

Surface Interaction of Montmorillonite Clay (mClays) and Cation Ex-changed Montmorillonite Clay in L-valine Amino acid

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Abstract - The adsorption of L-valine was systematically studied on the surface of montmorillonite and cation (Ca²⁺, Mg²⁺ and Ni²⁺) exchanged montmorillonites clay. Adsorption studies have been done in aqueous media as a function of pH (9.2 - 3.0) in the concentration range of (9.0 x 10⁻⁵M - 1.0 x 10⁻⁵M) and temperature of (20 - 25°C). The process of adsorption was monitored systematically by measuring the absorbance (optical density) of L-valine solution at the wavelength (λ_{max}) 216 nm. Percent binding of the amino acid is calculated in terms of their absorbance. The maximum amount of L-valine was adsorbed at neutral pH (7.0±0.01) and temperature 25°C. Langmuir type of adsorption isotherm was determined from the various concentration ranges. It was found that calcium-exchanged montmorillonite relatively is 'better adsorbent in all cases studied of L-valine. The adsorption parameters (K_L and X_m) have been calculated using L-isotherms as the plots linear with the correlation coefficient (r²) 0.9. A thermodynamically favourable exothermic reaction is observed, based on the values of Gibbs free energy (ΔG^0).

Thus, the present study suggests the possible importance of mineral clays in selection, concentration and condensation of important biomolecules during the chemical evolution of the prebiotic earth. This process could conceivably aid in the formation of polymers in fluctuating drying- wetting environments.

Index Terms - L-valine, Montmorillonite clay (mClays), Cation-exchanged clays, Adsorption.

1. INTRODUCTION

According to the theory put forward by Oparin [1-2] the abiotic synthesis of organic molecules and complex molecular compounds is one the most

important stages in the prebiotic earth. The increasing complexity of organic compounds thus formed was due to a variety of environmental factors one of which it seems, may have been the interaction of such significant biomolecules with the mineral surfaces, clays and rocks composing the outer stone shell of the primordial earth. Specifically, clay minerals and their changed aluminosilicate layered structure were envisioned as having the appropriate characteristics to harbour precursor organic molecules for the synthesis of significant biomolecules. In the nineteenth century, Bernal [3] suggested that clay minerals could have played a significant role in the prebiotic formation of biomolecules essential to life, has lined the pathway for series of experiments on the adsorption of different organic molecules. It has been emphasized that clays and metal oxides most widely spread over the earth's surfaces, at the site of seashores and the bottom of the sea before and during prebiotic and organic evolution, presumably after the formation of early hydrosphere [4-6]. During the period of chemical evolution, adsorption could have played an essential role in the concentration of reactant molecules and the protection of reaction products against hydrolysis. The involvement of clays and other inorganic minerals in adsorption and catalytic reactions of biomolecules in molecular and chemical evolution was first proposed by Bernal [7].

Few earlier workers [8-10] suggested that the clay minerals such as montmorillonite and kaolinite, which were common on earth during and before prebiotic evolution, could have been a suitable catalyst for concentrating bio-monomers on their surfaces.

Lawless and Levi [11] suggested that the clay minerals not only facilitate condensation of amino acids but also protect the resulting peptides against hydrolysis. Bujdak et al. [12] suggested that the role of clay and minerals was mainly in the chain – elongation of oligopeptides rather than the formation of peptides from amino acids. Hedges and Hare [13] have investigated the adsorption of amino acids on clay and Cu-exchanged clays whereas, Fripiat et al. [14] has reported the mechanism of amino acids adsorption through zwitterionic association. Kobayashi and Ponnampuruma [15] have proposed the presence of biologically essential transition metals in the primitive sea. Kamaluddin and co-workers [16-17] have been reported the interaction of ribose nucleotides with metal ferrocyanides and discussed in relevance in chemical evolution. Among the solid surfaces, montmorillonite clay has been reported to work as a condensing agent and multicatalytic system in the reactions of prebiotic significance [18]. Nagy et al. [19] systematically studied the adsorption of valine on the surface of cation exchanged montmorillonites by stability and surface complexion model.

