_2 was noted. Knowing the constant k, the viscosity was calculated.

The following sets were made for viscosity measurements:

A number of tubes were set up having H-bentonite of concentration 0.15 %. Solution of transfusion gelatin was then added to give a protein concentration of 6.0, 12.0, 20.0, 40.0, 50.0 gm. per 100 gm. clay. Buffer solution was added so that the pH of the mixture was 5.5. Before taking the observations the tubes were equilibrated for six hours. Similar sets having transfusion gelatin and H-bentonite were repeated having clay concentrations 0.25 %, 0.35 %, 0.4 %, 0.5 % .

Viscosity measurements of H-bentonite and egg albumin mixtures were done having protein concentrations as 2.0, 6.0, 8.0, 10.0, 12.0 gms. per 100 gms. clay and

clay concentration as 0.15%,0.25%,0.35%,0.4% at pH 4.5.

The entire set of observations with the two proteins were also repeated with Ca-bentonite and Al-bentonite. Tables 1 to 9 give the viscosity variations of H-bentonite (at various concentrations) in presence of different amounts of egg albumin and transfusion gelatin. The results are depicted in Figs.2 and 3.

Equation and the terms used.

The following viscosity terms have been used in this chapter.

η	Viscosity of the solution.
η_o	Viscosity of the pure solvent.
η/η_o	Relative viscosity.
$(\eta/\eta_o) - 1 = \eta_{sp}$	Specific viscosity.
$\frac{(\eta - \eta_o)}{\eta_o c} = \eta_{sp}/c$	Viscosity number.
$\eta_{sp}/c = (\eta)$ $c \to o$	Intrinsic viscosity.

(c = concentration of suspended particles in gm/ml).

The Schulz-Blaschke equation,

$$\frac{\eta_{sp}}{c} = k\,(\eta)\,\eta_{sp} + (\eta)$$

has been used to determine the value of 'k' which is taken to be the measure of the extent of particle-particle interaction of the system. It is named as 'Interaction Index' and is dimensionless.

—

Table No.1

Concentration of H-bentonite sol = 0.15%
= 0.0015 gm per ml.

pH of clay-protein suspension = 5.5

Concentration of transfusion gelatin in gms.per 100 gms. clay.	Absolute viscosity of the mixture in centipoises.	Specific viscosity.	Viscosity number.
0.0	0.8732	0.0906	60.4
6.0	0.8895	0.1110	74.0
12.0	0.9184	0.1470	98.0
20.0	0.9352	0.1680	112.0
40.0	0.9544	0.1920	128.0
50.0	0.9736	0.2160	144.0

Fig.4.

Table No.2

Concentration of H-bentonite sol = 0.25 %
= 0.0025 gms.per ml.

pH of clay-protein suspension = 5.5

Concentration of transfusion gelatin in gms. per 100 gms.clay.	Absolute viscosity in centipoises.	Specific viscosity.	Viscosity number.
0.0	0.8851	0.1055	70.2
6.0	0.9688	0.2100	84.0
12.0	1.0088	0.2600	104.0
20.0	1.0369	0.2950	118.0
40.0	1.0689	0.3350	134.0
50.0	1.2266	0.5320	152.0

Fig.4.

Table No.3

Concentration of H-bentonite sol = 0.35 %
= 0.0035 gms.per ml.

pH of clay-protein suspension = 5.5

Concentration of transfusion gelatin added in gms.per 100 gms.clay.	Absolute viscosity of the mixture in centipoises.	Specific viscosity.	Viscosity number.
0.0	1.025	0.2810	80.3
6.0	1.086	0.3325	95.0
12.0	1.108	0.3850	110.0
20.0	1.145	0.4305	123.0
40.0	1.193	0.4900	140.0
50.0	1.243	0.5530	158.0

Fig.4.

Table No.4

Concentration of H-bentonite sol = 0.4 %
= 0.004 gms.per ml.

pH of clay-protein suspension = 5.5

Concentration of transfusion gelatin in gms. per 100 gms.clay.	Absolute viscosity of the mixture in centipoises.	Specific viscosity.	Viscosity number.
0.0	1.076	0.3440	86.0
6.0	1.121	0.4000	100.0
12.0	1.165	0.4560	114.0
20.0	1.204	0.5040	126.0
40.0	1.255	0.5680	142.0
50.0	1.316	0.6440	161.0

Fig.4.

Table No.5

Concentration of H-bentonite sol = 0.5 %
= 0.005 gms.per ml.

pH of clay-protein suspension = 5.5

Concentration of transfusion gelatin in gms. per 100 gms.clay.	Absolute viscosity of the mixture in centipoises.	Specific viscosity.	Viscosity number.
0.0	1.1770	0.470	94.0
6.0	1.2410	0.550	110.0
12.0	1.2811	0.600	120.0
20.0	1.3291	0.660	132.0
40.0	1.3932	0.740	148.0
50.0	1.4732	0.840	168.0

Fig.4.

Table No.6

Concentration of H-bentonite sol =0.15 %
=0.0015 gms.per ml.

pH of clay-protein suspension =4.5

Concentration of egg albumin in gms.per 100 gms.clay.	Absolute viscosity of the mixture in centipoises.	Specific viscosity.	Viscosity number.
0.0	0.8935	0.1162	77.50
2.0	0.9015	0.1260	84.00
6.0	0.9184	0.1470	98.00
8.0	0.9328	0.1660	111.00
10.0	0.9448	0.1800	120.05
12.0	0.9544	0.1920	128.00

Fig.5.

Table No. 7.

Concentration of H-bentonite sol = 0.25 %
= 0.0025 gms.per ml.

pH of clay-protein suspension = 4.5

Concentration of egg albumin in gms.per 100 gms. clay.	Absolute viscosity of the mixture in centipoises.	Specific viscosity.	Viscosity number.
0.0	0.9588	0.1975	79.00
2.0	0.9723	0.2150	86.00
6.0	1.0000	0.2500	100.05
8.0	1.0270	0.2837	113.50
10.0	1.0440	0.3050	122.00
12.0	1.0609	0.3250	130.00

Fig.5.

Table No.8

Concentration of H-bentonite sol = 0.35 %
= 0.0035 gms.per ml.

pH of clay-protein suspension = 4.5

Concentration of egg albumin in gms.per 100 gms.clay.	Absolute viscosity of the mixture in centipoises.	Specific viscosity.	Viscosity number.
0.0	1.0276	0.2835	81.0
2.0	1.0473	0.3080	88.0
6.0	1.0888	0.3605	103.0
8.0	1.1250	0.4060	116.0
10.0	1.1480	0.4340	124.0
12.0	1.1670	0.4580	131.0

Fig. 5.

Table No.9

Concentration of H-bentonite sol = 0.4 %

= 0.004 gms.per ml.

pH of the clay-protein suspension= 4.5

Concentration of egg albumin in gms.per 100 gms.clay.	Absolute viscosity in centipoises.	Specific viscosity.	Viscosity number.
0.0	1.0633	0.3280	32.0
2.0	1.0857	0.3560	39.0
6.0	1.1350	0.4180	104.5
8.0	1.1750	0.4680	117.0
10.0	1.2010	0.5000	125.0
12.0	1.222	0.5280	132.0

Fig.5.

