SOIL HUMIC AND FULVIC ACIDS INTERACTION WITH CLAY-MINERAL SURFACES

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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DOCTOR OF PHILOSOPHY

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ANCA-MARIA TUGULEA

-abstract-

In natural environments, soil organic matter is intimately associated with mineral constituents of soils, mainly clays, producing a mineral-organic complex whose properties differ substantially from those of the component materials. The surface area of the organic phase attached to the mineral surface and the accessibility of the functional groups or hydrophobic domains implicated in the sorption of cations or organic pollutants have a considerable influence on the behaviour and fate of chemical contaminants in the environment.

An AFM (Atomic Force Microscopy) procedure for investigating the morphology of humic materials deposited on mica was developed. The AFM procedure was used to investigate the surface complexes formed by interaction between well-characterized soil humic fractions (IHSS Standard Soil HA/FA) and a model clay-surface (the muscovite surface) in aqueous solution under various pH, humic concentration and ionic-strength conditions. The degree of coverage of the mineral surface, the time scale and the relative importance of sorption/precipitation processes were studied. For the first time, a method (AFM) that can distinguish between precipitation-sedimentation and true adsorption is now available.

The AFM procedure was then applied to the study of complexes between the same soil humic fractions and a well-ordered kaolinite (KGa-1). The images obtained by AFM were compared with images of the same complex obtained by SEM. The AFM images were very similar to the SEM images, with a higher degree of resolution for the AFM images.

In a complementary experiment, the carbon content and $\delta\,^{13}C$ in clay/humic complexes, obtained from IHSS Standard Soil HA/FA and Source Clays, was determined for a wide range of humic concentrations.

Humic fractionation during interaction with clay surfaces has been predicted theoretically, but very little direct evidence was available until now. Indications of humic fractionation during the surface interaction were observed in AFM images. $\delta\,^{13}C$ data determined for the complexes of IHSS Standard Soil Humic Acid/Fulvic Acid with four Source Clays clearly demonstrate that a fractionation of the humic material occurs during sorption of the humic material on the clay minerals.

CHAPTER 1. INTRODUCTION

1.1. Clay-humic complexes

Many organic compounds, both simple and polymeric, form complexes with clay minerals (Theng, 1979). Humic substances are the most important class of organic materials in the environment in terms of both quantity and ubiquity. Although quantitative estimations differ widely, depending on the underlying assumptions, one aspect is beyond any doubt: the amount of organic carbon contained in humic substances far exceeds the amount of carbon contained in living organisms (Stevenson, 1982; Davies and Ghabbour, 1998). It comes as no surprise that humic substances were among the first organic compounds to be described as capable of bonding to clay minerals. As early as 1874, Schloesing observed that clays posses a tendency to unite with humates in soils (after Theng, 1979). More than 100 years later, soil scientists believe that "the most relevant studies of the nature and properties of humus-mineral complexes would use the clay-size fraction of soils" (Hayes and Himes, 1986). According to modern data, between 52% and 98 % of the organic carbon in soils is associated with clays (Stevenson, 1982).

1.2. Relevance of clay-humic complexes to soil and sediment-based processes

In soils and sediments, mineral and organic components are intimately associated, producing a mineral-organic complex whose properties differ substantially from those of the component materials. The interaction of humic substances with clays has a major influence on the physical, chemical and biological properties of sediments and soils.

Humus-mineral complexes are of great importance for pedogenesis (McKeague et al., 1986), the formation and stabilization of soil aggregates (Hayes and Himes, 1986; Emerson et al., 1986; Caillier and Visser, 1988), the turnover rates of soil organic-carbon (Martin and Haider, 1986), and soil enzymatic activity (Burns, 1986). Complexes between clays and humic fractions play a key role in soil processes involving metal cations (Hayes and Himes, 1986, Fairhurst et al., 1995; Huang and Yang, 1995; Takahashi et al., 1995), organic cations (Hayes and Himes, 1986), hydrophilic and hydrophobic organic compounds (Hayes and Himes, 1986; Margeson et al., 1989; Chester et al., 1989; Barriuso et al., 1991; DiToro et al., 1991; Rebhun et al., 1992; Pignatello et al., 1993; Pusino et al., 1994; Senseman et al., 1995; Celis et al., 1997), and ultimately determine the distribution of these compounds in soils and natural waters.

1.3. Surface interaction of clay-humic complexes with environmental contaminants

Adsorption of humic molecules on clay minerals provides an active organic surface for exchange with cations from solution and for sorption of organic compounds, giving clay-humic complexes an important role in the transport and deactivation of inorganic and organic contaminants. "There is a commonly held point of view that all environmental surfaces are either organic in nature or are mineral surfaces coated with organic, usually humic, substances" (Akim et al., 1998).

Experimental data suggest that interaction between humic substances and clay particles influences the conformation and sorptive properties of the humic acids. The orientation and configuration of humic molecules on mineral surfaces may affect the surface area of the organic phase and the accessibility of its functional groups or hydrophobic domains implicated in the sorption of cations or organic pollutants (Murphy et al., 1990; Murphy et al., 1994). Therefore, with regard to surface interactions, the aggregate properties of the humic-clay complex have a considerable influence on the behavior and fate of chemical contaminants in the environment (Akim et al., 1998).

1.4. Bioavailability and transport of xenobiotics in soils and sediments: a function of clay-humic complexes structure

The importance of sorption of organic compounds and heavy metals on soil components has long been recognized, from both an agricultural and an environmental perspective: sorption modifies all transport properties of the sorbed material. Sorption to soil organic matter (SOM) retards solute transport in ground water, but sorption on water-soluble SOM (WSSOM) can lead to unexpected contamination by runoff or lateral transport, or even preferential-flow phenomena in soils. Sorption on dissolved organic matter (DOM) can lead to underestimation of the total concentration of the contaminant as determined by traditional extraction procedures, and, although it is largely believed that the associated compounds are not bioavailable, they may become bioavailable with changes in some environmental parameters. As a consequence, sorption of contaminants to humic substances should be accounted for in establishing risk criteria for crop rotation, ground- and surface-water contamination, and sediment toxicity (Di Toro et al., 1991). At the same time, sorption of organic compounds to humic materials is expected to significantly affect procedures used in soil amendment or soil and groundwater remediation.

In natural environments, soil organic-matter is associated with mineral constituents of soils, mainly clays. Understanding the behavior of clay-humic complexes is relevant to both agricultural and environmental studies. Appropriate models for the behavior of organic and inorganic contaminants in the environment are important for the design of proper remediation technologies for contaminated sites, and for prediction of residue persistence and bioavailability in soils and sediments.

1.5. Understanding clay-humic associations: the complex character of humic mixtures and clay surfaces

It seems ironic that ubiquitous clay-organic complexes, which play important roles in natural processes, are the least well-characterized. This situation is primarily due to problems in defining humic fractions and understanding the structure and physico-chemical characteristics of these materials (Theng, 1979; McKeague et al, 1986). Humic fractions are very complex mixtures of naturally occurring organic compounds <u>which are operationally defined</u>. Clay minerals are better characterized than humic fractions, but the details of their reactive surfaces are less well-known. The surface interaction between these two types of materials is a complex process for which the relative importance of the most relevant parametres (e.g. the pH, the concentration of cations and the state of hydration of surfaces) is not well understood.

1.6. Research objectives

The objective of this work was to study the interaction between soil humic fractions and clay-mineral surfaces in aqueous solutions.

(1) The degree of coverage of the mineral surface and the thickness of the humic layer covering the mineral surface under various environmental conditions were investigated, in an attempt to improve our understanding of the sorptive properties of clay-humic complexes.

(2) The time scale and the relative importance of sorption/precipitation processes were studied. This is the first time such a discrimination has been attempted, mainly because it was believed imposible to achieve by classical adsorption-isotherm studies (Tombacz et al., 1988). A detailed understanding of the interaction processes between soil humic fractions and clay minerals will add new dimensions to our knowledge of soil microscopic structure, soil formation processes, carbon sequestration processes and soil sorptive properties and will enable us to develop more generally applicable models with improved predictive capabilities.

(3) Direct proof for humic fractionation during interaction with clay surfaces was also sought. The fractionation process has been predicted to occur theoretically, but very little direct evidence was available until now. Understanding the fractionation of organic material during clay-humic complex formation is essential to our understanding of soil formation and carbon-sequestration processes.

1.7. Thesis overview

Chapter 2 describes the properties of the precursors of clay-humic complexes which are relevant to complex formation, as well as the difficulties encountered in the study of clay–humic complexes.

Chapter 3 describes materials and methods used in the present research.

Chapter 4 describes the development of an AFM procedure for the investigation of humic materials. The procedure developed is then used to investigate the morphology of the surface complex formed by interaction between well-characterized soil humic fractions and a model clay-surface (the muscovite surface) in aqueous solution. Various pH, humic concentration and ionic-strength conditions are used. The degree of coverage of the mineral surface by the humic layer, the thickness of the organic layer, indications of humic fractionation during the surface interaction are determined.

Chapter 5 describes the use of the AFM procedure developed to study the kinetics of interaction between the soil humic fractions and the model clay-surface in order to determine the time scale and the relative importance of the processes involved in the surface interaction.

Chapter 6 describes the use of the AFM to study the complexes between the same soil humic fractions and a well-ordered kaolinite (KGa-1). The images obtained by AFM are then compared with images of the same complex obtained by SEM.

Chapter 7 discusses the C content of model clay-humic complexes obtained in batch experiments using well-characterized humic fractions and clays, for a range of humic concentrations.

Chapter 8 discusses the ^{13}C abundance variation in the model clay-humic complexes obtained in batch experiments using well-characterized humic fractions and clays.

Chapter 9 describes my conclusions and suggestions for future work.

CHAPTER 2. CLAY SURFACES AND HUMIC SUBSTANCES

2.1. Clay-surface chemistry

2.1.1. Clay minerals in soils

Clay minerals are generally defined as fine-grained silicate minerals characterized by a particle size less than 2 μm in diameter (beyond the limit of resolution of optical microscopy) (Velde, 1992). The small particle size of clay minerals is important in soils and sediments because the high surface-area makes them powerful adsorbents (Mortland, 1986). Most clays in natural environments have a phyllosilicate (sheet-silicate) structure. Other minerals which can be present in the clay-size fraction of soils include zeolites, quartz and oxide-hydroxides. Clay mineral groups of major importance in soils are montmorillonite, illite, kaolinite and vermicullite (Stevenson, 1982). The clay-size fraction of volcanic soils is composed mainly of highly disordered or non-crystalline hydrous aluminosilicates collectively named allophane. In some instances, a gel-like material of fine tubes, called imogolite, occurs in soils in conjunction with allophane (Theng, 1979). However, most geologists think of phyllosilicate minerals when they speak of "clay minerals" (Velde, 1992). The high surface area, due to the small size of the constant particles, and the cation-exchange capacity due to the negative charge of the surfaces, account for the high influence of the clay fraction on the physical and chemical properties of soils and sediments (in great disproportion to their concentration) (Mortland, 1986).

2.1.2. The structure of clay minerals.

The structure of clay minerals consists of layers containing one or two sheets of linked silicon-oxygen tetrahedra, one sheet of octahedrally coordinated cations (mainly Al^{3+}, Mg^{2+}, Fe^{2+}), associated H_2O and OH groups, and alkali and alkaline-earth cations. $(Si, Al)O_4$ aluminosilicate tetrahedra share oxygen atoms to form a sheet of tetrahedra. Octahedrally coordinated cations (Al^{3+}, Mg^{2+}, $Fe^{2+,}$ Fe^{3+}, Zn^{2+}, Ti^{4+}, Cr^{3+} and Mn^{2+}) are linked by shared oxygen atoms to form a sheet of octahedra. The cations in each octahedral sheet must have six positive charges for electroneutrality, leading to two types of occupancy: three divalent ions ($3\ R^{2+}$) [trioctahedral] or two trivalent ions ($2R^{3+}$) and one vacancy [dioctahedral] (Fig. 2.1).

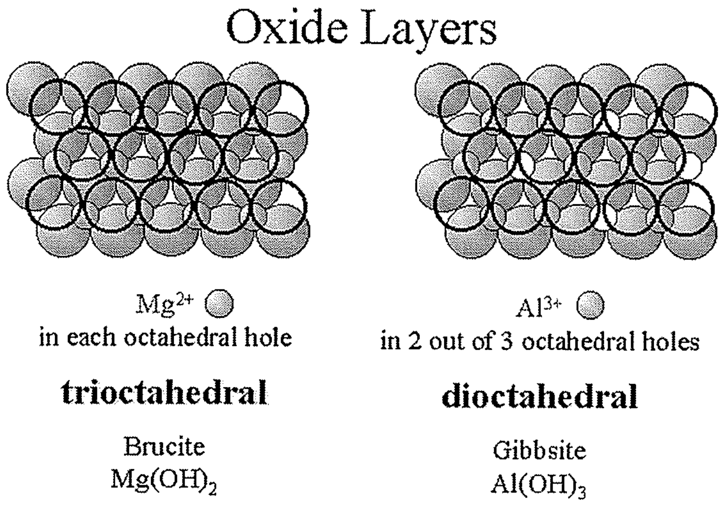


Figure 2.1. Cation distribution in octahedral sheets (www.unb.ca/courses)

The sheets of tetrahedra and octahedra may link together in two ways: (1) one sheet of tetrahedra (t) links to one sheet of octahedra (o) to form a t-o (1:1) layer; (2) a sheet of octahedra is sandwiched between two sheets of tetrahedra to form a t-o-t (2:1) layer (Fig.2.2).