It is plausible to believe that under fluctuating drying-wetting abiotic environment, amino acids, peptides and other organic compounds might have been trapped by montmorillonite, metal ion exchanged montmorillonite through adsorption and gave rise to biopolymers. Although, experiments concerning the adsorption of amino acid and their short peptides on clay surface under periodic conditions have been carried out in our laboratory [20-22] and elsewhere [23-25]. Therefore, it was considered of interest to study the explore the adsorption of L-valine in an aqueous environment in presence and absence of montmorillonite with or without coordinated divalent cations (Ca,2+ Mg2+ and Ni2+) for the preservation of significant bio-monomers which constitute the primary matrix of a living organism.

2.MATERIALS AND METHODS

Calcium (II) chloride, magnesium (II) chloride, nickel (II) chloride were obtained from BDH Co., USA. All chemicals were of analytical grade and used without further purification. Solutions were prepared in triply distilled water. Montmorillonite was purchased from E. Merck Chemical Company Mumbai and purified by the sedimentation of water and the purity of, <2µm

samples were tested by X-ray diffraction measurement. Homoionic clays (Ca, Mg, Ni) were prepared by saturation method [26] with 50ml of the 1M concentration of each metal chloride (CaCl₂, MgCl₂, NiCl₂). Excess of the salts was leached out by washing and centrifugation procedures were separated until chloride ion was no longer detected by AgNO₃ [27]. Finally, divalent cation exchanged forms thus obtained were dried under a vacuum at room temperature. The dried product was grounded and sieved to 125µm particle size.

The adsorption of amino acids on montmorillonite and cation exchanged montmorillonites in aqueous medium was studied as a function of pH, temperature and concentration of adsorbate. To obtain saturation point by adding an appropriate buffer to the amino acid containing montmorillonite / Ca / Mg / Ni incorporated montmorillonite keeping in mind, that the buffer should be very poor ligand so that suitable complex formation with clay could be avoided. Buffers used were 0.2M potassium chloride and 0.2M hydrochloric acid for pH1.0-2.0, 0.2M acetic acid and 0.2M sodium acetate for pH 3.0-8.0, 0.2M borax and 0.2M hydrochloric acid for pH 9.0 and 0.2M borax and 0.2 M sodium hydroxide for pH10.0 the pH of the solution was verified using a pH meter (Agronic digital-511). This was also verified by conductivity measurement, as there is no change in the inflection point by titration buffer against the salt solution.

10ml buffered amino acid solution was added into conical flasks containing 50mg of montmorillonite and cation exchanged montmorillonites and stirred mechanically for 15 min and allowed to stand at room temperature for 18hrs. Similar sets of varying concentrations of amino acid with various adsorbents were incubated over a temperature range 20-250C for varying periods and pH to find out the conditions of maximum adsorption. After about 18 hrs of incubation the suspension was centrifuged in Teflon-coated tubes at 3500rpm for 30min. The supernatant was decanted and the amino acid concentration of L-valine was measured spectrophotometrically at 216 nm at neutral pH7.0. Before the experiments the laboratory glass vessels were sterilized at 1600C for 30 min. Then covered with aluminium foil (Bacteria in the air can eat amino acid). The amount of amino acid adsorbed was calculated based on the difference in concentrations before and after the adsorption of each investigation. It was observed that additional

incubation time did not appreciably change the degree of adsorption. The absorbance of L-valine ($pK_{aCOOH}=2.3$, $pK_{bNH_3}=9.6$) was measured spectrophotometrically at corresponding λ_{max} 216nm. The equilibrium concentration and the amount adsorbed were used to obtain the adsorption isotherm (Indirect method). In the direct method absorption spectra were recorded using Spectrophotometer (Jasco-V550) for determining the quantity of amino acid adsorbed on clays which were further verified by desorption of amino acid from clay amino acid complexes formed after equilibrium and repeatedly washed by resuspending the centrifuged pellets in 1ml of double distilled water until no more amino acid was detected in the suspension (spot test, colour with ninhydrin reagent at 570 nm) and then the optical density was measured [20].