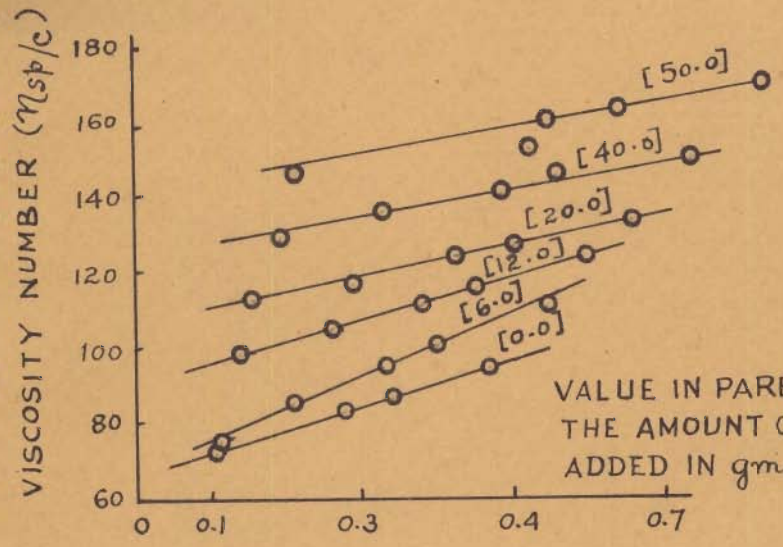


FIG. 2 SPECIFIC VISCOSITY (η_{sp})

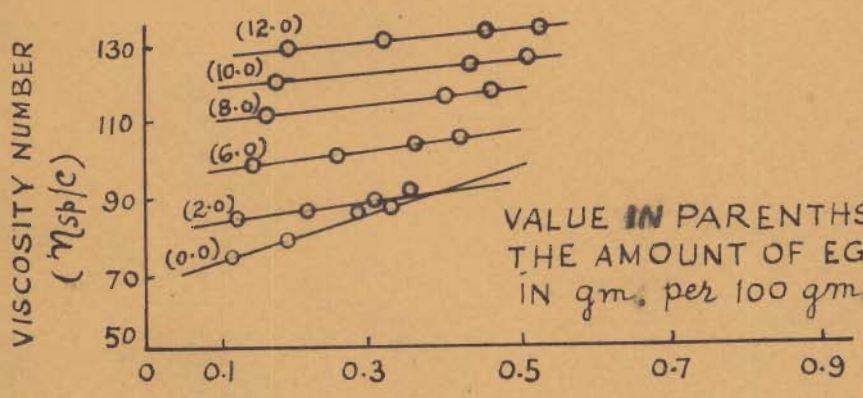


FIG. 3 SPECIFIC VISCOSITY (η_{sp})

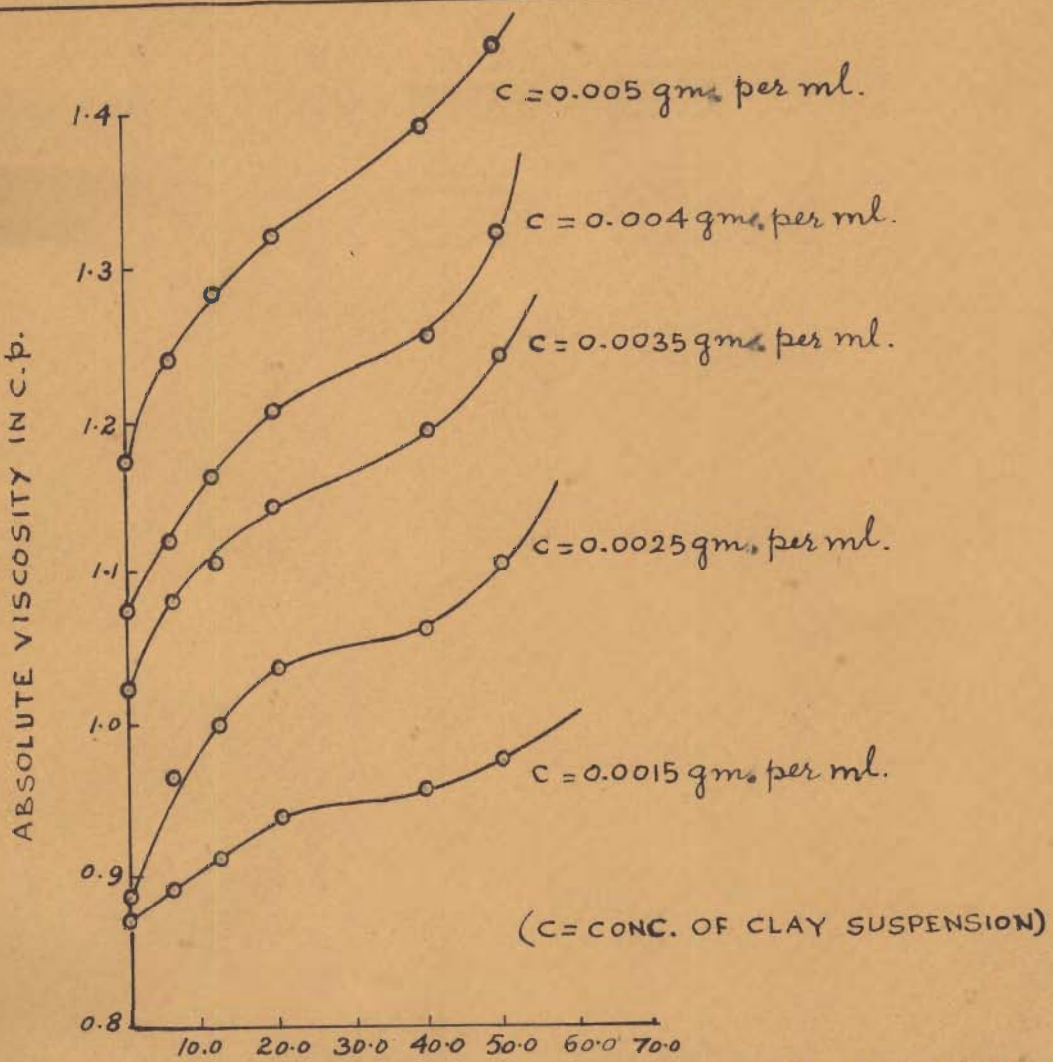


FIG. 4 CONCENTRATION OF TRANSFUSION GELATIN ADDED IN gms. per 100 gms. CLAY.