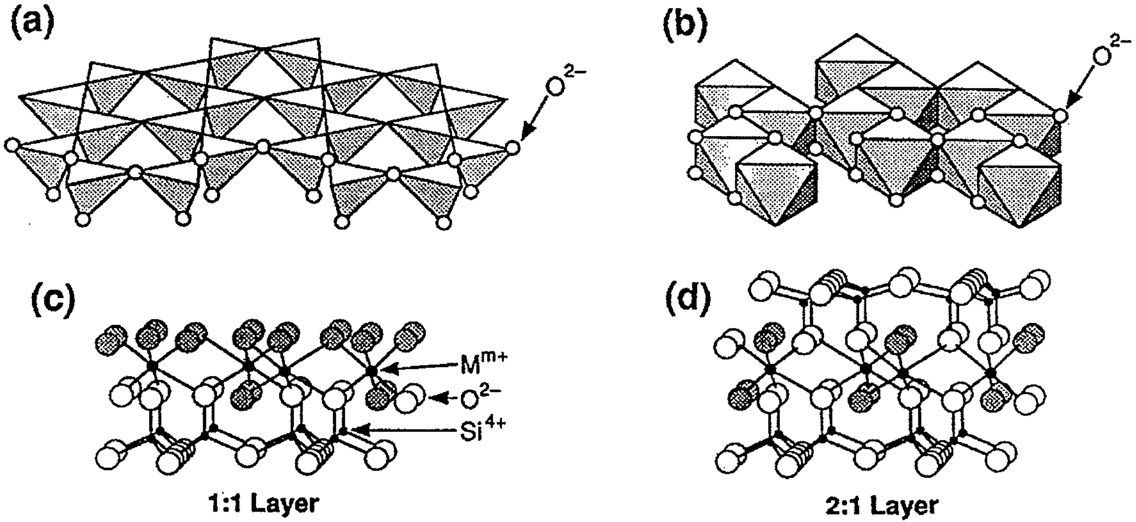


Figure 2.2. Clay structure. (a) tetrahedral sheet; (b) octahedral sheet; (c) 1:1 clay structure; (d) 2:1 clay structure. (Davies and Kent, 1990).

The (001) repeat distance is the distance between equivalent layers of basal oxygens or oxygen-hydroxyls in the clay structure, and is used to identify and characterize clay minerals using X-ray diffraction (Moore and Reynolds, 1989).

Adsorbed water occurs on the surface of clays (also called surface water) at defect sites or at sites of broken bonds on the silicate surface structure. Adsorbed water is present in small quantities, and is proportional to the surface area of the clays.

2.1.3. Adsorption on clay-mineral surfaces.

Clay minerals are important adsorbents in many systems of interest in aqueous geochemistry. Kaolinite and minerals of the smectite, vermiculite and illite groups are especially important because they often occur as extremely small particles with high surface area, and they are widespread.

Adsorption can be defined using the concept of relative surface excess (Davis and Kent, 1990). For the accumulation of the solute, *i,* at the mineral-water interface, the relative surface excess (or adsorption density) of solute, Γ_i, is defined as

$$\Gamma_i = n_i/A$$

where n_i is the number of moles of surface excess of solute *i* per unit mass of the mineral, and A is the specific surface area of the mineral. This definition assumes that the interaction between the solute and the mineral occurs only on the surface of the mineral. By contrast, the more general term *sorption* is used to describe more complex processes, which include surface precipitation and/or diffusion of solutes into porous material.

Experimental sorption data for clay-mineral surfaces have been traditionally described using partition coefficients or adsorption isotherms (graphs representing equilibrium concentrations of the solute on the sorbent versus equilibrium concentrations of the solute in solution, at a constant temperature). For small molecules, the shape of the adsorption isotherm and the initial slope of the curve are considered diagnostic of the mechanism underlying the adsorption process. Giles et al. (1974) described four types of adsorption of solutes to solids based on the specific shape of the adsorption isotherms of the system: S (describing the shape of the curve), L (for "Langmuir"), H (for "high affinity") and C (for "constant partition) (Fig. 2.3).

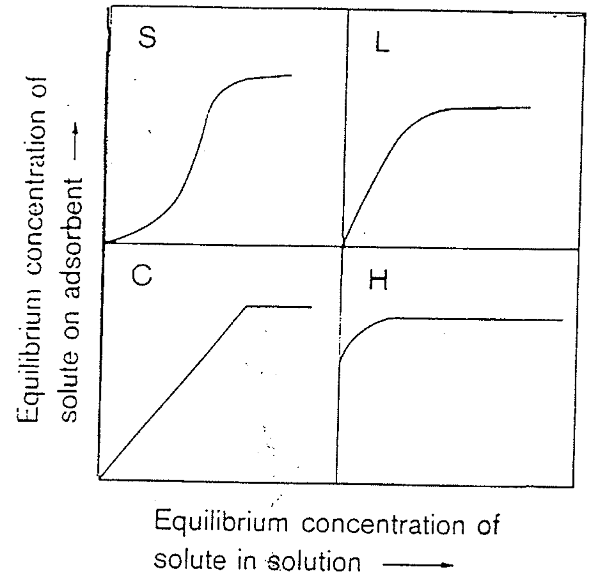


Figure 2.3. Types of adsorption curves (Giles et al., 1974).

For polymeric solutes however, determination of adsorption isotherms can only provide limited information about the structure of the adsorbed layer. The interpretation of the adsorption isotherms in such cases is difficult because the adsorption process is strongly influenced by polymer conformation (Theng, 1979).

2.1.4. Surface-complexation theory.

A modern approach, *surface complexation theory,* which describes adsorption in terms of complex-formation reactions between surface functional groups and solute molecules, seems to be particularly useful in describing sorption on clay-mineral surfaces because it can be coupled with appropriate aqueous-speciation models to describe adsorption equilibria in aqueous geochemical systems. The nature of the surface functional groups controls the stoichiometry of the adsorption reactions, and the variation in adsorption with the chemical composition of the solution (Davis and Kent, 1990).

2.1.4.1. Surface-complexation sites on 1:1 clay minerals: kaolinite and halloysite.

Clay minerals, hydrous-oxide minerals and humic materials have proton-bearing surface functional-groups, and consequently, adsorption on those solids is pH dependent. In solution, most kaolinites carry a pH-independent net-negative surface charge that is attributed mainly to isomorphous substitution within the structure (Bolland et al., 1976). The degree of cation substitution in kaolinite is very low and kaolinite has very low permanent charge.

The principal surface-complexation sites (the reactive surface functional groups) for kaolinite are the silanols, aluminols and Lewis acid-sites along the edge of the sheets.

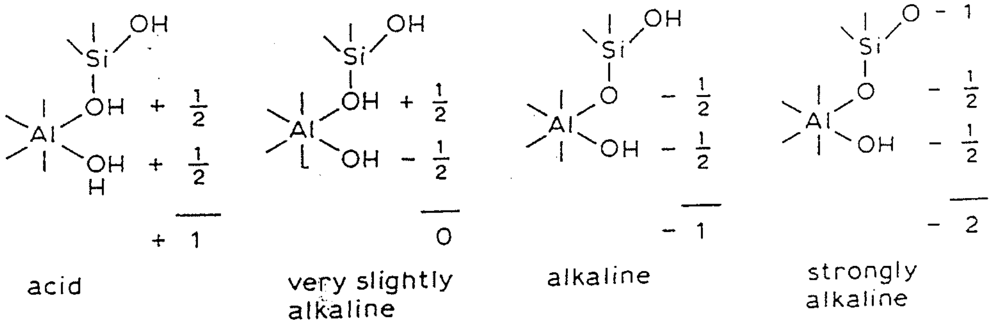
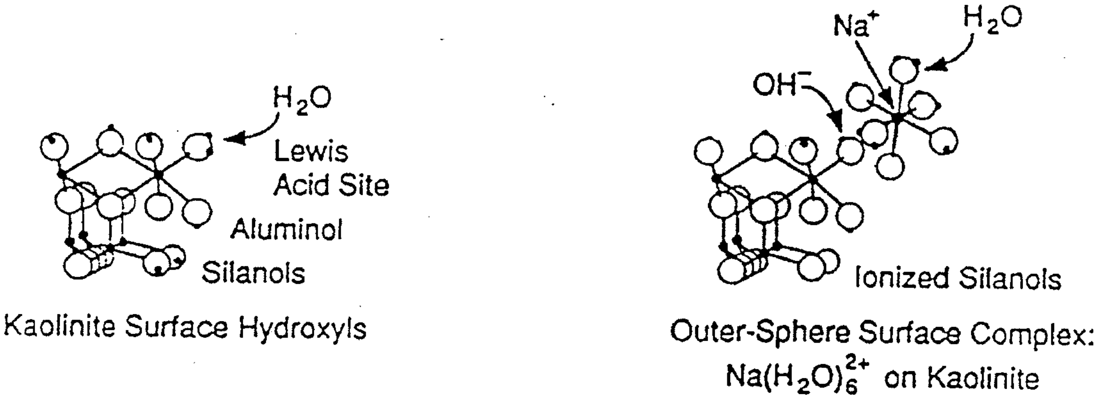


Figure 2.4. Functional groups along the edges of kaolinite sheets (after Theng, 1979)

Fig. 2.4. illustrates the changes in the electrical charge of functional groups as a function of pH.

A water molecule can be bound at a Lewis-acid site, generating a third type of OH group at the kaolinite surface (Fig. 2.5a). Outer-sphere surface-complexes can be formed between cations and singly ionized water molecules attached at a Lewis acid site (Fig. 2.5 b).



Kaolinite Surface Hydroxyls

Outer-Sphere Surface Complex: $Na(H_2O)_6^{2+}$ on Kaolinite

Figure 2.5. Complexation on kaolinite surface hydroxyls (after Davies and Kent, 1990)

These three types of functional groups are proton donors and can complex only cations. Only the aluminols are proton acceptors that can complex anions. A number of methods can be used to determine the densities of proton-donor and proton-acceptor sites.

For kaolinite, the edge surface occupies about 10-20 % of the total surface area (of about 15-40 m^2/g) (Theng, 1979).

Hydrated halloysite and some kaolinites may intercalate organic species such as alcohols and amines, forming complexes where H_2O is displaced and an organic species takes its place. H-bonding to surface hydroxyls and oxygens is thought to be mainly responsible for stabilization of such complexes. The interlamellar surfaces in intercalated kaolinites are a network of oxygen atoms belonging to the base of a sheet of silicon tetrahedra and, opposing that, a sheet of hydroxyl groups belonging to the octahedral sheet (Mortland, 1986).

2.1.4.2. Surface-complexation sites on smectites.

Smectites, vermiculites and illitic micas all have significant permanent charge resulting from isomorphous substitution; this charge is independent of pH. In smectites, the charge results from substitution of divalent cations (e.g., Mg^{2+}, Fe^{2+}) for trivalent cations (e.g., Al^{3+}, Fe^{3+}) in the octahedral sheet (Mortland, 1986). The permanent charge for smectites is 1-2 μequiv. /m^2 of the total sheet area, depending on the degree of substitution, and is diffusely distributed in the ditrigonal cavities (Fig. 2.6).

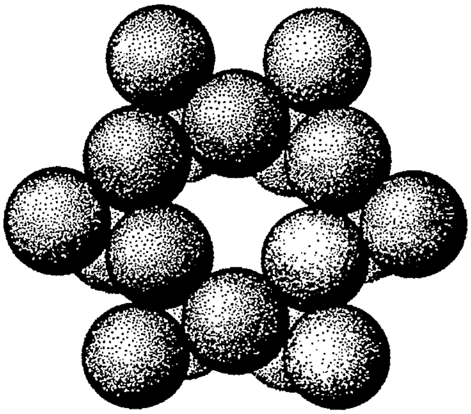


Figure 2.6. The siloxane ditrigonal cavity (Davis and Kent, 1990).

The charge resulting from one substitution is distributed between the 10 surface oxygens of the 4 tetrahedra linked to the substituted site, favoring formation of outer-sphere complexes (complexes in which there is at least one layer of solvent molecules between the complexing ion and the functional group). Fig. 2.7. shows the formation of such a complex on a smectite clay, montmorillonite.

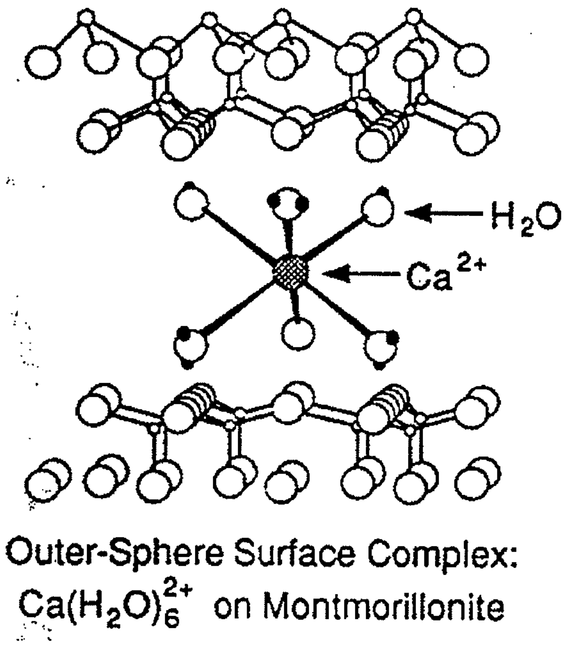


Fig. 2.7. Complex formation on the surface of smectites (after Davies and Kent, 1990).

The site density of the edges of montmorillonite has been reported to be similar to that of kaolinite (Bar-Yosef and Meek, 1987, quoted by Davis and Kent, 1990). The specific surface area for montmorillonite is about 700-800 m^2/g. Billingham et al., 1996 have proposed a method for determination of Bronsted/Lewis acidity of surface groups in montmorillonite exchanged with different cations by recording the infrared (IR) spectrum of the surface reaction of the clay mineral with a probe base (pyridine). Their data suggest that both types of acidic sites are present and their distribution depends on the exchange cation present.

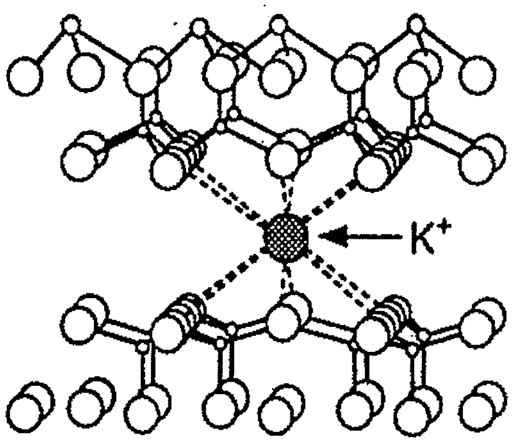
In addition to the negative structural charges, the exchange cations provide sites for interaction with polar molecules through ion-dipole or coordination-type processes. The nature of the exchange cation and its electron-accepting abilities are crucial in this case (Mortland, 1986).