3.RESULT AND DISCUSSION

The adsorption of L-valine on montmorillonite clay and cation (Ca, Mg, Ni) exchanged clays in aqueous medium was studied the pH 3.0-9.2 temperature range 20-250C and concentration range $9.0 \times 10^{-5}M$ - $1.0 \times 10^{-5}M$ of the adsorbate to find out the conditions of maximum adsorption. Moreover, the main aim is to study the adsorption of L-valine on montmorillonite clay with or without divalent cation (Ca, Mg, Ni) as an adsorbent for preferential adsorption under prebiotic conditions believed to have existed near the lithosphere-hydrosphere boundary of the primitive sea or on the bottom of the sea. The study of adsorption as a function of temperature showed maximum adsorption at 250C. Preliminary studies have shown that the amount of L-valine adsorbed on montmorillonite clay separately was depends on the pH of the solution, and it was maximum at pH 7.0. Subsequent investigations were therefore performed at pH 7.0 and temperature 250C (the constant temperature was maintained by an ultrathermostate) in all case studied related to the adsorption of L-valine. The effects of pH on the adsorption of L-valine on montmorillonite clay at a temperature at 250C are shown in Fig.1 and results recorded in Table1.

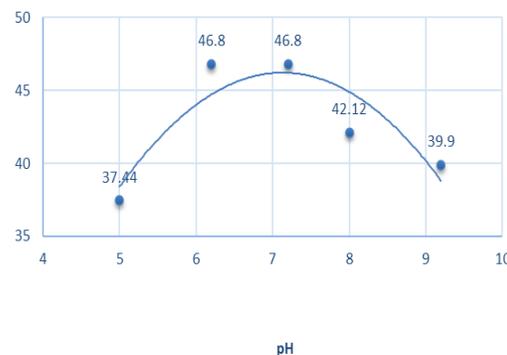


Fig.1. Adsorption of L-valine on montmorillonite clay as a function of pH, temp. 250C

Adsorption studies of L-valine as a function of pH indicating that initially upto pH7.0 the adsorption was increased and decreases after that as shown in Fig.1, this trend appears in all cases studied of L-valine. Therefore, neutral pH was chosen to run the maximum adsorption of L-valine. Therefore, neutral (pH=7.0) was chosen to run the maximum adsorption of L-valine in a wide concentration range because the neutral pH is physiologically significant and most of the redox reactions in biologically systems take place in a neutral medium. Though, the inherent zwitterionic nature of amino acid also provides some insight into the pH effects. Under acidic conditions amino acid occurs as cations however as the pH of the solution tend towards to the neutrality the zwitterionic form exists. Note that the L-valine has the isoelectronic point value of 6.0. The more substantial adsorption of amino acid observed at pH above the isoelectric point (>6.0) indicating that the adsorption occurs in the form of anions [28]. The higher adsorption of L-valine on clay and cation exchanged clays could be due to interactions of amino acid with replaceable divalent cations: Ca (II), Mg (II) and Ni (II) present in the clays. At higher pH (>7.0) considerable decrease in adsorption of L-valine on montmorillonite with or without cation exchanged clay was observed. This may be because at higher pH, coordination of available hydroxide with divalent cations (viz, Ca, Mg and Ni) becomes competitive with that of adsorbate molecules. Effects of pH on the adsorption of L-valine studies showed that L-valine is adsorbed an anionic form on adsorbents. It may be due to the presence of the amino group, a carboxylic group and a hydrophobic isopropyl group, which acts as a site for interaction with the clay surface. The percent binding was calculated with

$$\% \text{ Binding} = (C_{bf} - C_{af}) / C_{bf} \times 100$$

Where, C_{bf} and C_{af} are concentration before and after adsorption, respectively. The value of maximum uptake of L-valine was observed on montmorillonite and cation exchanged clays are in the following trend: $M\text{-Ca}^{2+} > M\text{-Ni}^{2+} > M\text{-Mg}^{2+} > M\text{-Clay}$

The adsorption isotherms C_{eq} (equilibrium adsorbate concentration) as a function of concentration, i.e., the adsorption of L-valine on clay with or without cation exchanged clays exhibit a linear relationship. At lower concentration of amino acid, the adsorption increases rapidly however at higher concentration saturation appeared rendering to more adsorption. The asymptotic nature of adsorption isotherm was shown in Fig. 2 and suggested Langmuir type of adsorption or monolayer formation. Langmuir plots exhibit the amount of amino acid (L-valine) adsorbed as a function of their equilibrium concentration. The adsorption of amino acid on adsorbents follows the Langmuir equation [29-30].