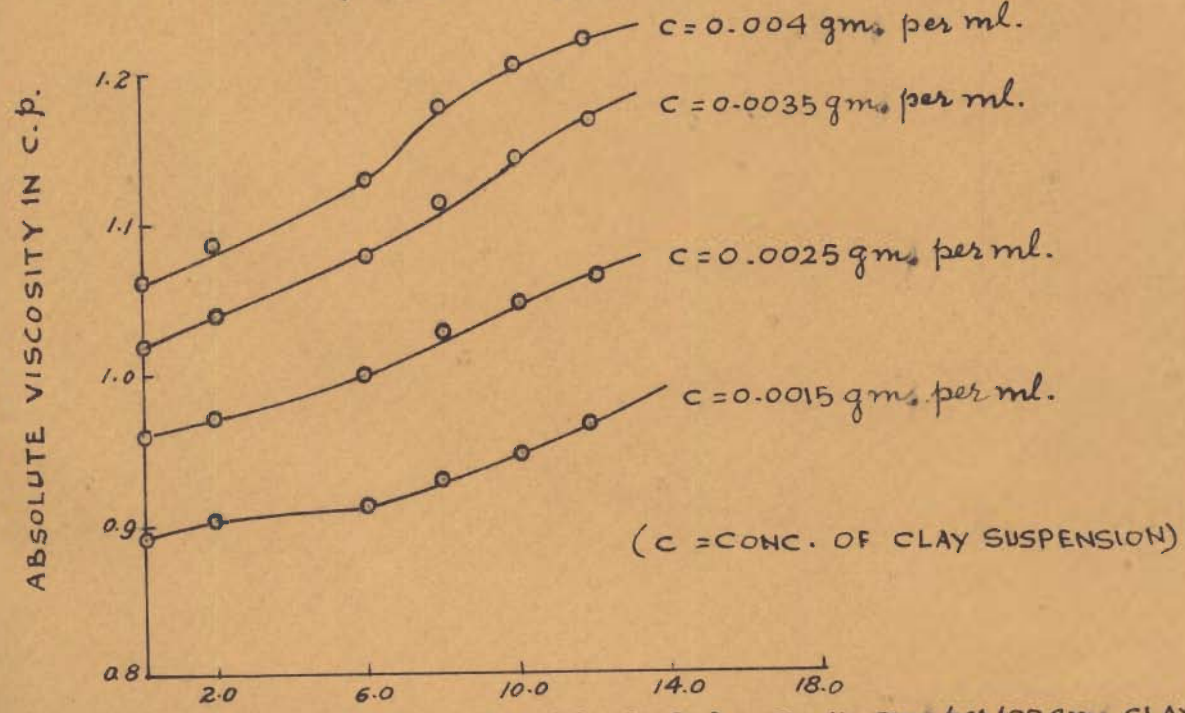


FIG. 5 CONCENTRATION OF EGG ELBUMIN IN gms. per 100 gms. CLAY.

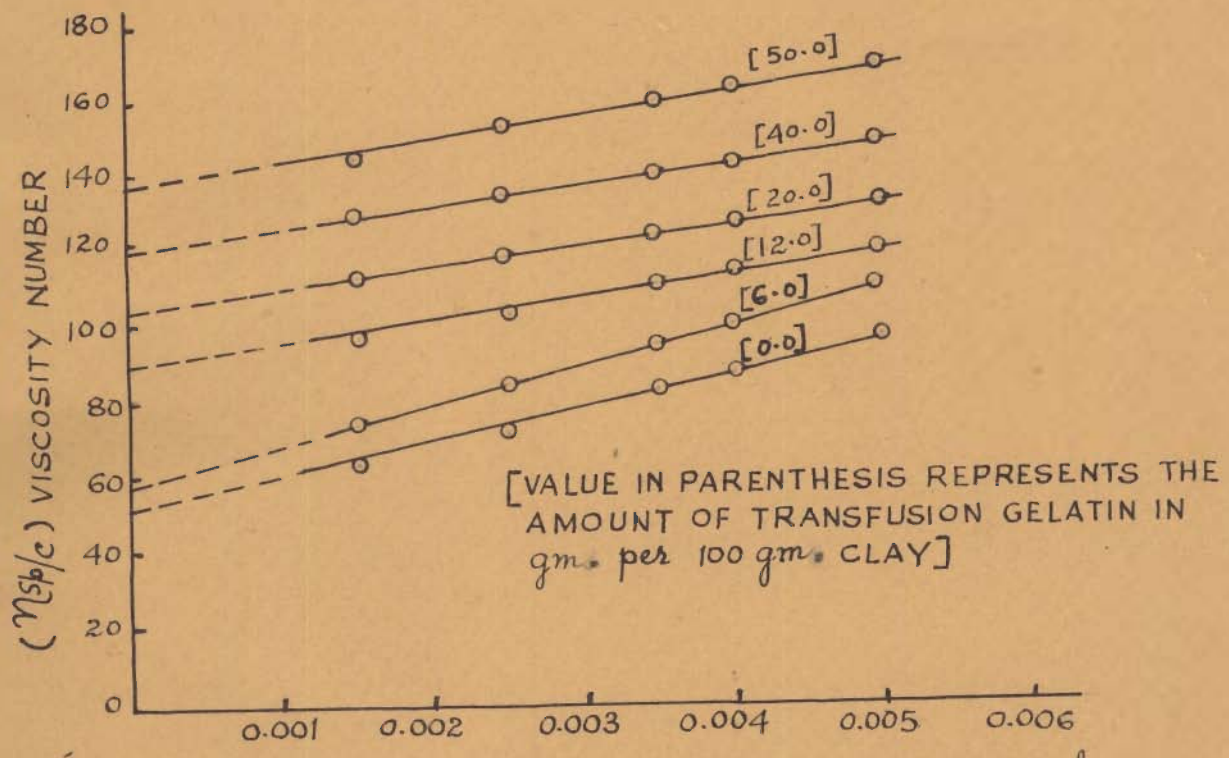

180
160
140
120
100
80
60
40
20
0
(ηsp/c) VISCOSITY NUMBER
[50.0]
[40.0]
[20.0]
[12.0]
[6.0]
[0.0]
[VALUE IN PARENTHESIS REPRESENTS THE AMOUNT OF TRANSFUSION GELATIN IN gm. per 100 gm. CLAY]
0.001
0.002
0.003
0.004
0.005
0.006
CONCENTRATION OF H-CLAY IN gm. per ml.

FIG. 6

Table No.10

Concentration of clay in gms.per ml. (c)	Specific viscosity η_{sp}	Viscosity number $\eta_{sp/c}$	Intrinsic viscosity (η)	Interaction index (k)
	(Transfusion gelatin 0.0 gms.per 100 gms.clay)			
0.0015	0.0906	60.4	52.0	1.9
0.0025	0.1055	70.2	-	-
0.0035	0.2810	80.3	-	-
0.0040	0.3440	86.0	-	-
0.0050	0.4700	94.0	-	-
	(Transfusion gelatin 6.0 gms.per 100 gms.clay)			
0.0015	0.1110	74.0	58.0	1.8
0.0025	0.2100	84.0	-	-
0.0035	0.3325	95.0	-	-
0.0040	0.4000	100.0	-	-
0.0050	0.5500	110.0	-	-
	(Transfusion gelatin 12.0 gms.per 100 gms.clay)			
0.0015	0.1470	98.0	90.0	0.58
0.0025	0.2600	104.0	-	-
0.0035	0.3850	110.0	-	-
0.0040	0.4560	114.0	-	-
0.0050	0.6000	120.0	-	-
	(Transfusion gelatin 20.0 gms.per 100 gms.clay)			
0.0015	0.1680	112.0	104.0	0.45
0.0025	0.2950	118.0	-	-
0.0035	0.4305	123.0	-	-
0.0040	0.5040	126.0	-	-
0.0050	0.6600	132.0	-	-
	(Transfusion gelatin 40.0 gms.per 100 gms.clay)			
0.0015	0.1920	128.0	120.0	0.34
0.0025	0.3350	134.0	-	-
0.0035	0.4900	140.0	-	-
0.0040	0.5680	142.0	-	-
0.0050	0.7400	148.0	-	-

Contd...