Solute molecules can also be bound to the smectite surfaces by H bonding of the appropriate functional groups from organic molecules to the surface oxygen atoms of the silicate seets (Mortland, 1986).

2.1.4.3. Surface-complexation sites on vermiculites.

In illitic micas and vermiculite, the permanent charge results mainly from substitution of Al^{3+} and Fe^{3+} for Si^{4+} in the tetrahedral layer. The resulting negative charge is much less diffuse because it is only distributed between the three surface oxygens of the tetrahedra involved. A higher degree of substitution (for illitic micas) and a higher density of charge in the ditrigonal cavities favor formation of inner-sphere complexes (complexes in which the cation is directly linked to the functional group) (see Fig. 2.8). The K^+ ion represents a special case because it fits exactly into the ditrigonal cavity, forming an extremely stable surface complex.

Vermicullites have a lower degree of substitution than illitic micas, and consequently they have lower permanent charge. They can form both inner- and outer-sphere complexes. The relative importance of ditrigonal cavities and surface hydroxyl groups depends on the reaction system. Cations tend to bind predominantly at the ditrigonal cavities and the majority of ditrigonal cavities are located in the interlayer region. Anion binding on clay minerals occurs predominantly at surface hydroxyl groups along edges of the clay crystallites.



Inner-Sphere Surface Complex:
K^+ on Vermiculite

Figure 2.8. Complex formation on the surface of vermiculite (after Davies and Kent, 1990).

Kubicki et al., 1997 have proposed a model for adsorption of bidentate-ligand salicylic acid to the illite surface, based on a ligand-exchange mechanism with Al $(OH_2)^+$ groups. They have been able to construct a minimum-energy molecular model of the complex and to calculate the corresponding infrared-absorption frequencies. The IR spectrum predicted for the Al-bound bidentate complex shows good correlation with experimental Fourrier-Transform Infrared Spectroscopy (FTIR) measurements.

2.1.5. Surface-complexation models for clay minerals.

A number of surface-complexation models have been developed in the last two decades: the Constant Capacitance Model (CCM), the Diffuse Double-Layer Model (DDLM), and the Triple-Layer Model (TLM). The models differ by the interfacial structure they propose, resulting in the consideration of different kinds of surface reactions and electrostatic correction-factors to mass-law equations. Davis and Kent (1990) review the models as they apply to the surface of hydrous oxides. The authors suggest that these models can be extended to other minerals that develop amphoteric surface charge, including clay minerals.

Ward and Brady, 1998 have modeled pH and ionic-strength-dependent proton and hydroxyl adsorption onto kaolinite using the triple-layer model (TLM). They proposed diagrams for kaolinite surface speciation as a function of pH at constant ionic strength and temperature. Their experimental determinations indicate that TLM provides a reasonably good estimation for surface-charge distribution. They have also studied adsorption of small organic acids on kaolinite, quartz and corundum, and the experimental data seem to confirm that the interaction between organic-acid molecules and the clay surface occurs mainly at exposed Al sites.

2.1.6. Surface-complexation sites of naturally occurring clay minerals.

There are important differences between adsorption onto ideal mineral surfaces (used in surface-complexation models) and surfaces of well-characterized clay minerals, and adsorption on complex naturally occurring clay minerals. The materials involved in adsorption processes in natural systems are composite materials; they consist of mixtures of various minerals. Moreover, their surface chemical properties have been affected by weathering reactions, diagenetic processes and microbiological activity. During such transformations, leaching of the surface layers of minerals, and deposition of fine-grained poorly crystalline materials (e.g., amorphous Al and Fe hydroxide) may occur (Davis and Kent, 1990) (see Fig. 2.9). In some cases, secondary minerals and coatings that constitute only a minor fraction of the sample (by weight) dominate the surficial chemical properties of natural materials.

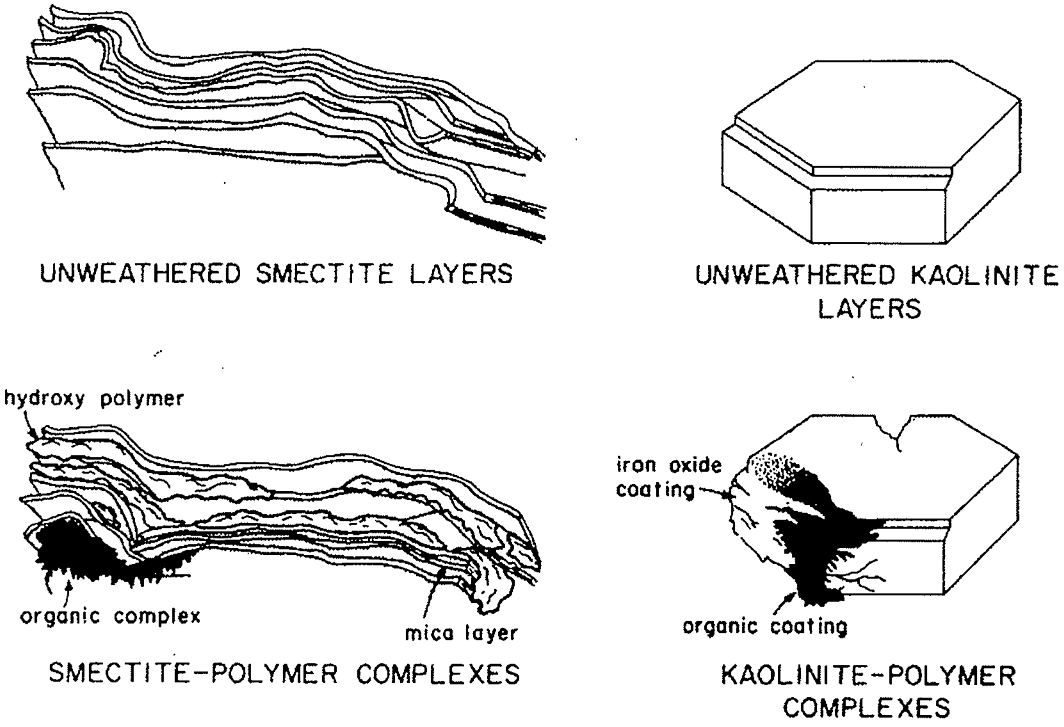


Figure 2.9. Weathered "real" clay surfaces (after Davies and Kent, 1990).

2.1.7. Specific surface area of clay minerals.

A crucial parameter in any discussion of adsorption on clay minerals is specific surface area. The SI units for specific surface area are m^2/kg or cm^2/g. Evaluation of specific surface area for clays from gas-adsorption methods is complicated by the microporosity of the interlayer regions. Empirical methods which use the retention of a variety of polar organic compounds such as glycerol (van Olphen, 1970), ethylene glycol-EG (Rawson, 1969) or ethylene glycolmonoethyl ether-EGME (Eltantawy and Arnold, 1973) have been developed for clays. These small, polar organic molecules can penetrate in the interlayer region of expandable clays, and are more useful to determine the specific surface area of phyllosilicate materials (Davis and Kent, 1990).

2.2. Humic materials: structure and chemical properties.

2.2.1. Humic substances: definition and classification.

Humic substances (after Aiken, 1985) are a class of naturally occurring biogenic heterogeneous organic substances that can be generally characterized as being yellow to black, of high molecular weight, and refractory. MacCarthy et al., 1990 observe that this definition is more a description of humic substances than a definition, and is typical of the nonspecificity that is prevalent in the study of humic substances. Humic substances are organic materials which are formed by alteration of organic matter of animal, vegetal or microbial origin in a natural environment. They are composed of “complex mixtures, which, to date, have defied all attempts at separation into even reasonably pure fractions" (MacCarthy et al., 1990).

Due to their marked heterogeneity, most researchers agree that "humic fractions" can only be defined operationally, that is, by the separation procedure used to obtain them. "Humic substances are Nature's least understood materials." (Davies and Ghabbour, 1998). A major problem in the development of research in the field of humic substances has been the lack of consistent terminology.

Humic substances are operationally classified by their aqueous solubility as humic acid (HA), fulvic acid (FA) and humin. This is an arbitrary classification because humic-substance solubility depends on their molecular weight, their state of aggregation, and their metal and mineral contents (Davies and Ghabbour, 1998).

Humic acid (HA) is defined as the fraction of humic substances that is not soluble in water under acidic conditions ($pH < 2$) but is soluble at higher pH values.

Fulvic acid (FA) is defined as the fraction of humic substances that is soluble in water under all pH conditions.

Humin is defined as the fraction of humic substances that is not soluble in water at any pH value.

Alkaline-extraction procedures are by far the most widely used for separating humic fractions, but they are also criticized for some undesirable characteristics: oxidation occurs if the extraction is not conducted in an inert atmosphere, hydrolysis of ester or amide bonds may occur (Stevenson, 1982). The researcher should always keep in mind the limitations of the particular extraction procedure used when discussing the characteristics of the humic fractions used.

2.2.2. Humic substances: structure and molecular properties

Soil Organic Matter (SOM) is frequently described as consisting of humic substances and non-humic substances. The situation is complicated by the fact that some identifiable organic compounds are covalently linked to humic material and can be released from it by hydrolysis during extraction and fractionation procedures (Schnitzer and Khan, 1972).

The structure of humic substances reflects, to a certain extent, the original material from which they were derived. Analysis of the aminoacid components of humic acids and/or the alkanes in humic fractions allows determination of the source of the material (Thurman and Malcolm, 1994). This can become especially important for determining the source of aquatic humic substances and sediments: one can distinguish between autochthonous humic substances and allochtonous humics.

In accordance with data provided by chemical analysis of humic materials (elemental analysis, functional-group analysis, degradation studies), researchers have concluded that humic substances are heterogeneous mixtures of compounds for which no single formula can be written. Molecular weights of humic substances vary from a few hundred Daltons for fulvic acids up to several hundred-thousand Daltons for humic acids. Most recorded values represent average molecular weights. Nevertheless, humic macromolecules have many common features, and numerous attempts have been made to devise a structural formula representative of humic substances.

2.2.2.1. Classical models of humic molecules: macromolecular aromatic polyelectrolytes.

No model is widely accepted by researchers, but most favor a model of micelles of polymeric nature, with a basic aromatic structure of a di-or trihydroxy-phenol, bridged by ether, methylene, secondary amine, imine or sulfide groups, and containing free COOH, OH and quinone groups (Stevenson, 1982). In the natural state, peptidic or carbohydrate molecules can be attached to the humic polymer (Fig. 2.10).

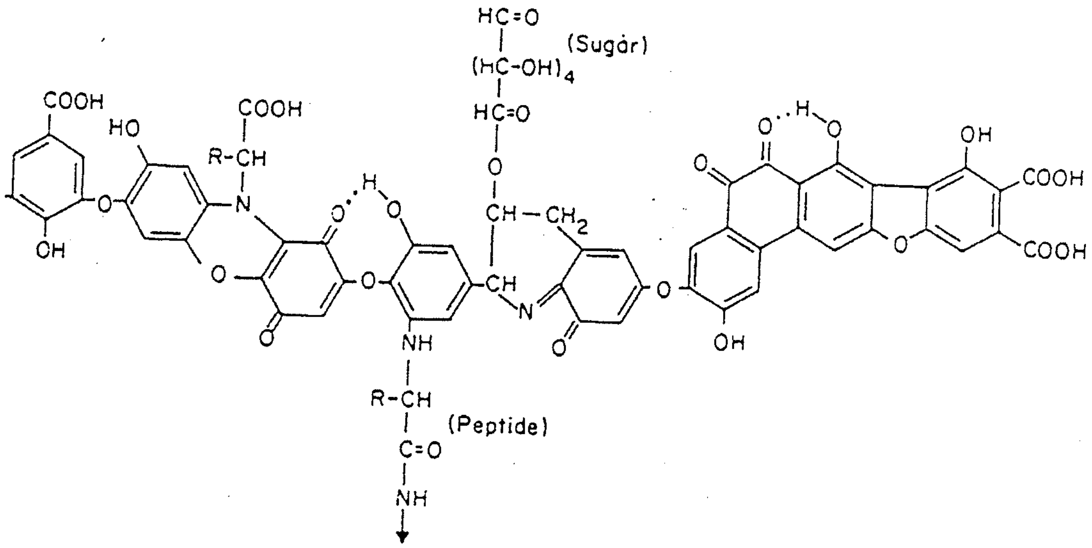


Figure 2.10. Humic molecule structure (after Stevenson, 1982).

An important feature of humic structure is that the molecule forms voids with different dimensions and charge characteristics, which can trap low-molecular-weight organic compounds or metal ions.

2.2.2.2. New models for humic substances structure: the Swanee River fulvic-acid structure.

The detailed analysis of the Swannee River fulvic-acid fraction published by the U.S. Geological Survey brings some new refinements to the traditionally accepted model of humic substances. Elemental composition and heat of combustion for the standard Swannee River fulvic-acid were determined by Reddy et al., 1994. Their conclusion is that the elemental analysis of this reference fulvic-acid of aquatic origin is consistent with a partly oxidized hydrocarbon that contains small quantities of nitrogen and sulfur. The authors propose the following molecular formula, based on a molecular weight of 750 Daltons: $C_{34.3\ (+/-\ 0.2)}H_{33.1\ (+/-\ 0.7)}O_{19.2\ (+/-0.3)}$.

C/N ratios for Swanee River humic substances have large values (107:1 for fulvic acid and 55: 1 for humic acid). On average, data indicate one nitrogen atom per three molecules of fulvic acid, and one nitrogen atom per molecule of humic acid (Thurman and Malcolm, 1994). The carboxyl content of the Swannee River fulvic acid has been estimated at 6.0 mmol/g, and, using a molecular weight of 800 Daltons, an average content of 4.8 carboxyl functional groups per molecule results. This means that the fulvic-acid molecules are not really polyelectrolytes and cannot be modeled as such (Aiken et al., 1994). This idea contradicts the established model of fulvic molecules but may not be as difficult to accept as it seems at first. The classical model of fulvic molecules was one of polymeric polyelectrolytes, based on a much larger molecular weight (as determined by gel-permeation studies) and a high water solubility. If we accept a lower value for the molecular mass, a much smaller number of carboxylic groups can account for the high water solubility of the fulvic molecules.