$$\frac{C_{eq}}{X_e} = \frac{C_{eq}}{X_m} + \frac{1}{K_L X_m}$$

Where, C_{eq} is the equilibrium concentration of the amino acid in solution, X_e the amount (mg) of solute adsorbed per gram weight of adsorbent and X_m is the amount (mg) of solute required per gram weight of adsorbent for complete monolayer formation. K_L a constant related to enthalpy (ΔH) of adsorption ($K_L \propto e^{-\Delta H/RT}$) the parameter K_L reflects the steepness of the approach to saturation, more precisely; the K_L value is the reciprocal of concentration at which half of the saturation of the adsorbent is attained. “ K_L ” is a constant, which is a function of adsorption energy.

Table 01

Percent binding and Langmuir constants of adsorption of L-valine on MClays with or without cations at a temperature of 25°C

L-valine (Ip=6.0)	Adsorbents	%Binding	X_m (m $g g^{-1}$)	K_L (l $m g^{-1}$)	$r^2(L)$
	M-Clay	23.33	49.14	-3633.50	0.99
	M-Ca	27.80	58.49	-1245.47	1.00
	M-Mg	25.55	53.18	-3489.06	0.99
	M-Ni	26.70	56.15	-369.46	0.99

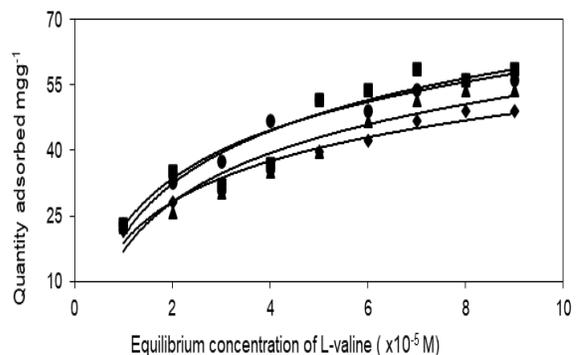


Fig.2. Adsorption isotherm of L-valine on montmorillonite in presence and absence of different cations

◆ Clay ■ Ca²⁺ ▲ Mg²⁺ ● Ni²⁺

The appropriate Langmuir adsorption parameters X_m and K_L were calculated from the slope and the intercept obtained from the graph of C_{eq}/X_e versus C_{eq} . X_e can be calculated asymptotically from the Fig.3 on extrapolating the adsorption curve towards Y-axis when saturation phenomenon occurs. It was observed that the adsorption trend (% binding and Langmuir constants) of adsorbate adsorbed on montmorillonite clay (M) with or without cations largely depend on the nature of adsorbate as well as an adsorbent.

Results of adsorption of L-valine revealed that the addition of divalent cations such as Ca^{2+} , Ni^{2+} and Mg^{2+} increase the adsorption throughout the entire concentration range of isotherm. This shows that the increase in ionic strength of clay increases the attractive force between the amino acids and a clay surface. The role of inorganic cations in the adsorption of amino acids on clay may be accounted for the neutralization of negatively charged surface of montmorillonite clay (aluminosilicate) by intercalation of divalent cations in between. The divalent metal cations act as a bridge or mediator cations in between the two negative charges and thus due to stronger electrostatic forces of attraction the complex becomes more suitable adsorbent in the aqueous environment of primitive seabed or seashore. In some case, the K_L value, which is characteristic of measure of energy, associated with adsorption show the negative intercept and thus the adsorption process is facilitated with the release of energy i.e. exothermic reaction.