Concentration of clay in gms.per ml. (c)	Specific viscosity η_{sp}	Viscosity number $\eta_{sp/c}$	Intrinsic viscosity (η)	Interaction index (k)
(Transfusion gelatin 50.0 gms.per 100 gms.clay)				
0.0015	0.2160	144.0	136.0	0.27
0.0025	0.5320	152.0	-	-
0.0035	0.5530	158.0	-	-
0.0040	0.6440	161.0	-	-
0.0050	0.8400	168.0	-	-

Table No.11

Concentration of clay in gms.per ml. (c)	Specific viscosity (η_{sp})	Viscosity number ($\eta_{sp/c}$)	Intrinsic viscosity (η)	Interaction index. (k)
Egg albumin 0.0 gms.per 100 gms.clay.				
0.0015	0.1162	77.5	75.0	0.27
0.0025	0.1975	79.0	-	-
0.0035	0.2835	81.0	-	-
0.0040	0.3280	82.0	-	-
Egg albumin 2.0 gms.per 100 gms.clay.				
0.0015	0.1260	84.0	81.5	0.24
0.0025	0.2150	86.0	-	-
0.0035	0.3080	88.0	-	-
0.0040	0.3560	89.0	-	-
Egg albumin 6.0 gms.per 100 gms.clay.				
0.0015	0.1470	98.00	94.5	0.22
0.0025	0.2500	100.05	-	-
0.0035	0.3605	103.00	-	-
0.0040	0.4180	104.00	-	-
Egg albumin 8.0 gms.per 100 gms.clay.				
0.0015	0.1660	111.0	108.0	0.17
0.0025	0.2837	113.5	-	-
0.0035	0.4060	116.0	-	-
0.0040	0.4680	117.0	-	-
Egg albumin 10.0 gms.per 100 gms.clay.				
0.0015	0.1800	120.05	118.0	0.11
0.0025	0.3050	122.00	-	-
0.0035	0.4340	124.00	-	-
0.0040	0.5000	125.00	-	-
Egg albumin 12.0 gms.per 100 gms.clay.				
0.0015	0.1920	128.0	126.0	0.08
0.0025	0.3250	130.0	-	-
0.0035	0.4580	131.0	-	-
0.0040	0.5280	132.0	-	-

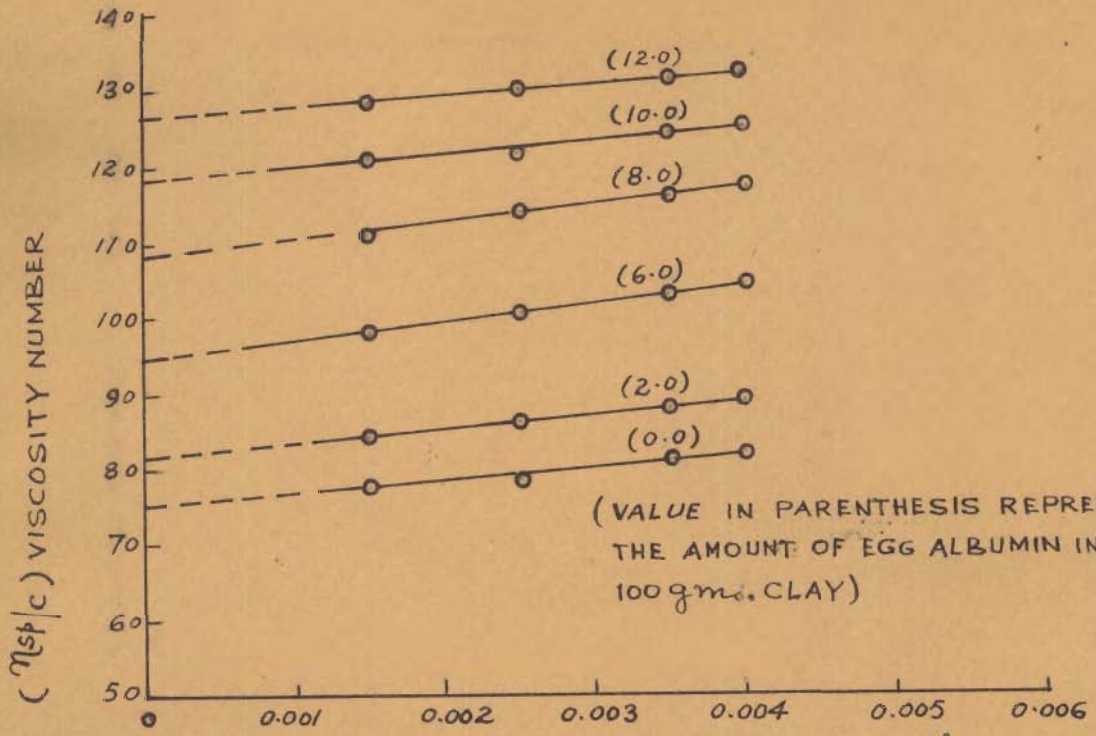


FIG. 7 CONCENTRATION OF H-CLAY IN gm. per ml.

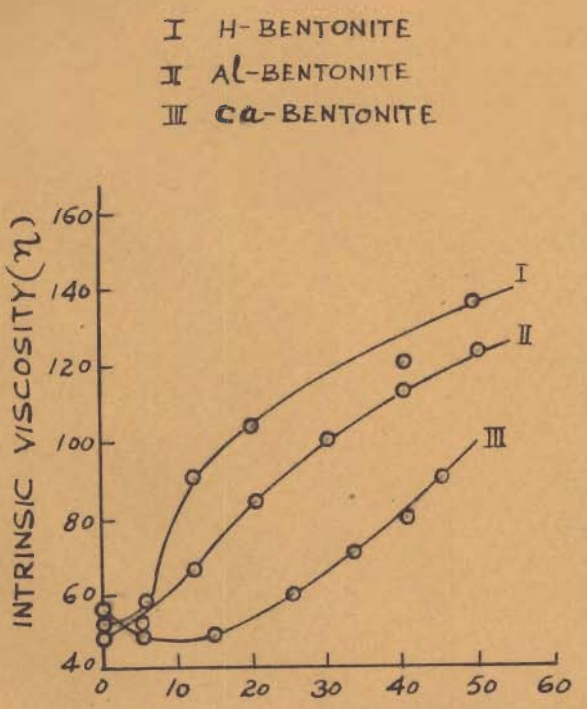


FIG. 8 AMOUNT OF TRANSFUSION GELATIN ADDED IN gm. per 100 gm. OF CLAY.

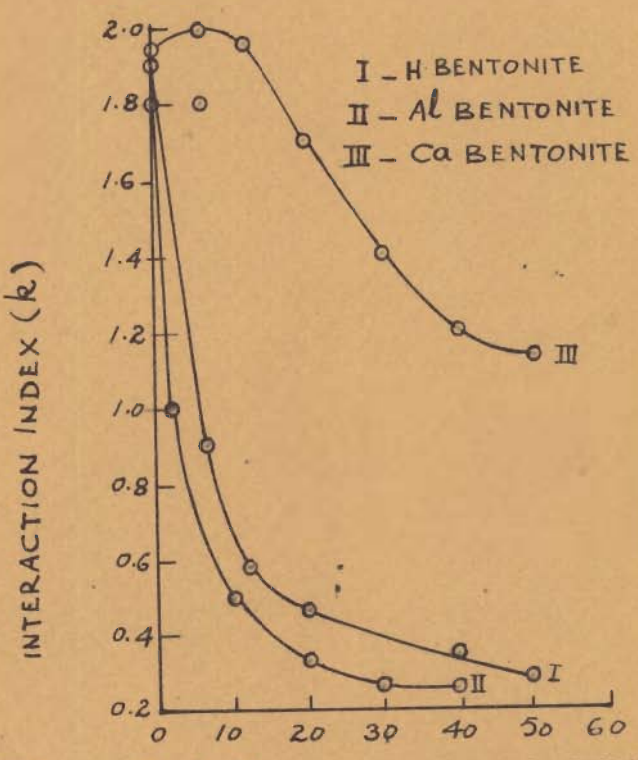


FIG. 9 AMOUNT OF TRANSFUSION GELATIN ADDED IN gm. per 100 gm. CLAY.