Structural analysis and model development for the Swannee River fulvic acid is described by Leenheer et al., 1994. Three average structural models (corresponding to the major molecular characteristic and a number of other molecular models corresponding to minor requirements) are proposed for the Swanee River fulvic acid (Fig. 2.11).

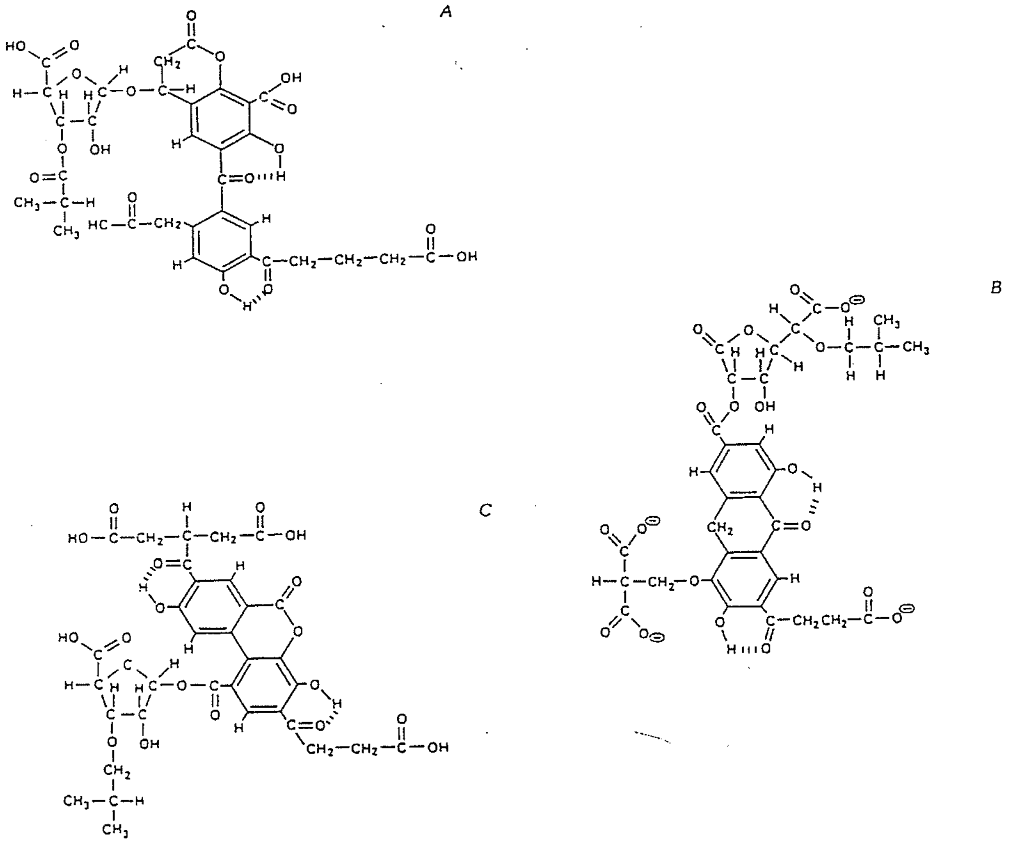


Figure 2.11. Model molecular structures of the Swanee River fulvic acid (Leenheer et al., 1994).

All the proposed models show the importance of spatial arrangements of functional groups (potential for lactone formation, intramolecular hydrogen-bonding, negative-charge potential on the exterior of molecules).

Many models can be constructed for subfractions of fulvic and humic acids, based on different criteria: size, functional groups, spectroscopic data, origin and diagenetic differences. A critical review of methods applicable to structural analysis of fulvic and humic acids (Leenheer, 1994) concludes that: "The absence of definitive quantitative methods for certain measurements... requires that circumstantial evidence be provided by convergence of semiquantitative data or by convergence of indirectly related data".

An example of humic molecules of different origins (as proposed by Kolla et al., 1998) is shown in Fig. 2.12.

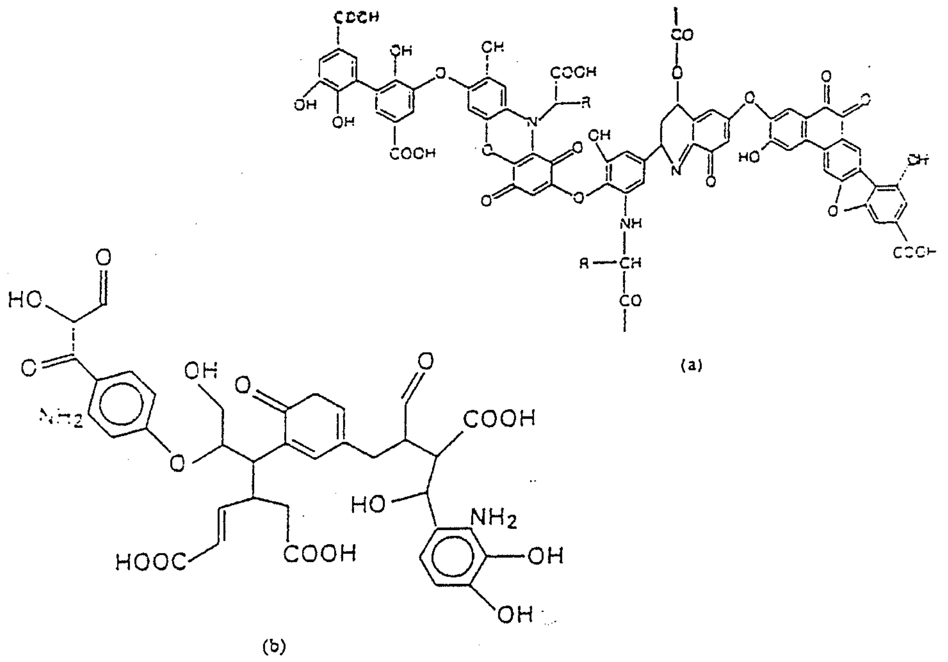


Figure 2.12. Model humic-acid structures: coal-derived (a) and plant-derived (b) (after Kolla et al., 1998).

Recently, for the first time (Stenson et al., 2003), molecular formulas have been determined for a large and representative number of individual fulvic acid molecules. In all, approximately 5000 formulas have been identified in a sample of Swanee River fulvic acid (SRFA) by ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS). Those formulas are consistent with expected molecular formulas for degraded lignins, one of the main constituents of SRFA. The data also show that SRFA is a complex but highly ordered system with an almost polymeric character. This observation is consistent with the broad, continuous separation profiles that are observed in liquid-phase separation of humic mixtures. Once developed, this new method is relatively easy to apply to a multitude of samples: it should be possible, therefore, to base all future comparisons between humics on direct molecular information.

A promising new approach in the study of humic fractions combines the results of the various 2D NMR experiments (Cook et al., 2003) and proposes covalent networks corresponding to a certain NMR signature. The main advantage of this approach is being able to trace out covalent bond systems, which allows one to determine specific structures rather than simply propose generic classes. Using this approach, entities consistent with phloridzin, hesperidin, 2-hydroxy-2-methylsuccinic acid, 2-hydroxy-3-methylbutyric acid, 3-(p-methoxybenzoyl)propionic acid, 3-butene-1, 2,3-tricarboxylic acid, menthoxyacetic acid, and/or benzylmalonic acid have been found in Laurentian Fulvic Acid (LFA). The authors note that 2D NMR methods strongly bias the data in favor of small molecules over large molecules.

2.2.3. Acidic properties of humic molecules.

Many organic functional groups are present in humic substances. The acidic groups seem to be both the most frequent and the most important in determining properties of humic substances. Carboxylic groups, phenolic OH groups, enolic OH groups and alcoholic OH groups occur in humic materials. Many of these acidic functional groups are believed to form vicinal dicarboxylic structures or vicinal carboxyl-hydroxyl structures (salicylic structures). Such structures are important in chelation processes and in determining the conformation of humic polymers in solution.

Polycarboxylic high-molecular-mass polymeric acids exhibit a series of dissociation constants. The values of the dissociation constants are influenced by the vicinity of other polar functional groups to the acidic groups, and decrease as successive protons dissociate because of electrostatic-charge accumulation. The acidity of poly-electrolytic structures increases with dilution of the polymeric acid and is a function of the supporting electrolyte. Buffering occurs over a wide range in pH. Because of the many acidic groups with different but overlapping ionization constants, end-point determination becomes difficult. Specific methods have been developed for titration and end-point determination of humic acids (Gamble, 1972; Bowles et al., 1994).

Weakly acidic functional groups are responsible for the high buffering capacity of humic substances and, by ionization, provide the negatively charged sites which account for the cation-exchange capacity of humic compounds. As much as 80% of soil CEC (cation-exchange capacity) can be caused by soil organic matter (Stevenson, 1982).

2.2.4. Humic molecules aggregation.

2.2.4.1. Colloidal models.

Humic substances are a mixture of molecules that vary in both molecular weight and chemical composition. Molecules interact in solution through H-bonds or cation bridging, forming aggregates with large molecular weights.

The degree of aggregation depends upon factors such as concentration of humic compound, presence of monovalent or polyvalent cations, pH and the ionic strength of the solution (Hayes and Himes, 1986; Aiken et al., 1994; Brown and Leenheer, 1994).

Humic substances are hydrophilic colloids. Low-molecular-weight fulvic acids are probably too small to be classified as colloids, but most humic substances fall within the size range of colloids. Their model is a mixture of coiled long-chain molecules or two- and three-dimensional cross-linked molecules whose negative charges originate in the ionization of acidic groups (mostly COOH) distributed on the humic colloid particles. In their protonated forms, humic macromolecules are supposed to be in a mostly contracted state. When the acidic functional groups are completely dissociated, the molecules are believed to assume a stretched configuration due to mutual repulsion of the negatively charged groups (e.g., COO^-). The presence of many negatively charged sites results in mutual repulsion of the humic aggregates and enhances the stability of the colloid (Stevenson, 1982).

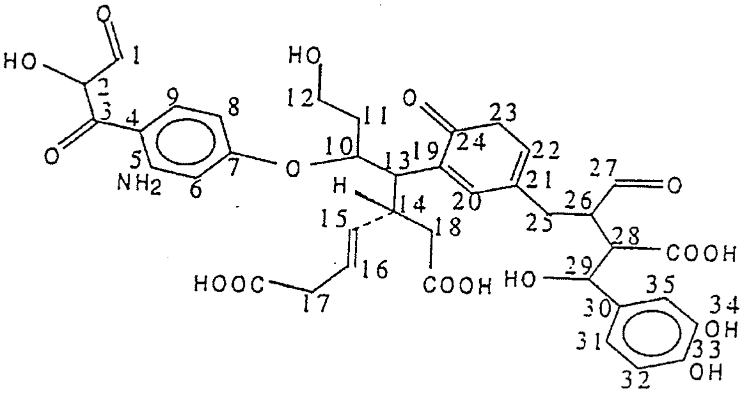
Wershaw (1993) suggested a micellar model for humic aggregates in solution, based on an analogy with cellular membranes: the hydrophobic domains of the humic molecules are oriented toward the interior of the micelle and the polar functional groups toward the surrounding water.

Gu and Doner, 1992, describe humic aggregates observed by freeze-fracture TEM. At pH 7, the humic-acid molecules form long sheets or fibers with a parallel arrangement. Such a long sheet or fiber is believed to be a bundle of several tens (50-100) of H-bonded molecules which can form a fiber 50-100 nm thick and several micrometers long. At pH 2, the HA was precipitated or aggregated and appeared as a massive short-rod structure.

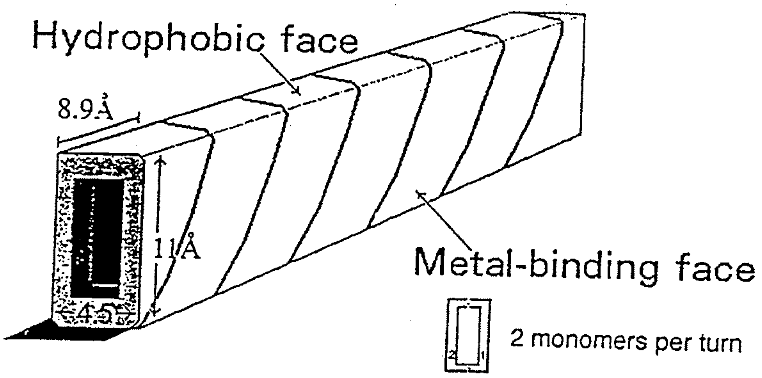
The colloidal character of humic-water mixtures contributes to the unique properties of humic materials: chemical reactions and physical processes are greatly influenced by the relatively large surface areas associated with the small particles in colloidal systems. An interesting aspect of interactions between FA and HA molecules is the tendency for smaller humic molecules to sorb onto larger humic molecules, forming aggregates which exhibit higher hydrophobicity (Wang, 1989). Such processes are expected to be important in the formation of thick organic coatings on mineral particles.