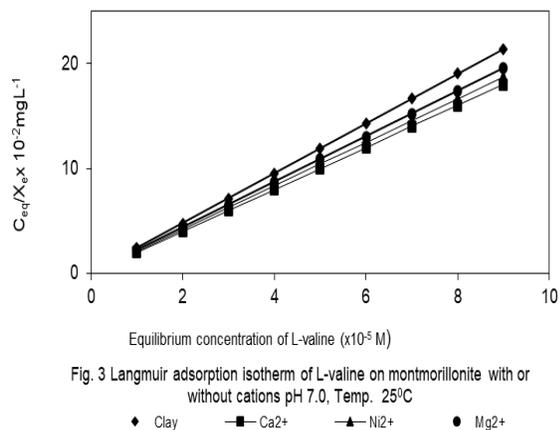


Fig. 3 Langmuir adsorption isotherm of L-valine on montmorillonite with or without cations pH 7.0, Temp. 25°C

The above results and facts were further supported by the thermodynamic free energy (ΔG^0) considerations, calculated, by the following equations [20, 31].

$$\Delta G^0 = -RT \ln K^0 \dots\dots\dots (i)$$

$$\ln \frac{K_{T2}}{K_{T1}} = \frac{-\Delta H^0}{R} \left[\frac{1}{T_2} - \frac{1}{T_1} \right] \dots\dots\dots (ii)$$

Where, K_0 is the thermodynamic equilibrium constants calculated from the slope of linear Langmuir adsorption curves plotted C_{eq}/X_e in Y-axis and equilibrium concentration C_{eq} in X-axis R is the gas constant and T is absolute temperature. With the equilibrium constant K_0 the changes in standard Gibbs free energy (ΔG^0) of adsorption of L-valine were calculated in the range of 1.33 to - 1.75 kJ mol⁻¹ at 25°C. The negative values of ΔG^0 for adsorption of amino acid on montmorillonite clay with or without cations (Ca^{2+} , Mg^{2+} , Ni^{2+}) suggested a thermodynamically favourable exothermic reaction. All the calculated values are summarized in Table 02

Table 02

Equilibrium constants (K_0) and free energies (ΔG^0) for L-valine adsorbed on mClays with or without divalent cations at 25°C

L-valine (Ip=6.0)	Adsorbents	lnK ₀	ΔG^0 (kJmol ⁻¹)
	M-Clay	2.03	-1.75
	M-Ca	1.71	-1.33
	M-Mg	1.78	-1.73
	M-Ni	1.86	-1.54

Observations, the clay montmorillonite provides a higher number of adsorption sites per mass unit than the other clays and its cation exchange capacity (CEC) is 60-150 meq/g, is attributed to their large areas of the external surface, accessible to hydrated cations [32-

33]. This shows that the added divalent metal cations either first gets involved in complex formation with L-valine or then gets adsorbed on clay surfaces resulting in the lower value of K_L . The function of the exchangeable cation seemed to be paramount in the different interactions, including direct coordination of polar groups (carboxyl, carbonyl, amino) to the exchangeable cation through a water bridge. Also, the protonated amino (group NH_3^+) is an excellent hydrogen bond donor [34] and it can form a hydrogen bond with the structural oxygen of siloxane surfaces [35]. The carboxylate groups of valinate were apparently bound to the hydrated Ca, Mg, Ni by water bridge through H-bonding in an outer sphere complex.

4. CONCLUSIONS

The adsorption of L-valine on montmorillonite with or without metal cations substitution is maximum at neutral pH and 25°C, these values are of physiological important as they also correspond to the ideal conditions for various biochemical reactions in living systems.

The adsorption of L-valine on all the four forms of montmorillonite follows Langmuir type adsorption, which indicates monolayer formation of adsorbate (L-valine) on the clay surface. The percent binding and the X_m values recorded in Table 01 revealed that the effect of metal cations incorporated clays was relatively more significant for adsorption of L-valine. Result reported in this communication show that Ca^{2+} - exchanged montmorillonite clay has better adsorption properties as compared to Ni^{2+} , Mg^{2+} and montmorillonite without cations. The thermodynamics studies of montmorillonite - amino acid complexes have shown that the equilibrium constant K_0 , the changes in negative values of standard Gibbs free energy (ΔG^0) of adsorption of L-valine as montmorillonite in presence and absence of different cations a thermodynamically favourable exothermic reactions in the forward direction. In a primeval sea experiencing a fluctuating environment (wetting-drying cycle) montmorillonite and cation exchanged clays could have acted as an active surface for concentrating biomolecules. Further investigations on adsorption of L-aspartic acid, nitrogenous bases and sugars on montmorillonite clay with or without divalent cations may be considered based on those above.

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