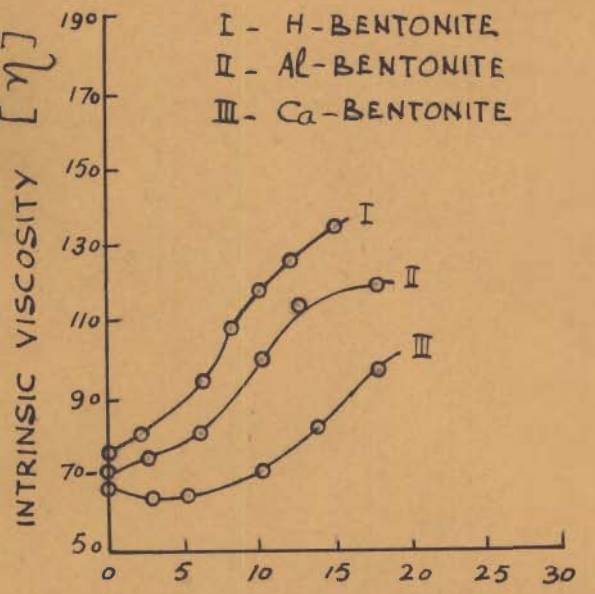


FIG. 10 AMOUNT OF EGG ALBUMIN ADDED IN gm. per 100 gms. OF CLAY

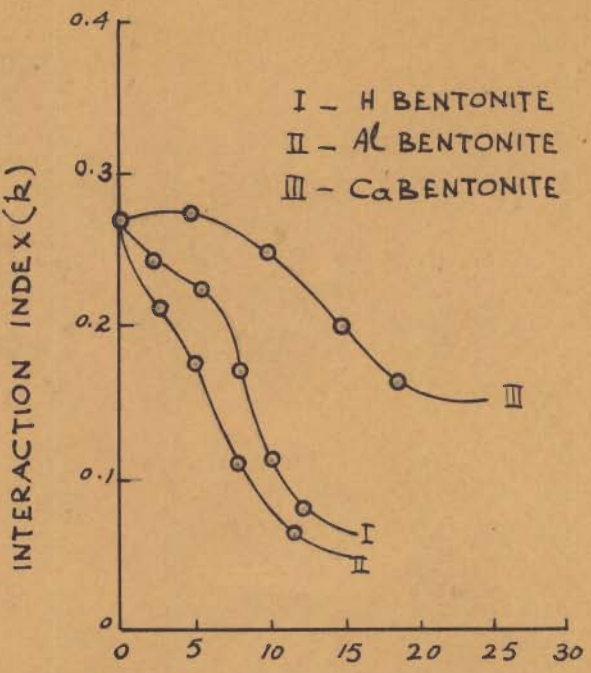


FIG. 11 AMOUNT OF EGG ALBUMIN ADDED IN gm. per 100 gm. OF CLAY.

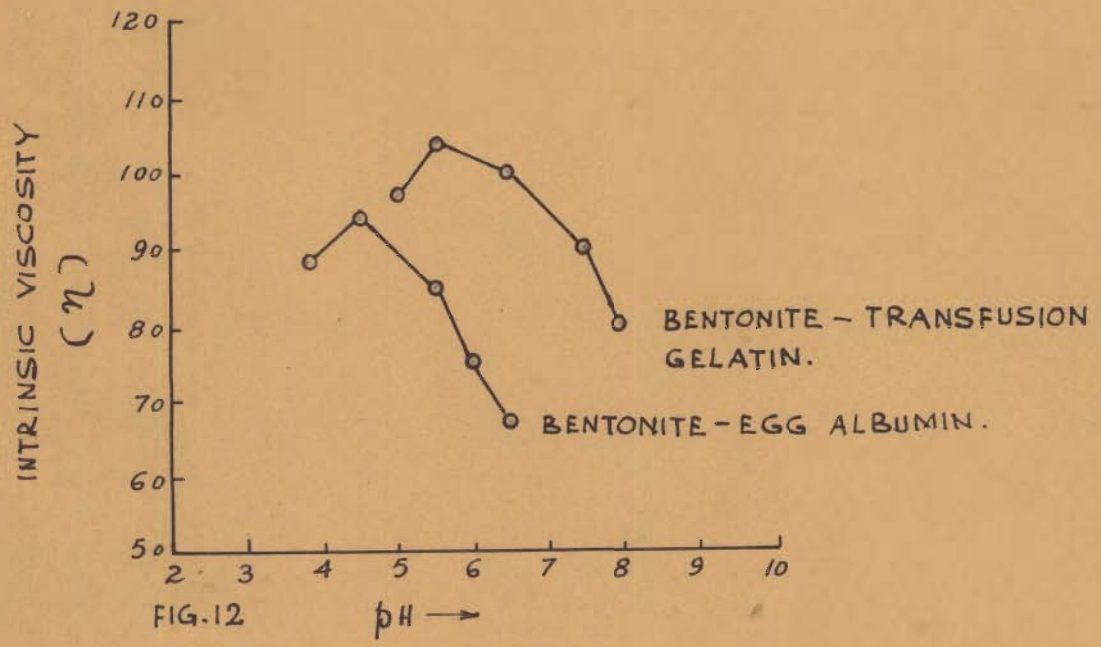


FIG. 12

RESULTS AND DISCUSSION

Before determining the influence of protein concentration on the viscosity of clay suspensions and evaluating various constants like intrinsic viscosity and interaction index it was considered necessary to know whether the suspensions under investigation exhibited a Newtonian flow or not. For this purpose the viscosity numbers were plotted against specific viscosity. Linear plots were obtained thereby indicating that our suspensions exhibited Newtonian flow. The results in the case of H-bentonite are shown in Figs.2 and 3.

Figs.4 and 5 show a plot of the viscosity values of H-bentonite with increasing concentration of transfusion gelatin and egg albumin. It is observed that the viscosity goes on increasing along with the addition of protein although the rise is more steep in the beginning in the case of transfusion gelatin than in the higher concentration range of protein. The initial decrease in viscosity (due to peptisation) on adding alkali, which is the main characteristic of all the minerals,is not observed here,instead a continuous rise occurs over the whole concentration range. These results indicate

flocculation of clay particles on adding proteins to the clay.

A better understanding of the nature of protein clay interaction could not be,however,obtained from the above data. In order to know this the intrinsic viscosity was determined in each case (Figs.6 and 7) and the values of interaction index were calculated by Schulz-Blaschke equation. The values of intrinsic viscosity and the interaction indices are given in Tables 10 and 11. These constants were then plotted against protein concentrations(Figs.8-11). A comparison of the values of the two viscometric constants in the case of H and Al clays would reveal that these constants are higher in the case of H-clay than Al clay. Since the intrinsic viscosity of a system is also a measure of their dis-symmetry it can be concluded that H-bentonite -protein complexes have higher dis-symmetry as compared to Al complex.

The difference in the interaction index values can be explained in terms of factors like electrical interaction,hydrodynamic interaction,extent of dis-symmetry etc.. Here again the H clays show a higher electrical and hydrodynamic interaction than Al clays.

It has been shown by Mehta[18] that the presence of a polyvalent cation on the clay surface leads to the

formation of a bond like clay-Al-OOC. Existence of such bonds can also be contemplated in the case of clay-protein interaction. In the pH range 4.5 and 5.5 an appreciable number of carboxyl groups will offer sites for reaction with the clay particles resulting in the formation of Al-OOC bonds with either of the two proteins. These results confirm the view point that variations in k are due to electrical factors because the formation of these bonds will always be accompanied by decrease in electrical interaction. Moreover there is a greater decrease in the value of k with Al complex than the corresponding H complex. This is obvious because the H bentonite would provide sites for the formation of Al-OOC bonds at the broken edges only with the result that the value of k will be higher in the case of H bentonite than Al bentonite. Moreover under these conditions H bentonite-protein particles would form larger units of higher dis-symmetry.

It naturally goes to show that H bentonite and protein particles would link to form larger units of higher dis-symmetry through the lattice Al present at broken edges extending the unit also in the direction of b axis along with the interplanar

adsorption.

In the case of Al bentonite and proteins an additional site exists at the flat surface apart from edges thus causing vertical face to face stacking of units to form blocky aggregates of reduced dissymmetry.