2.2.4.2. Helicoidal models of humic molecules.

A rectangular-helix model for the secondary structure of humic acids (HA) was proposed by Paciolla et al., 1998. The proposed shape of the aggregate is based on arguments involving stereochemical and van der Waals interactions in a model humic molecule with the molecular formula: $C_{36}H_{39}O_{15}N_2$ and the following proposed structural formula (Fig. 2.13):



a)



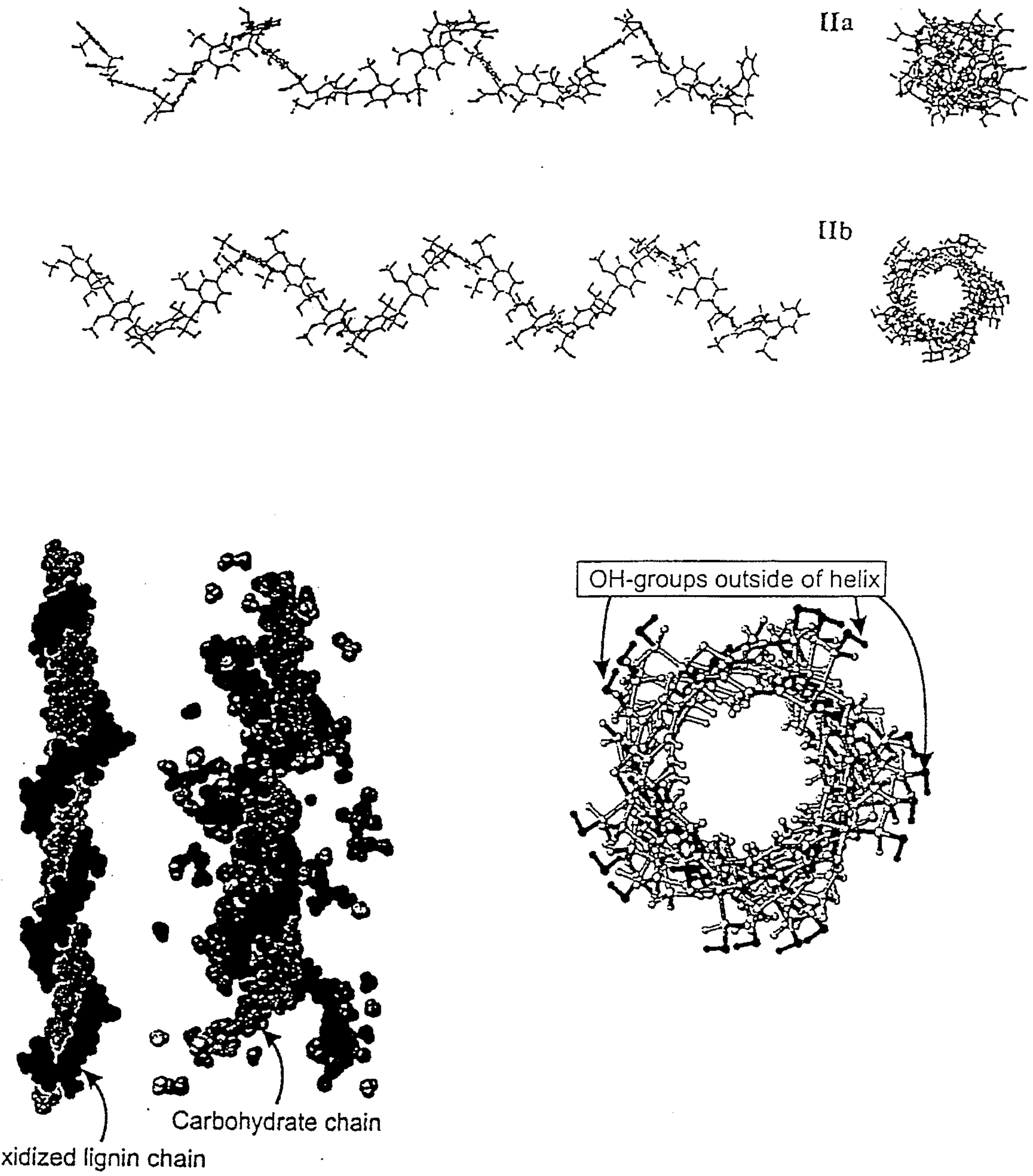
b)

Figure 2.13. Model molecular structure and representation of HA secondary structure (after Paciolla et al., 1998).

Hydrophobic groups remain in the interior of the coil, and hydrophilic photo-active quinoids and metal-binding functional groups are exposed on the HA surface (Fig. 2.13 b) and therefore can be active in metal binding and radical chemistry. The structure of the model molecule does not include the aminoacid and sugar components of the humic acid, which are bound on the surface of the humic helix, generating a more complex structure.

Akim et al., (1998) developed a computer model for humic macromolecules based on an oxidized lignin-carbohydrate complex. Classic theories of the genesis of humic materials consider lignin, a component of vegetal cell walls, the main starting material. Humic substances are believed to be formed by partial oxidation of the lignin macromolecule and subsequent reactions of the oxidized product with other components of the cells (sugars, proteins or their hydrolyses products). The authors of this model chose a broad-helix lignin structure and a cellulose ribbon structure as starting materials to computer-simulate the oxidation process and the integration of the components in the final inclusion complex (Fig. 2.14).

(a) Optimized lignin chain conformations: IIa-extended helix; IIb-broad helix.



(b) oxidized ionized lignin chain before (left) and after (right) simulated annealing with one hydration shell.

(c) Side view of broad helix

Figure 2.14.Helix-type structure of humic molecule based on an oxidized lignin computer-generated model (after Akim et al., 1998).

2.3. Development of reference clay and humic materials.

2.3.1. The Source-Clays Project.

By the early 1970's, the need for sources of homogeneous clay samples had become apparent, and *The Clay Minerals Society* set up the Source-Clays Project. The Society's *Source-Clay Repository* offers two series of materials, Source Clays and Special Clays.

Source Clays are large and reasonably homogenous samples of clay. Data on these reference materials can be compared, with confidence that the clay used in the experiments is the same in all cases (http://web.missouri.edu/~geoscjy/Clay/ref.html). Samples have been carefully selected from the source deposits to minimize *in-situ* variation. Because any beneficiation technique can potentially cause changes in properties, pre-treatment usually involves only low-temperature steam-fired tray-drying, and imp or Raymond-mill pulverization. Each original sample consisted of one metric ton of clay. Literature pertaining to the source clays may be obtained from the Clay Minerals Society (http://web.missouri.edu/~geoscjy/Clay/chem.html).

In the last two decades, many authors have used source clays in their studies of the structure and sorption properties of clay-humic complexes, allowing data comparison with reasonable confidence that the clay used was the same in all cases. Source Clays which have been previously used in the study of clay-humic interaction include: KGa-1, kaolinite, well-ordered, Washington County, Georgia, USA. and SWy-1, Ca-montmorillonite, Crook County, Wyoming, USA.

2.3.2. Differences in structure and properties between "commercial humic acids" and soil-derived humic acids.

In the last 20 years, suppliers of chemical reagents have offered researchers "off-the-shelf" humic acids (e.g., Aldrich humic acid, Fluka humic acid), and humic acids in large amounts are commercially available for industrial purposes (soil amendment or environmental-cleaning procedures). However, many studies comparing sorption properties of commercial humic substances with sorption properties of natural humic material have shown a tendency for measurements on the commercial humic acids to overestimate the sorption capacity of natural humic acids (Malcolm and McCarthy, 1986; MacCarthy and Malcolm, 1989). Some studies also suggest a lower affinity of commercial humic acids (Fluka) for clay minerals such as Ca montmorillonite CaSwy (Celis et al., 1997).

MacCarthy and Malcolm (1989) have done a very thorough study of the elemental composition and spectral properties of commercial humic acids and have compared them with humic acids extracted from several sources. They concluded that commercial humic acids are probably extracted from coal, and have a high degree of aromaticity, which probably accounts for their high sorption capacity. They also point out that no information is provided to the researcher about the source of the material or the method of extraction-fractionation used in the manufacturing, and strongly advise against using such materials to model soil- and sediment-humic acids in environmental studies.

Gonzalez-Vila et al. (1996) have compared the characteristics of humic acids extracted from commercial fertilizers to those of soil-derived humic acids in order to evaluate the potential agronomic quality of those fertilizers. They used Fourier-Transform Infrared Spectroscopy (FTIR) and solid-state Nuclear Magnetic-Resonance Spectroscopy (CPMAS ^{13}CNMR) for this purpose, and have found major differences between the spectra of soil humic acids and commercial fertilizer-derived humic acids. These differences in spectroscopic characteristics suggest a higher content of fulvic acids in the commercial fertilizers, a higher aromatic-C content, and a lower N content for the commercial fertilizers than for soil-derived humic acids.

2.3.3. Standard humic fractions.

The lack of standard materials for interlaboratory comparison of experimental data and for method calibration was a long-standing difficulty in the study of such heterogeneous materials as humic substances. This problem was addressed by the International Humic Substances Society (IHSS) which has established a series of standard humic materials extracted from soil, peat, coal (leonardite) and surface water (McCarthy et al., 1986). The society also maintains a stock of homogenized starting materials available to investigators interested in comparing different extraction methods (McCarthy et al., 1986). Many characteristics of standard and reference humic materials have been determined and are provided by IHSS with the reference samples (http://www.ihss.gatech.edu/). This saves time and resources, and allows better reproducibility and data interpretation. An increasing number of experiments on clay-humic complexes use standard or reference humic fractions provided by IHSS.

2.4. Clay-humic complexes

Clay-humic complexes are components of the conglomerate soil colloids in which clays, oxyhydroxides and humus materials associate (Hayes and Himes, 1986). They have been studied in the past mainly in connection with their influence on the physical properties of soils (aggregate stability, wet ability) (Chaney and Swift, 1984; Emerson et al., 1986). The dynamics of organic matter in soils is believed to be strongly related to soil mineralogy and to the degree of association of the organic and inorganic components of soil. Clay-humic complexes constitute the most stable form of organic matter in soil, and elucidation of their structure and formation is essential for understanding sequestration of C in soils. The long-term impact of soil and organic-residue management depends on whether the management system encourages or discourages formation of the highly recalcitrant clay-humic complexes. The sequestration of organic carbon has recently become an area of high interest, due to hopes to reduce net C emissions by prudent management of agricultural soils in the USA and Canada (Laird, 2001).

2.4.1. Directions in the study of clay-humic complexes

The study of structure and properties of clay-humic complexes can involve two different approaches: (1) isolation and separation of complexes from soil, based on size and density; (2) synthesis of model complexes by the interaction of clay and humic compounds in solution, under controlled conditions (Theng, 1979).

In the present study we refer to clay-humic complexes as they have been described in Theng, 1979. In Theng's review, the term synthetic clay-humic acids is used for the solid phase associations between humic fractions and clay materials obtained in an aqueous solution.

2.4.1.1. "Natural" clay-humic complexes versus model clay-humic complexes.

The study of naturally-occurring clay-humic complexes is difficult due to the complexity of the mineral and organic materials implicated and the variability of environmental conditions. The number of variables implicated in the organic-mineral interaction can be reduced if model clay-humic complexes are synthesized under laboratory conditions from humic materials and clay specimens. The reactants can then be characterized and interaction conditions can be controlled. This approach has resulted in the most meaningful information in terms of bonding mechanisms and structure (Theng, 1979). However, it is difficult to compare such studies due to the huge number of parameters involved in the adsorption processes.

2.4.1.2. Challenges in separating "natural" clay-humic complexes.

Soil organic matter is present as both clay-humic complexes and biological material: living organisms and partly decomposed remains of plants, fungi or microbes. Distinguishing between humified and unhumified organic material is a challenge in studies involving clay-humic complexes separated from soils. Flotation of unbound material using a liquid of density intermediate between the free material and clay-humic complexes (relative density = 1.8-2) is often done prior to soil fractionation (Stevenson, 1982).

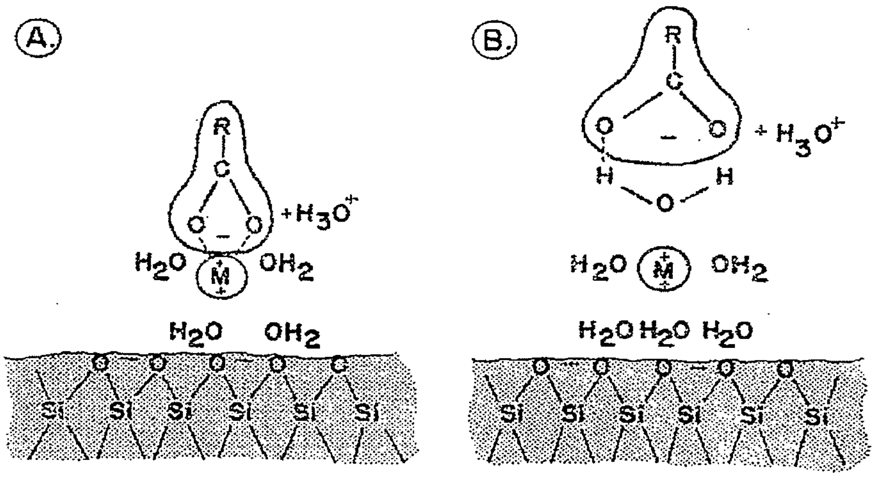
Fractionation of soil components according to particle size can be done by sedimentation, flotation and centrifugation, with or without ultrasonic dispersion (Theng, 1979). In order to fractionate naturally occurring clay-humic complexes according to particle size, large aggregates of soil have to be disrupted to separate primary particles from aggregates of similar size. Ultrasonic vibration has been used in particle-size fractionation since it was first proposed by Edwards and Bremner, 1967a. Christensen,1992 proposed that the optimum energy for fractionation is the energy of maximum disruption of the sand-size fraction (the energy value beyond which no further change occurs in the recovered sand-size fraction). The optimum disruption energy is considered to correspond to complete dissociation of the macroaggregates present in soil to form more stable microaggregates.

The greatest advantage of ultrasonication is the possibility to quantify the amount of energy put into the soil suspension. A method was developed to standardize the energy input in ultrasonic disruption by calibrating the equipment calorimetrically, measuring the temperature increase due to ultrasonic emission for a certain mass of water per unit time (North, 1976).

The quantitative (Christensen, 1992; Balesdent and Mariotti, 1996; Roscoe et al., 2000) and qualitative (Barriuso et al., 1991; Ford et al, 2001; Laird et al., 1994, 2001 a, b) distribution of soil organic matter between the different particle-size fractions of soil (as well as its degree and mode of association with the mineral part of soil) were studied in order to understand the formation process and the dynamics of the most recalcitrant organic compounds in soil-humic substances.