Effect of pH on intrinsic viscosity.

Fig.12 shows the effect of pH on the intrinsic viscosity of H bentonite protein complexes at concentrations 20.0 gm. of transfusion gelatin and 6.0 gm.of egg albumin per 100 gm.of clay. It is observed that the intrinsic viscosity is maximum at pH 4.5 in case of egg albumin and at pH 5.5 in case of transfusion gelatin. It decreases with an increase in pH showing thereby a lesser dissymmetry at higher pH values which may be a result of repulsion between clay particles and protein since both of them got negatively charged at high pH.

—

R_E_F_E_R_E_N_C_E_S

1. Kruyt,H.R.,Colloids.p.83 John Wiley and Sons.New York 1927.

2. Pauli,W.,Colloid Chemistry of Proteins. J.and A. Churchil Ltd.,London.

3. Marshall,C.E., J.Soc.Chem.Ind., 50,457,1931.

4. Einstein,A.,Ann Physik, 19,301,1906.

5. Smoluchowski,M., Kolloid Zeit, 18,190,1916.

6. Krawmer,E.O., Ind.Eng.Chem.30,1200,1938.

7. Schulz,G.V. and Blaschke,F., J.Prakt.Chem., 158,130, 1941.

8. Granquist,W.T., Clays and Clay Minerals 6,207,1959.

9. Vander Watt,H.,Ph.D.Thesis,Univ.of California.

10. Scarpa,Gazzeta. 40,271,1910.

11. Farrow., J.Ind.Chem.Soc., 101,347,1912.

12. Joshi., J.Ind.Chem.Soc., 10,323,1933.

13. Mehta,N.C.,Ph.D.Thesis.Univ.of California.

—

R_E_S_U_M_E

When the organic cations interact with clay minerals,other types of adsorption besides the exchange adsorption can exist and these cations may be held to the clay surface by Van der Waal forces also. It was observed that the exchange is quantitative with small organic molecules but big molecules might show a cover up effect. The amount of the organic substance adsorbed would also depend,on the pH of the medium,concentration of the adsorbate,and on whether the compound is taken as such or its aqueous suspension has been used. The existing literature provides little information on these points, especially in the case of big molecules. It was,therefore, considered worthwhile to carry out investigations in this direction.

The big molecules chosen for these studies were substances which themselves exist in the colloidal form, namely,dyes and proteins. The interaction of dyes with clays was studied by colorimetric and polarographic techniques while that of proteins was studied by viscometric and u.v. spectrophotometric methods. In the appendix a few data on adsorption studies by radioisotopic technique have been included.

A resume of the results obtained on the basis of the above mentioned studies is presented below:

Interactions of dyes with clays.

Clay dye reactions have mostly been qualitatively

used to identify clay minerals. However,these reactions are used to determine the base exchange capacity. Uniform values are not obtained. They differ from dye to dye. A critical study of the adsorption of three dyes existing in different states of aggregation was undertaken in order to explain this discrepancy. The dyes used were methylene blue,crystal violet and rhodamine 6G.

The adsorption studies give isotherm curves which are concave to the concentration axis in the case of all the three dyes. The data fits in well in the Langmuir's adsorption equation thereby indicating that multilayer films of the dye are not formed on the clay surface at least in the lower concentration range (1×10^{-5} M to 4×10^{-5} M).

The cation exchange capacity values calculated from dye adsorption are given below. The standard values of cation exchange capacity obtained from the ammonium acetate method are also listed for the sake of comparison.

c.e.c. values in m.e.per 100 gm.clay as obtained from:

	Methylene blue adsorption.	Crystal violet adsorption.	Rhodamine 6G adsorption.	Ammonium acetate method.
Bentonite	110	102	94	99
Illite	28	26.3	21.0	26
Kaolinite	4.3	6.3	4.3	6.3

The values of cation exchange capacity of bentonite and

illite obtained in the case of methylene blue are higher while those obtained from rhodamine 6G are lesser than the standard value. In the case of kaolinite both these dyes methylene blue and rhodamine 6G give lower values than the standard one. However most reasonable values are obtained in the case of crystal violet.

The difference in c.e.c. values can be explained in terms of area occupied by one dye molecule on the clay surface (Total surface area/Number of dye molecules adsorbed) and area per exchange site (Total surface area/Number of exchange cations). The surface area of clay minerals as determined by glycerol retention method is 750.4 m^2/gm. for bentonite, 94.00 m^2/gm. for illite and 18.12 m^2/gm. for kaolinite. The values are given below:

	Area per exchange site in A^{o2}	Area associated/methylene blue molecule in A^{o2}	Area associated per crystal violet molecule in A^{o2}	Area associated per rhodamine 6G molecule in A^{o2}
Bentonite	125.8	113.2	122.2	132.4
Illite	60.02	55.8	59.6	74.3
Kaolinite	47.4	56.7	47.1	53.7

Since the area occupied by one rhodamine 6G molecule is more than the area per exchange site, each dye molecule appears to cover up more than one exchange

site with the result that smaller c.e.c. values are obtained. On the other hand the higher c.e.c. values with methylene blue are due to the fact that more than one dye molecule attaches itself to one exchange site. This behaviour is however restricted to three layer clay minerals and not to kaolinite where the adsorption pattern is similar to rhodamine 6G.

Assuming the total dye adsorption capacity as equal to the sum of exchange adsorption and physical adsorption (due to Van der Wall forces) , the surface concentration of physical adsorption sites were calculated by dividing the total dye adsorbed by the exchange capacity. The values obtained in the case of bentonite and illite are 5.0 and 5.9 m.e. per cm^2. These values agree well with the data obtained for the adsorption of dyes on silica surface. There the adsorption is purely physical and the value is 5.2 which is not far from the calculated values given above.

It is further observed that the adsorption of dye increases with the increase in pH of the medium. This behaviour can only be explained if we assume that another double layer also exists in clay minerals besides the usual negative layer. This layer would be formed at the broken edges due to the disruption of -SiO tetrahedral

and AlO octahedral bonds. The clay would then behave as a hydrophobic colloid on the edge surface, the charge of whose double layer would be solely governed by potential determining ions. The double layer at the edge surface would therefore be positive in the acidic media due to adsorption of H ions or negative in the alkaline range due to adsorption of hydroxyl ions or the dissociation of SiONa into SiO^- and Na^+. Under these conditions a large amount of the cationic dye will be adsorbed in the alkaline medium whereas lesser amount would be adsorbed in the acidic medium due to electrostatic attraction and repulsion forces. Two distinct stages of adsorption 98 to 115 m.e. and 115 to 125 m.e. for bentonite and 24 to 31 and 31 to 34 m.e. for illite are realised in the pH range 2 to 10(Page 42).

An approximate idea of the amount of dye bound to the clay mineral was obtained by carrying out colorimetric studies with the dilute clay-dye complex suspensions. The calculations were made on the basis of Klotz's equation and assuming the formula of H-bentonite unit reacting with the dye as,

$$(Si_{3.91}Al_{0.09})\ (Al_{1.32}Fe_{0.13}Mg_{0.29})\ O_{10}(OH)_2$$

$$\downarrow$$

$$(H_{0.25})$$

with a weight of 356.46.

The following conclusions were arrived at on the basis of these studies (Page 75 to 78).

1. A fraction of the clay unit offers site for reaction with the dye.

2. Since Klotz's equation, which is applicable to macromolecular solutions has been applied it can be said that 100 to 110 clay units form the colloidal micelle which would interact with 1 mole of dye.

The dye-clay adsorption studies can be put to another analytical use namely, the estimation of the mineral content in a particular soil-sample. On carrying out adsorption experiments with clay samples containing varying amounts of powdered silica, it was found that the amount of dye adsorbed is linearly related to the percentage of mineral content in the sample (Page 43-46)

The clay-dye interaction was also investigated using polarographic technique. Methylene blue dye gave a well defined wave at pH 2.9, 4.9 and 9.2 with a $E_{1/2}$ as -0.02, -0.28 and -0.32 volts respectively. A prewave with $E_{1/2}$ as -0.01 volts was realised at a pH 4.9 only (Page 94). The normal reduction wave disappears below a concentration of 6×10^{-5} M and so the concentration maintained in these studies was 8×10^{-5} M. It was observed that the diffusion current of the dye was considerably reduced in presence of clay minerals. The $E_{1/2}$ however did not change. The diffusion current reduces

from 33 x 8 x 10^{-2} microamperes to 16 x 8 x 10^{-2} microamperes, 28.8 x 8 x10^{-2} microamperes and 31.6 x 8 x10^{-2} microamperes in presence of the equal amounts of bentonite, illite and kaolinite; so the order for the decrease in diffusion current is bentonite > illite > kaolinite.

Further a linear relationship has been observed between the decrease in wave height ($i_{do}-i_d$) and the amount of clay mineral present (vide Tables 1 to 7 Chapter III) and this can possibly be utilised for estimating the clay fraction of a soil sample. On taking the polarograms of methylene blue in presence of clay samples of varying cation-exchange capacity a linear relationship is observed between the decrease in the diffusion current and the cation exchange capacity of the mineral. Thus this technique can be used as a very rapid method of determining the c.e.c. of clay samples.

Clay-protein interactions.

Controversy exists about the state in which nitrogenous components are present in the soil. Although some amino acids have been identified in soil hydrolysates, there is very little evidence of the presence of proteins as such in clays. A postulate which has found favour in recent years, is that the protein forms a complex with the clay and in a few cases microbial

degradation of clay-protein complexes has been investigated. The available references,however,do not throw any light on the physico-chemical aspect of the reaction between these two substances. This problem was investigated firstly by carrying out adsorption studies and secondly by studying the flow properties of clay-protein complexes.

The isotherms obtained on the basis of adsorption studies do not follow either Langmuir or Freundlich adsorption equation (Pages 118,119). On the other hand the amount of protein adsorbed is so high as to cover up both the surface layers and interlamellar spaces. In the initial stages the adsorption is rapid,due to binding of the protein to the random sites on the clay surface. Like dyes here too the adsorption is physical as well as exchange with the only difference that the protein gets randomly bound.

Unlike dyes the pH of the medium would influence the charge of both the protein as well as clay. In the acidic medium,below the iso-electric-pH,the protein gets positively charged and above the iso-electric pH it will start acquiring negative charge becoming almost negative at high pH. The clay is negatively charged in fairly acidic medium but would acquire a positive charge due to adsorption of H ions at the broken edges in highly acidic medium. The adsorption of the protein will therefore be

small at low pH but would go on increasing with pH due to lesser positive charge on the edges. Maximum adsorption is however achieved when the protein molecule is uncharged and electrostatic attraction and repulsion forces are not at all operative. Above the iso-electric point the protein is negatively charged and the clay is predominantly negative. This would result in a decrease in adsorption above the iso-electric pH. As the pH is further increased the adsorption would continue to decrease and the protein which is now anionic may still contain some localised sites of positive charges and gets attached to the clay surface through these sites.

The role of the iso-electric point in the adsorption phenomenon is also evident from the desorption experiments. It is observed that the amounts of protein released at different pH are different, for instance, 42.5 % protein is released in the case of bentonite complex and 85 % in the case of illite complex when the reequilibration is carried out at a pH higher than the iso-electric-point. Such a high release go to show that the forces of repulsion are so strong that even the protein present at interlamellar sites is also released. On the other hand in the pH range below the iso-electric

point the desorbed protein is much smaller (3.5% in the case of bentonite complex and 7.7 % in the case of illite complex) thereby showing that the forces of repulsion exist between protein molecules only and are not strong enough to remove the protein from interlamellar spaces. This explanation finds support by the fact that the adsorption at pH below the iso-electric point increases in presence of an electrolyte like KCl whose chloride ions form an ionic atmosphere around the positively charged protein molecule thereby reducing its charge density and consequently increasing the protein adsorption.

Viscosity variations of protein-bentonite suspensions have been measured with a veiw to have an idea of particle-particle interaction. Viscosimetric constants like intrinsic viscosity and interaction index have been calculated using Schulz-Blaschke equation

$$\eta_{sp/c} = k\,(\eta)\,\eta_{sp} + (\eta)$$ where η_{sp} is specific viscosity, (η) is intrinsic viscosity, c is the concentration in gm. per ml. of the suspended particles and k is the interaction index. These constants have been calculated for H-bentonite, Al-bentonite and Ca-bentonite protein complexes (vide tables 10 and 11 Chapter V).

The intrinsic viscosity of the system is a measure

of dissymmetry and hydrodynamic interaction between the particles. It has been found that the intrinsic viscosity of H-clay protein complex is more than Al-clay-protein complex showing thereby a larger amount of dissymmetry in the case of H-complex than in the case of Al-complex. Likewise the value of k (interaction index) is higher in the case of H-complex showing a larger amount of electrical interaction in this clay complex in comparision to the Al-clay complex.

The large dissymmetry in the case of hydrogen clay protein complexes can be attributed to stacking through the Al ions present at the edges. This results in aggregation along the direction of b axis. In Al-bentonite the Al ions are also present in the flat surface with the result that vertical face to face stacking takes place. This results in the formation of aggregates of much reduced dissymmetry.

—

APPENDIX

Edges as the site for adsorption.

I_N_T_R_O_D_U_C_T_I_O_N

Clay suspensions move towards the positive electrode of the electrophoresis tube and are, therefore, considered to be negatively charged. The existence of this charge is attributed to isomorphic substitution in the flat layer surfaces. Recently the existence of another double layer of comparatively very small magnitude, carrying a positive charge, has been postulated[1]. Its existence has not been unambiguously established although the electromicrograph of kaolinite sols flocculated by negatively charged gold sol[2], adsorption of anions on the clay surface, possible reversal of charge by changing the medium from highly acidic to highly alkaline, go in support of such a postulate. Evidence on this point is also forthcoming on the dye adsorption and protein adsorption studies described in Chapter I and Chapter IV of this thesis. In spite of all these evidences the need of a more direct and subtle method for proving the existence of a positive double layer was felt.

The most straight forward proof for showing the existence of positive electrical double layer can be had from adsorption experiments. However, since the adsorption will be very small a far more sensitive method to register it has to be employed. It was

considered that a radioisotopic method using a suitable isotope as adsorbate could be of some value. In the following pages the results of the preliminary studies carried on H-bentonite using Cd^{115} as adsorbate are described.

—

E X P E R I M E N T A L

Reagents.

A suspension of H-bentonite (Standard Mineral from Wards) of known concentration was prepared by usual procedure.

A stock solution of cadmium sulphate (3 $CdSO_4.8H_2O$) was prepared in double distilled water.

Cd-115m (specific activity 5.5 mc/g) in the form of cadmium nitrate in dilute nitric acid solution was obtained from the Isotope Division, Bhabha Atomic Research Centre Bombay. The tracer was sufficiently diluted so as to give activity of the order of 3000 counts per min.

Solutions of NaoH and cetyl pyridinium bromide were used for flocculating the clay.

Apparatus.

A G.M. counter was employed for beta counting measurements. The slope of the G.M. tube employed for activity measurements was 0.065/100 V. and its length 150 volts. For all activity measurements, G.M. counter was operated at 1400 volts. A standard beta source Ra D-E was employed to check the response of the counter for day to day work.