2.4.1.3. Relevant reaction parameters for synthesis of model clay-humic complexes.

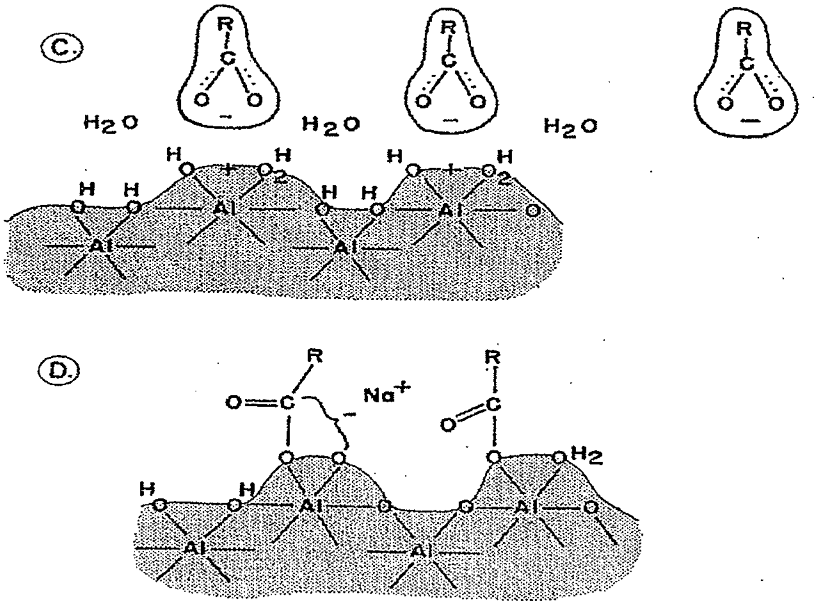
The possible mechanisms involved in the interaction of humic materials with clay surfaces have been developed based on knowledge of sorption of small organic molecules on clay surfaces (Greenland, 1971)-Fig. 2.15.



Interaction of anions with mica-type clay surfaces through polyvalent cation bridges.

Ⓐ anion associated directly with cation

Ⓑ anion associated with cation via a water bridge (Yariv, Russell and Farmer, (61))



Suggested mechanism of interaction of humic anions with hydrous aluminous oxide surface,

Ⓒ by anion exchange.

Ⓓ by ligand exchange.

R is remainder of humic molecule which may also be associated with the surface.

Fig. 2.15. Possible clay-humic interaction mechanisms (Greenland, 1971)

The diversity of the mechanisms implicated in the formation of clay-humic complexes is reflected by the large number of environmental conditions which influence this process. Probably the most lasting legacy of the early studies of humic material adsorption on clay surfaces is the long list of parameters which affect adsorption of humic materials on clays: pH, ionic strength, nature and concentration of cations present, temperature, and the nature, hydration state and degree of aggregation of both the humic materials and the clay minerals (Theng, 1979).

2.4.1.3.1. Nature of reactants

2.4.1.3.1.1. Clay minerals

Studies using montmorillonite as the adsorbent by far outnumber studies involving other clay minerals (Theng, 1979). There are few studies in which the adsorption properties of different clays with respect to humic materials are compared, and the results reported are contradictory. Evans and Russel (1959) reported different shapes of the adsorption isotherms for systems involving montmorillonite and kaolinite. The maximum amount of C sorbed/100 g clay was lower in all cases for kaolinite than for similar systems involving montmorillonite. Abo El-Fadl et al., 1994a found the highest humic/fulvic acid amount sorbed among non-expanding clays for vermiculite, followed by illite and kaolinite. In a follow-up study (Abo El-Fadl, 1994b) in which the adsorbent used was montmorillonite (exchanged with Na^+, Ca^{2+} or Al^{3+} cations), the amount of humic/fulvic acid sorbed was determined by the exchange cation and the type of humic material (HA/FA). Angle and Hamza (1991) found the following order for adsorption excess of HA (mg/g) on different clays:

Fe-kaol ~ Fe-mont > Ca-kaol > illite > Ca-mont ~ Na-mont > Na-kaol ~ 0

(15.6+/-0.11 > 10.3+/- 0.43 > 9.5+/-0.83 > 2.3+/-0.42 > 0.9+/-0.08 > 0.7+/-0.32 > 0)

Filip and Alberts (1994) report very high and similar adsorption degrees for kaolinite (80.2-84.6 %) and for Ca-montmorillonite (79.9-98.9 %).

Experimental observations suggest that pre-treatment of the clay before the adsorption experiment plays an important role in its consequent surface reactivity toward humic mixtures (Evans and Russel,1959; Schulthess and Huang,1991).These observations are in accord with the prediction that the "real" surfaces of natural clays can be significantly modified from the ideal mineral surfaces (see 2.1.6).

Evans and Russel (1959) were the first to investigate differential sorption of humic fraction constituents on clay surfaces. Chassin et al. (1977) found selective adsorption of lower-molecular-mass components of humic fractions. Filip and Alberts (1994) observed preferential adsorption of aliphatic structures from a marsh-extracted humic acid on montmorillonite using FTIR spectroscopy.

2.4.1.3.1.2. Humic materials

Experimental results are no less contradictory regarding the adsorption properties of HA versus FA. A number of studies report higher adsorption excesses for HA on various clays (Evans and Russel,1959; Theng and Scharpenseel,1975 compared to data from Theng,1976; Murphy et al.,1990). Other authors report higher levels of adsorption for the fulvic fraction (Chassin et al., 1977 and 1978; Abo-El Fadl et al., 1994, a and b; Khalil et al., 1995; Tombacz et al., 1988 compared to data from Tombacz et al., 1990).

The available experimental data seem contradictory, but in most cases they are not directly comparable. The composition and properties of humic fractions used vary greatly with origin and extraction procedure used in their preparation. Materials as diverse as water-soluble soil humic fractions (Evans and Russel, 1959), brown-coal humic acid (Tombacz et al., 1988), Aldrich humic acid (probably also of coal origin) (Chassin et al., 1977; 1978), as well as materials obtained by a variety of chemical and biochemical processes from pine bark (Tan and McCreery, 1975), straw (Abo El-Fadl, 1994), marsh vegetation (Filip and Alberts, 1994) or even galic acid, have been used as "humic acid" or "fulvic acid" fractions. Ghabbour et al. (1998) found significant differences between two humic fractions of different origin in terms of adsorption on kaolinite.

2.4.1.3.2. Concentration of the humic fraction

The influence of the humic-fraction concentration is reflected by the adsorption isotherms of humic substances on clay minerals. Linear isotherms were reported for many humic-clay systems (Evans and Russel,1959; Theng and Scharpenseel, 1975;Theng, 1976), as well as isotherms reflecting saturation of the clay surface for some conditions (Evans and Russel,1959; Chassin et al.,1977; Tombacz et al. (1988); Tombacz et al., 1990; Abo El-Fadl et al., 1994, a and b).

2.4.1.3.3. Solution pH

Humic materials are weak polyelectrolytes and at least part of the surface charge of clays is pH dependent (see 2.2.3), so pH is expected to be a major environmental factor affecting clay-humic interactions. The humic and fulvic fractions behave similarly with respect to variation in pH: adsorption is highest at low pH, corresponding to a low level of ionization of the acid functional groups of the humic components, adsorption decreases when pH is raised and the negative charge of the humic molecules increases (Evans and Russel,1959; Tombacz et al., 1988; Tombacz et al., 1990; Abo El-Fadl et al., 1994, a and b; Khalil et al., 1995).

Schnitzer and Kodama (1967) observed interlamellar adsorption of a soil-extracted fulvic acid on montmorillonite at $pH = 2.5$. Most of the fulvic acid was expelled by adjusting the pH to 6.0. The high adsorption of fulvic acid in the interlamellar space of 2:1 clays observed at very high fulvic-acid concentrations (14 g/L) and low pH values ($pH < 2.5$) suggest that fulvic acid molecules behave like uncharged molecules in this process (Kodama and Schnitzer, 1968).

2.4.1.3.4. Polyvalent cations

Polyvalent cations are expected to play a major role in adsorption mechanisms, especially by “bridging” between negatively charged species: the humic polymer and the clay surface are predominantly negatively charged at environmentally significant pH values (Theng, 1979). Evans and Russel,1959 observed that the extent to which a humic fraction was adsorbed on clays (both bentonite and kaolinite) depends on the dominant cation in the system: K^+ systems have lower adsorption compared to systems in which H^+ and Ca^{2+} dominate. Theng and Scharpenseel, 1975 reported adsorption isotherms for Na humate at pH 7 on montmorillonite saturated with 11 different monovalent, divalent and trivalent cations. For all systems examined, the authors report C-type isotherms and a proportional relation between the ionic potential (z/r) of the saturating cation of the clay and $\log_{10}$ of the slope of the adsorption isotherm for polyvalent cations, and a less pronounced inverse relation for monovalent cations. Theng, 1976 found similar trends for adsorption of a fulvic acid on montmorillonite saturated with different cations.

Iron- and aluminum-chelate complexes with humic molecules have long been believed to be the determining factor in the formation of podzols (Stevenson, 1982) although this is not the only current hypothesis (McKeague et al., 1986). Theng and Scharpenseel, 1975 found complexation between humic acid and Al^{3+} and Fe^{3+} decreased the pH of the reaction system containing montmorillonite and Na-humate. Leita et al.,1998 found significant stability and solubility, especially for Fe^{3+} complexes with humic fractions. Singer and Huang,1990 showed that at pH 4.8, Al^{3+} was partially complexed with humic acid and the presence of humic acid in the solution interfered with the crystallization of Al-hydroxide polymorphs. Al^{3+} and Fe^{3+} complexes with HA and FA are chelate complexes, stable in aqueous solutions. The formation of such stable species may interfere with the formation of clay-humic complexes which are believed to be formed by much weaker surface complexation forces.

2.4.1.3.5. Ionic strength

The effect of neutral electrolytes (e.g. NaCl) on the clay-humic system has been less studied until recently. In many studies, the results reported are influenced by both the pH and the ionic strength of the solution and it is impossible to distinguish between these two factors. Tombacz et al., 1988 described a particularly large favorable effect that low concentrations of NaCl (10-20 mmol/L) had on the adsorption of the humic acid on Na-montmorillonite. Tombacz et al.,1990a reported a similar, but less pronounced, behavior for fulvic-acid adsorption on montmorillonite. The favorable effect of neutral-electrolyte concentration on adsorption of humic molecules on clay surfaces is common to other polymeric systems (e.g. polyacrylamide), but is contrary to the effect of neutral electrolyte concentration on adsorption of small acidic molecules (e.g. salicylic acid). The authors explaine the effect they observed by the decrease in size of the random coil due to the charge-screening effect of the electrolyte.

2.4.1.4. Critical interpretation of data from adsorption studies

The vast literature dedicated to adsorption of humic materials on clay minerals has some characteristics which make the data difficult to compare and the results difficult to reproduce or extrapolate for use in developing models of soil or sediment aggregates.

Measurement of the concentration of humic substances in solution by UV absorption spectroscopy can be a source of error: absorption at a certain wavelength is known to be a function of the composition of the humic material (Kumada, 1968) and particle-size distribution in the suspension (Chen et al., 1977). Variation of humic fraction composition during adsorption experiments (due to differential sorption on the clay surface-Evans and Russel, 1959; Murphy et al.,1990; Fillip and Alberts,1994) and the incomplete separation of fine clay particles in suspension are other possible sources of error in concentration measurements based on UV absorption. The use of different wavelength and pH values of the solution by different investigators make data comparison and interpretation difficult.

Separation of solution and solid phase after reaction is done by various centrifuge and filtration methods, which leads to significant differences in the definition of the soluble phase. Terms like "solution" and "suspension" are often difficult to define in these particular circumstances, and material balances are difficult to achieve.

Tombacz et al., 1988, proposed that what we call adsorption on the clay surface may, in fact, be a combination of real adsorption and coagulation of the humic colloids or even heterocoagulation of clay and humic colloids. Experimental separation of adsorption and coagulation processes is not possible. The high tendency of association between humic molecules (Wang, 1989) (which is dependent on their nature and concentration in solution but is also likely to be affected by the adsorption excess on the clay surface) was rarely discussed. Solubilization of clay minerals during the reaction with the humic material, although recognized (Kodama and Schnitzer 1972, Theng, 1979, Filip and Alberts, 1994), has not been accounted for in data interpretation.

2.4.1.5. Diversity of humic materials and clay specimens used

In many studies, the humic fractions used were only characterized by their molecular-mass distribution and identified by their origin, making experimental data impossible to reproduce. Extraction methods for humic material and preparation of suspensions or “solutions” used are specific to each research team and, in many cases, discriminate toward the most soluble components in the humic or fulvic fractions (e. g. Evans and Russel, 1959). This is the main reason why a collection of Standard Humic Fractions was established by the IHSS (see 2.3.3.) and an increasing number of studies use material from this collection.

2.4.2. Morphology of clay-humic complexes

Study of the soil clay fractions by SEM (Laird, 2001) revealed the existence of two distinct types of clay-associated humic substances: the first type exists as diffuse filamentous films associated with the surfaces of 2:1 phyllosilicates in the fine and medium clay fractions, and the second type exists as discrete particles of high-density metal-humic complexes (containing also Fe and presumably other metals) in the coarse clay fraction.

Clay-humic complexes from the Athabasca oil sands were studied by SEM (Margeson et all., 1989). Comparison between images and Si and Al count frequency of the material, after heating at 25 and 775^0C, show an agglomeration of fine, amorphous material coating the sand particles at the lower temperature. Trace elements (Al, Fe, Ti, K, S, Sb, V, Cu) were highly concentrated in the humic-clay material.

Ohashi and Nakazava, 1996 found a high similarity between the morphology of the initial clay and the synthesized humic acid-montmorillonite complex using Transmission Electron Microscopy (TEM). Rothe et al., 2000 found a specific form of montmorillonite-HA particle agglomeration at near-neutral pH, in fully hydrated form, using Soft X-ray Spectromicroscopy (STXM). No separate clustering of HA was found under these experimental conditions.

2.4.3. Chemical composition of clay-humic complexes

2.4.3.1. The relation between soil-clay mineralogy and humic substances.

Considerable evidence seems to indicate that humic substances associated with different clay minerals are functionally different and seem to have a different degree of association with the mineral component of the fraction. Chemical, biochemical and spectroscopic analysis of soil fractions revealed unique characteristics for the organic matter associated with each mineralogically significant fraction (Laird et al., 2001). The organic matter associated with the coarse fraction has characteristics which are believed to be indications of a less humified, newer biological material: higher C/N ratio, lower levels of extractable aminoacids, fatty acids, monosaccharides, aminosugars and stronger carboxyl and O-alkyl ^{13}C-NMR peaks relative to the organic matter associated with the fine and medium clay fractions. Previous experiments (Laird et al, 1994) on the same clay fractions indicate that the affinity of the coarse clay fraction for atrazine is much higher than the affinity of the fine clay fraction, and the affinity of the coarse clay fraction decreases dramatically following treatement by H_2O_2, whereas the same property decreases only marginally for the fine clay fraction. Wu et al.,1999, using similar procedures, found that the coarse clay fraction of a soil similar with the one investigated by Laird et al., 1994, has a much higher affinity for Cu than the medium and fine clay fractions.