Procedure.

Requisite amount of cadmium solution was taken

in a number of pyrex boiling tubes and 0.5 ml.of the diluted tracer solution was added in each case. The solutions were mechanically stirred for one hour to bring about complete isotopic exchange. Clay suspension was added to each tube so as to have an effective concentration 0.06%. Final volume was made upto 10 ml. The tubes were again stirred for 1 hour and left for 24 hours to attain equilibrium. The contents of each tube were centrifuged and 0.5 ml. of the supernatant liquid were with drawn and dried on stainless steel planchet under infra red lamp. The activity was counted for 5 minutes and three such counting measurements were done in each case. In order to evaluate total activity added a blank (containing no clay suspension) was also simultaneously run. Since the counting rate was small coincidence loss corrections were not applied.

The clay suspension was then flocculated by adding NaoH and cetyl pyridinium bromide (cationic surfactant) successively and pH was maintained at 6.0. Two more adsorption sets were run by the same procedure using this clay as adsorbent.

The observations are reported in Tables 1-3.

—

Table No.1

Adsorption of cadmium on H-bentonite at pH 3.2.

Concentration of H-bentonite = 0.06 %

Total volume in each tube = 10 ml.

BLANK

Observed counts per min.	Average counts per min.	Background counts per min.	Net counts per min. (A)
3841 3702 3255	3599	45	3554

Initial conc. of cadmium solution.	Observed counts per min.	Average counts per min.	Net counts per min. (A').
M/10000	900 920 922	914	869
M/8000	1670 1680 1692	1680	1635
M/5000	1804 1932 1939	1891	1846
M/2000	2825 2818 2800	2785	2740
M/1000	2725 2845 2836	2802	2757
M/500	3319 3417 3382	3372	3327

Table No.2

Adsorption of cadmium on H-bentonite flocculated by NaoH at pH 6.0

Concentration of H-bentonite = 0.06 %

Total volume in each tube = 10 ml.

BLANK

Observed counts per min.	Average counts per min.	Background counts per min.	Net counts per min. (A).
2962 2792 2890	2881	44	2837

Initial conc. of cadmium solution.	Observed counts per min.	Average counts per min.	Net counts per min. (A)
M/12000	1840 1920 1832	1864	1820
M/10000	1910 1950 2059	1973	1929
M/8000	1970 2032 2035	2029	1985
M/6000	2187 2120 2122	2143	2099
M/5000	2205 2273 2290	2259	2215

Table No.3

Adsorption of cadmium on H-bentonite flocculated by a cationic surfactant at pH 6.0.

Concentration of H-bentonite = 0.06 %

Total volume in each tube = 10 ml.

BLANK

Observed counts per min.	Average counts per min.	Back ground counts per min.	Net counts per min. (A).
2460 2520 2453	2478	42	2436

Initial conc. of cadmium solution.	Observed counts per min.	Average counts per min.	Net counts per min. (A').
M/12000	1900 1990 2104	1998	1956
M/10000	1907 2120 2090	2039	1997
M/8000	2097 2060 2137	2098	2056
M/6000	2160 2190 2076	2142	2100
M/5000	2205 2200 2150	2185	2143

RESULTS AND DISCUSSION

If a radioisotope is mixed with an inactive isotope of the same element, then because of their virtual identity in physical and chemical properties, the two isotopes will always remain in the same proportion in solution, that is, the specific activity (activity per unit wt.) will remain same, even if total amount of the isotope (inactive active both) is different.

Specific activity of Cd^{115m}.

Let the total activity present in 0.5 ml. of different solutions be A counter per min. (from blank).

Let total amount of Cd present in 0.5 ml. of different solutions before adsorption be W gm.

Specific activity = A/W counts per min./gm. of Cd.

Calculation of equilibrium concentration.

Let the amount of Cd present in 0.5 ml. after adsorption be W' gm. and its activity be A' counts per min.

Specific activity = A'/W'

Since the specific activities should be same

$$\frac{A}{W} = \frac{A'}{W'}$$

$$\text{or } W' = \frac{A' \times W}{A} \text{ gm.}$$

Therefore the amount of cadmium present in total volume (10 ml.) after adsorption,

$$= \frac{A' \times W \times 10}{A \times 0.5}$$

$$= \frac{A'}{A} \times W \times 20 \text{ gm.}$$

Table No.4

Amount of cadmium adsorbed at pH 3.2

Initial conc. of cadmium ($x10^{-3}$) gm.	Concentration of cadmium in equilibrium ($x10^{-3}$) gm.	Amount adsorbed ($x10^{-3}$) gm.	Amount adsorbed in m.e./100 gm. clay.
0.346	0.0830	0.263	65.0
0.429	0.1470	0.282	75.0
0.683	0.3480	0.335	83.0
1.690	1.3030	0.388	100.0
3.379	2.9590	0.420	112.0
6.740	6.400	0.340	100.0

Table No.5

Amount of cadmium adsorbed at pH 6.0 (NaoH)

Initial conc.of cadmium ($x10^{-3}$) gm.	Concentration of cadmium in equilibrium ($x10^{-3}$) gm.	Amount adsorbed ($x10^{-3}$) gm.	Amount adsorbed in m.e./100 gm. clay.
0.290	0.185	0.105	25.0
0.346	0.235	0.111	31.0
0.430	0.301	0.129	35.0
0.571	0.422	0.149	42.0
0.683	0.532	0.151	44.0

Table No.6

Amount of cadmium adsorbed at pH 6.0(surfactant).

Initial conc. of cadmium ($x10^{-3}$) gm.	Concentration of cadmium in equilibrium ($x10^{-3}$) gm.	Amount adsorbed ($x10^{-3}$) gm.	Amount adsorbed in m.e./ 100 gm.clay.
0.290	0.232	0.058	15.0
0.346	0.283	0.063	17.0
0.430	0.361	0.069	20.0
0.571	0.491	0.080	23.0
0.683	0.599	0.084	23.4

On the basis of these calculations the amounts of cadmium adsorbed in various cases has been calculated and tabulated in Tables 4-6.

It can be seen from the values of Table 4,that the amount of cadmium adsorbed increases with the concentration of cadium solution and the maximum value (112 m.e./100 gm.clay) shows that a complete exchange of H ions with cadmium takes place and basically this would be through exchange adsorption.

Now on repeating the adsorption experiments with the flocculated clay the adsorption of Cd^{115} would take place on broken edges only. However,the amount adsorbed will be very much less. The results given in Tables 5 and 6 show that very little cadmium is adsorbed in the case when the negative charge on clay surface has been neutralised by the cationic surfactant or NaoH.

Further it is observed that the amount of cadmium adsorbed when the clay has been flocculated with NaoH is more than in the case when it has been flocculated by the surfactant.This may be due to the greater coverage of the surfactant cation on the neutralised clay leaving smaller number of sites for adsorption.

—

R E F E R E N C E S

1. van Olphen,H., Clay Colloid Chemistry,Interscience Publishers New York p. 89-93.

2. Thiessen,P.A.,Z.Elektrochem., 48,675,1942.

—

LIST OF PAPERS PUBLISHED AND COMMUNICATED

1. On the constitution of metal hydrous oxide sols as determined by electrometric titrations.
   Published in the Proc.of the National Academy of Sciences,India,36,299,1966.
2. Studies on the interactions of proteins with kaolinites.
   In press.Bulletin Chemical Society of Japan.
3. Viscosity variations of H-bentonite suspensions in presence of Surface Active Compounds.
   In press. ''Clay Minerals'' Journal U.K.
4. Adsorption of cationic dyes on three layer minerals.
   In press.Indian Journal of Chemistry.

—