2.4.3.2. Degree of humification of organic matter in soil particle-size fractions reflected by C/N ratios and $\delta\ ^{13}C$ values.

C/N ratios and ^{13}C abundance have been determined for particle-size fractions of soil because they are believed to describe the dynamics of organic matter in association with different mineral components of soils. Roscoe et al., 2000 found a decrease in C/N ratios from litter (35.9), to light organic matter (26.4), to whole soil (C/N ratio = 14.8) in a Dark Red Latosol (Typic Haplustox) from the Cerrado region in Brazil. These data illustrate a typical trend of C/N ratio decrease with humification progress. C/N ratios also decreased with particle size, suggesting the presence of a more humified material in the finest fractions. The ^{13}C abundance value significantly increased from litter to soil. The ^{13}C values were larger in the clay fraction than in the other fractions at all energies used for fractionation. With increased energy, the ^{13}C abundance value in the silt and sand fractions decreased logarithmically. In the clay fraction, ^{13}C abundance values were almost constant. This suggested that the organic matter leaving the coarse fractions and accumulating in the clay fraction had ^{13}C abundance values very close to those of this fraction. The authors also observed that the transferred organic matter had a small C/N ratio, suggesting a high degree of humification.

2.4.4. Isotopic composition of clay-humic complexes. Selective incorporation of fresh plant residue into humic fractions.

Increase in δ^{13}C from litter to soil organic matter was reported by Balesdent and Mariotti, 1996 and could be related to selective persistence of compounds rich in ^{13}C or to isotopic fractionation during microbial respiration. Natelhoffer and Fry (1988) proposed that isotope fractionation during decomposition results in accumulation of ^{13}C in more humified material. The abundance of ^{13}C (δ^{13}C) in litter, light organic matter and whole soil for a Brazilian Dark Red Latosol (Typic Haplustox) suggested isotopic enrichment during decomposition (Roscoe et al., 2000). A latitudinal gradient in the δ^{13}C value of surface soils under C_3 vegetation (Bird et al., 1996) links the decrease in δ^{13}C values at lower latitudes to the latitudinal gradient in the residence time of soil organic matter measured by the ^{14}C signature. Naturally ^{13}C-enriched plants , such as those employing a C_4 pathway, are expected to influence the isotopic distribution in organic matter associated with the different fractions of soils (Spaccini et al., 2000).

CHAPTER 3. MATERIALS AND METHODS.

3.1. Morphology of the clay-humic complexes

The morphology of clay-humic complexes is considered to be the main factor determining the sorptive properties of clay-humic complexes (Rebhun et al., 1992; Murphy et al., 1990, Murphy et al., 1994). Atomic Force Microscopy (AFM) was selected as the main investigative method in this study because it provides more flexibility in sample preparation and for better image resolution than electron microscopy. The three-dimensional images obtained were used to derive information about the shape, dimensions, degree of aggregation of humic particles, and the degree of coverage of the mineral surface under different sorption conditions.

3.1.1. AFM study

Freshly cleaved muscovite plates were immersed in soil HA/FA solutions at different humic concentrations, solution pH values, with or without 0.01 M NaCl as a background electrolyte, in an attempt to model adsorption of a natural humic/fulvic-acid fraction to clay minerals. Contact-mode AFM was used to obtain images of the resulting surface complexes and describe their morphology as a function of the environmental conditions.

3.1.1.1. Sample preparation

3.1.1.1.1. Solutions of Standard Soil HA/FA (IHSS)

Standard Soil Humic Acid (HA) and Standard Soil Fulvic Acid (FA) (see Apendix 2), extracted using NaOH solution from Elliot silt loam soil (Joliet, IL, Mollic Horizon) from the IHSS collection (McCarthy et al., 1986) were used. Stock solutions with a concentration of 400 mg/L humic material in double-distilled water were prepared and kept under continuous stirring. The stock solutions were stirred at least 5 days before using them for preparation of working solutions, in order to avoid any possible artifacts due to incomplete hydration of the larger, more hydrophobic humic components. The concentration of the HA/FA in the working solutions was 0.4, 4 and 40 mg/L. When required by the experiment design, the pH of the working solutions was adjusted to 1.5 with a few drops of HCl solution 1M, and to 7 and 10.5 with a few drops of 1 M NaOH solution. When required by the experiment design the working solutions were adjusted to 0.01 M NaCl concentration using solid NaCl. The ionic strength of the NaCl solution was chosen because it lies within the normal range of the ionic strength of natural soil solutions.

3.1.1.1.2. Mica (muscovite) plates

Mica (muscovite), commercially available from Asheville-Schoonmaker Mica Company, VA was used. A fresh mica surface (1 cm/1cm) was prepared by removing the old surface using clear adhesive tape, then it was immediately immersed in the working solution.

Blanks were prepared using freshly cleaved mica plates immersed for 24 h in double distilled-water at all pH values used in the experiment.

3.1.1.1.3. HA/FA adsorption on the mica surface

The mica plates, with one surface covered with adhesive tape, were suspended vertically in 5 mL glass beakers containing 4 mL of the working solution. In some of the experiments, a second mica plate, with one surface covered with adhesive tape, was placed horizontally in the beaker. The plates were removed from the solution after 24 h and the excess solution drained as well as possible by capillarity. The samples were air dried for 2-4 h.

3.1.1.1.4. Kinetic experiments

For each experiment, seven beakers containing 4 mL each of the working solution (HA or FA 0.4 mg/L, unadjusted pH) were prepared. For HA experiments the solution was adjusted to 0.01 M NaCl content using solid NaCl. In each beaker, one freshly-cleaned mica plate was suspended vertically, with one of the surfaces covered with adhesive tape, and a second mica plate was placed horizontally. After 5 minutes, 20 minutes, 2 h, 3.5 h, 6 h, 18 h, and 24 h, respectively, the plates were removed from the solution, the excess solution was drained as well as possible by capillarity and the mica plates were air dried for 2-4 h.

3.1.1.1.5. Well-ordered kaolinite (KGa-1)samples

Well-ordered kaolinite (KGa-1: see Appendix 1) from the Source Clay Collection (http://web.missouri.edu/~geoscjy/Source Clay/ref.html) was suspended in distilled water and a few drops of the clay suspension were deposited on thin glass slides (slide covers used for optical microscopy) using a Pasteur pipette. Excess water was drained by capillarity and the samples were air dried for 2-4 h.

3.1.1.1.6. Well-ordered kaolinite-HA Complexes Samples

Well-ordered kaolinite (KGa-1) from the Source Clay Collection (http://web.missouri.edu /~geoscjy/Source Clay/ref.html) was mixed in a 10: 1 ratio with 4 mg/L humic-acid solution at the pH of the humic-acid solution (aprox. pH=6).

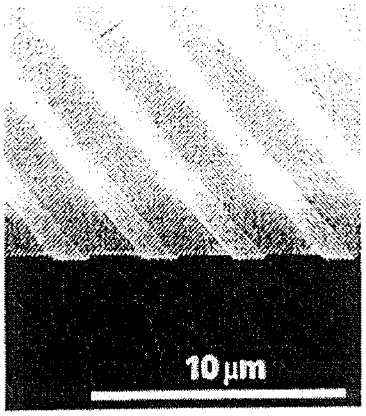
After mixing for 24 h, the suspension was collected using a Pasteur pipette, and a few drops were deposited on a thin glass slide (slide cover used for optical microscopy). Excess water was drained by capillarity and the samples were air dried for 2-4 h.

3.1.1.2. AFM imaging

Images were obtained using a contact-mode, constant-force scanning probe microscope (SPM). A Park Scientific Instruments (http://www.hylab.fi/psi2.html) SPM, operating at ambient room temperature and atmospheric conditions, was used throughout this investigation.

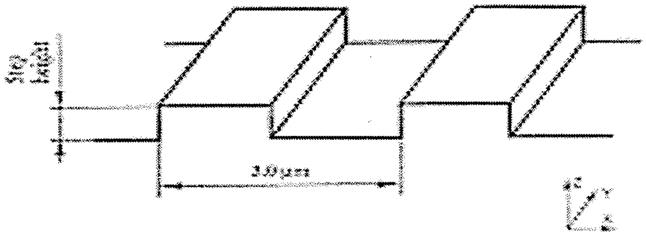
The instrument was calibrated using a standard calibration grating (Ultrasharp/Silicon-MTD Ltd. TGZ02) which has a period of 3 μm (xy) and a step height of 104 nm (z). NT-MTD SCS12/W2C cantilevers were used in all experiments.

SEM image of silicon grating TGZ02 ►



Silicon gratings TGZ01, TGZ02, TGZ03

Active area: 3x3 mm array
Height of steps:
- TGZ01 - 25.0 nm
- TGZ02 - 104 nm
- TGZ03 - 515 nm



Silicon calibration grating of TGZ series provide the calibration value of the step height on the whole sample area. The accuracy of step height for heights up to 30 nm is 1.0 nm, for step heights more than 30 nm - 1.5 nm. It is intended for Z-axis calibration and nonlinearity measurements.

During SPM operation, the probe is brought into contact with the surface and this contact is maintained at a constant force while the probe is scanned in a raster pattern across the sample. The probe is mounted on a rectangular cantilever which bends as the force is applied. During topography measurements, a constant probe-sample contact-force was maintained by detecting variations in the position of a laser beam reflected from the upper surface of the cantilever. The SPM controller adjusts the z position of the sample to compensate for these variations. This technique and instrument are capable of achieving topographic resolution of less than 1 nm. A general description of this (and similar) techniques may be found in Wiesendanger, 1994.

The images obtained via the data-acquisition boards (Lemus, 1996) and the software interface (Shimizu, 1998) were analyzed and formatted for presentation using Image SXM software (NIH Image, 2001). On each sample, between 6 and 12 different locations were imaged in order to account for spatial variability of the sample.

3.1.2. SEM study

Complexes between soil HA/FA and four clays were prepared and their morphology studied by Scanning Electron Microscopy (SEM). The KGa-1 and KGa-1/HA images were compared with the equivalent images obtained by AFM.

3.1.2.1. Sample preparation

3.1.2.1.1. Clay standards

Four Source Clays from the Source-Clays Project of the Clay Minerals Society were used as supplied: well-ordered kaolinite (KGa-1), poorly ordered kaolinite (KGa-2), Ca-rich montmorillonite (STx-1) and Na-rich montmorillonite (Swy-2). The clay particles were glued to round, aluminum sample holders using carbon paint and subsequently coated with Pd-Au.

3.1.2.1.2. Clay-Humic Complexes.

Standard Soil Humic Acid (HA) and Standard Soil Fulvic Acid (FA), extracted using a NaOH solution from Elliot silt loam soil (Joliet, IL, Mollic Horizon) from the IHSS collection, were used. Stock solutions with a concentration of 400 mg/L in double-distilled water were prepared and kept under continuous stirring. The stock solutions were stirred at least 5 days before use for preparation of working solutions. Working solutions containing 200 mg/L were prepared using equal volumes of stock solution and double-distilled water, and mixed for two hours before the clay was added. The pH of the working solutions was unadjusted (pH = 3.5 for FA solution and pH = 6.1 for HA solution).

Four Source Clays from the Source-Clays Project of the Clay Minerals Society were used as supplied: well-ordered kaolinite (KGa-1), poorly ordered kaolinite (KGa-2), Ca-rich montmorillonite (STx-1) and Na-rich montmorillonite (SWy-2) (see Appendix 1).

20 mg of clay were mixed with 500 ml of the 4 mg/L (2 mg humic fraction) humic solution for 24 h, in an attempt to attain equilibrium. The pH of the solutions was dictated by the dissolved humic fraction. The clay-humic mass ratio was 10:1. Different cations (H^+, Na^+, Ca^{2+}) originating from the clays or the humic fractions were expected to be present in the solution at very low concentrations. The pH of the system was measured at the beginning and at the end of the contact time; no significant variation was found. The liquid and the solid phase were separated by filtration using 0.45 µm membrane filters (type HA from Millipore Corp., Billerica, MA), the operationally defined limit between solid particles and solution colloids. The solid residue was air dried for 24 h and ground using an agate mortar before SEM samples were prepared.

The clay-humic particles were glued to round, aluminum sample holders using carbon paint and subsequently coated with Pd-Au.

3.1.2.2. SEM imaging

Samples were observed using a Cambridge Instruments Scanning Electron Microscope with beam energy of 20 keV and a Secondary Electron Detector, at various magnifications up to 15000 x. An EDS detector was used to record spectra of the surfaces.

3.2. Carbon content of clay-humic complexes

3.2.1. Equilibrium experiments

The precursors used were Standard Soil Humic Acid and Standard Soil Fulvic Acid (IHSS), extracted using a NaOH solution from Elliot silt loam soil (Joliet, IL, Mollic Horizon) and four Source Clays (Clay Minerals Society): well-ordered kaolinite (KGa-1), poorly ordered kaolinite (KGa-2), Ca-rich montmorillonite (STx-1) and Na-rich montmorillonite (Swy-2).

The starting mineral (200 mg clay samples) and organic components (HA/FA) were contacted in aqueous solution at seven different concentrations of the humic fraction (2 mg/L; 4 mg/L; 10 mg/L; 40 mg/L; 100 mg/L; 200 mg/L; 400 mg/L) for 24 h, in an attempt to attain equilibrium. The pH of the solutions was dictated by the dissolved humic fraction. The clay-humic mass ratio was constant (10:1). Different cations (H^+, Na^+, Ca^{2+}) originating from the clays or the humic fractions were expected to be present in the solution at very low concentrations. The pH of the system was measured at the beginning and at the end of the contact time and no significant difference was observed. The liquid and the solid phase were separated by filtration using 0.45 μm membrane filters (type HA from Millipore Corp., Billerica, MA), the operationally defined limit between solid particles and solution colloids. The solid residue was air-dried for 24 h.

The well-ordered kaolinite-humic acid (KGa-1/HA) complexes were synthesized in three identical repetitions in order to evaluate data reproducibility.

The carbon content was determined by elemental analysis for all solid samples and for some of the filtered solutions. $^{13}C/^{12}C$ ratios were also determined.

3.2.2. Elemental analysis

Carbon content was determined using a continous-flow system (Carlo Erba NCS 2500 Elemental Analyzer coupled with a Finnigan Matt 252 Mass Spectrometer). The carrier gas was He. The samples were packed in tin capsules (Microanalysis, from Elemental Microanalysis Ltd.).

3.2.3. Stable-isotope analysis

$^{13}C/^{12}C$ ratio was determined using a continuous-flow system (Carlo Erba NCS 2500 Elemental Analyzer coupled with a Finnigan Matt 252 Mass Spectrometer). The carrier gas was He. The samples were packed in tin capsules (Microanalysis, from Elemental Microanalysis Ltd.). The carbon content and the $^{13}C/^{12}C$ ratio were determined in the same run for each sample.

CHAPTER 4. SORPTION OF SOIL HA/FA AT THE MICA-WATER INTERFACE: A MODEL FOR CLAY-HUMIC INTERACTIONS

Sorption of humic molecules on clay minerals improves their geochemical stability and provides an active surface for cation exchange and sorption of organic compounds (Theng, 1979; Stevenson, 1982; Wershaw, 1993). Experimental data suggest that the interaction between humic substances and clay particles influences the conformation of humic molecules, and consequently, the sorptive properties of the clay-humic complex (Murphy et al, 1990; Murphy et al., 1994). Very little direct information is available on the mechanism of humic-fraction surface-interaction with clays and the structural characteristics of the resulting reaction products. Relevant information about clay-humic complexes includes the topography of clay-humic surface-complexes (degree of coverage of the clay surface; thickness of the organic layer) and the influence of the environmental parameters (humic-fraction concentration, pH, ionic strength, presence of complexing cations) on the topography of the clay-humic complexes.

In this study, IHSS Standard Soil Humic/Fulvic Acid (McCarthy et al., 1986) and freshly cleaved muscovite were used to model adsorption of a natural humic/fulvic-acid fraction to clay minerals and describe the morphology of the resulting surface complexes.

It is well documented, both experimentally and theoretically, that in clay-humic mixtures, both precipitation of the clay and humic colloids and adsorption of humic aggregates on the clay surface may occur simmultaneously (Tombacz et al., 1990). In order to avoid deposition of humic aggregates on the mica surface by sedimentation and study only the humic material which interacts with the mica surface, sorption was done with the mica plate immersed vertically in the humic solution. Samples immersed vertically in solution were used previously in AFM studies of humic-mica interactions (Plaschke et al., 1999), but no data to support this decision were presented.

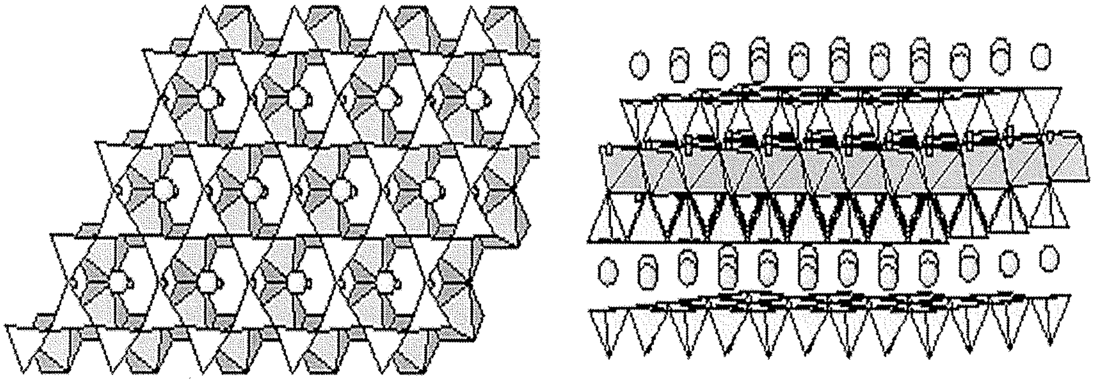
In an attempt to study the system under equilibrium conditions ("steady state"- Zhou et al., 2001), the contact time selected was 24 h. There are no previously existing experimental data about reaction time in these systems.

Sorption experiments were done at various humic-material concentration, pH and ionic strength values. Contact-mode AFM was used for the imaging of the dried mica surface.

4.1. Mica (muscovite) as a model for the surface of clay minerals

Mica (muscovite) can be used as a model for clay mineral surfaces in AFM studies (Plaschke et al., 1999; Liu et al., 2000). Muscovite plates are commercially available for microscopy use. They are inexpensive and easy to cleave in order to produce a fresh clean surface. Freshly cleaved mica provides an atomically smooth surface: flatness better than 0.3 nm over areas of tens of square microns as proved by AFM observations (Liu et al., 2000) or molecularly flat in the dimension of the X-ray coherence length, as confirmed by X-ray reflectivity studies (Cheng et al., 2001). A variety of atomically-flat surfaces have been used as solid support for morphological studies on humic materials: graphite (Shevchenco et al., 1998) or glass (Liu and Huang, 1999), but these surfaces do not have chemical characteristics similar to clay-mineral surfaces and, consequently, are not appropriate models for clay-mineral/humic-acid interactions.

The mica surface is similar to some surfaces of phyllosilicate minerals frequently found in the clay fractions of soils and sediments. The muscovite surface is simple, negatively charged at intermediate pH values, with uniform characteristics on a relatively large scale and little intrinsic topographic variation. X-ray reflectivity data suggest an ionic exchange between the K^+ ions of the mica surface and the H_3O^+ ions of water at the mica-water interface, which produces a water profile with noticeable density modulations above the interface (Cheng et al., 2001). This particular structure of the mica-water interface can have important implications for humic-fraction sorption on the mica surface from aqueous solutions.



Muscovite structure

The mica surface (an aluminosilicate surface) is expected to be reactive under extreme pH conditions, and the purity of double-distilled water and inorganic reagents may be insufficient at the sensitivity level of the analytical method used (AFM). In order to account for any changes in mica-surface morphology not related to the interaction with humic colloids, blanks were prepared by immersing freshly cleaved mica plates for 24 h in the electrolyte solutions, with no humic material added. The variability of the mica surfaces treated this way was found to be an order of magnitude higher than the variability of the freshly cleaved surface (Fig. 4.2 a, b, c). Similar observations were reported for mica immersed in 0.1 M NaCl at pH values 3-8 by Plaschke et al. (1999). There seems to be little difference in variability between blank samples with respect to pH, but this may be due to insufficient sensitivity of the method. The higher variability of electrolyte-treated mica surfaces may be due to chemical reaction of the mineral surface or to deposition of small undetectable amounts of colloids present in the purified water. The observation that the topographic variability of the blank mica samples is highest at low pH (which represents optimum sorption conditions for organic colloids of the humic type) and decreases dramatically at high pH values (for which an alumino-silicate surface is expected to be less chemically stable) suggests that the higher variability of the electrolyte-treated mica surface as compared to the freshly cleaved mica surface is due to organic material deposition from the water, rather than chemical reactions at the mica surface. More research is necessary in order to determine which is the cause of the increased background variability in blank samples immersed in electrolyte solutions, but the results may prove valuable, because the background variability of the samples may be avoided by using organic-free water in experiments involving mica-water systems.

In order to distinguish between adsorbed humic colloids and background variability, each sample was compared to the appropriate blank. This situation makes it difficult to distinguish small aggregates (height smaller than 4 nm) and to estimate the degree of coverage of the mineral surface by humic aggregates.

4.2. Atomic Force Microscopy (AFM) in the study of humic- and fulvic-acid morphology

Atomic Force Microscopy (AFM) is a relatively new imaging technique used in the study of humic- and fulvic-acid morphology (Schevchencko et al., 1998; Plaschke et al., 1999; Liu and Huang, 1999; Liu et al., 2000). Compared with electron microscopy, AFM provides more flexibility in sample preparation, three-dimensional images and, in most cases, better image resolution (Plaschke et al., 1999). The three-dimensional images can be used to derive information about the degree of coverage of the mineral surface as well as the shape, dimensions, and degree of aggregation of humic particles, under different sorption conditions. Contact-mode AFM (Schevchencko et al., 1998; Liu and Huang, 1999; Liu et al., 2000), as well as tapping-mode AFM (Plaschke et al., 1999), has been used to study humic-fractions morphology. Both methods have advantages and disadvantages when used on fragile humic samples. In an attempt to provide relevant environmental information for samples in their normal hydrated state, liquid humic solution-mica samples have also been studied (Plaschke et al., 1999).

In the present experiments, contact-mode AFM was used for imaging of air-dried mica surfaces after adsorption.

4.3. Results

4.3.1. Mica blanks

A freshly cleaved mica surface was scanned and the variability in surface relief was found to be under 0.2 nm (Fig. 4.1)

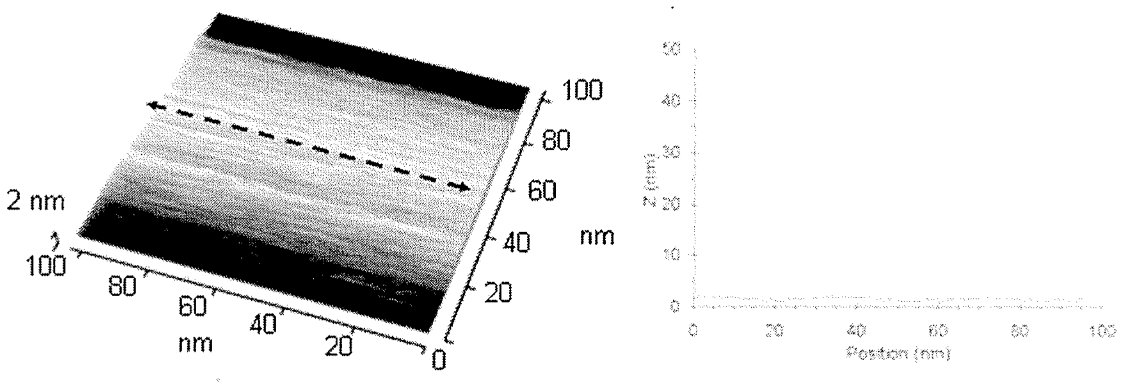


Fig. 4.1. *Topography of a freshly cleaved mica surface-3 D image and line profile.*

The variability of the mica surfaces treated by immersing freshly cleaved mica plates for 24 h in background electrolyte solutions, with no humic material added, was an order of magnitude higher than the variability of the freshly cleaved surface (Fig.4.2 a, b, c).

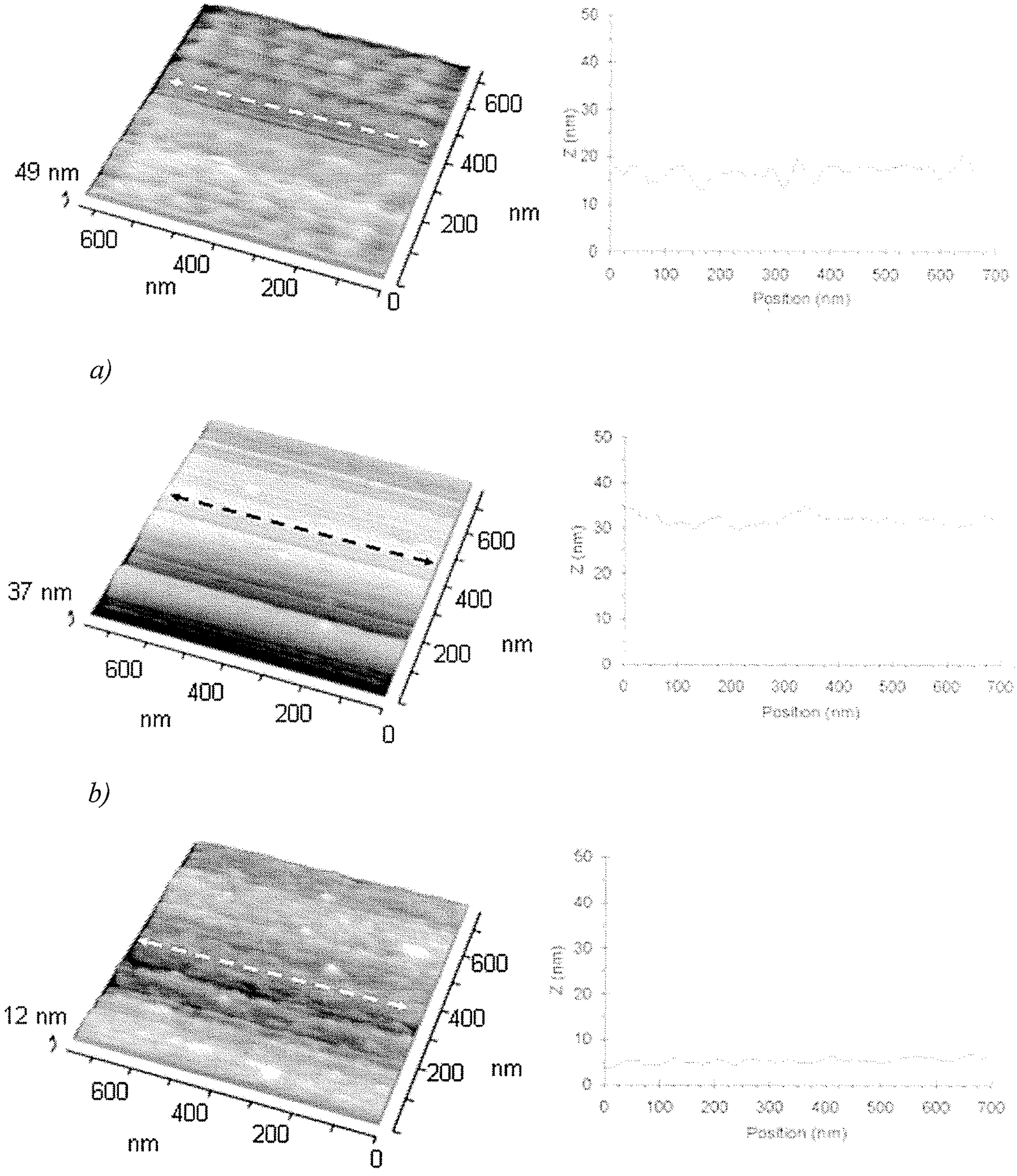
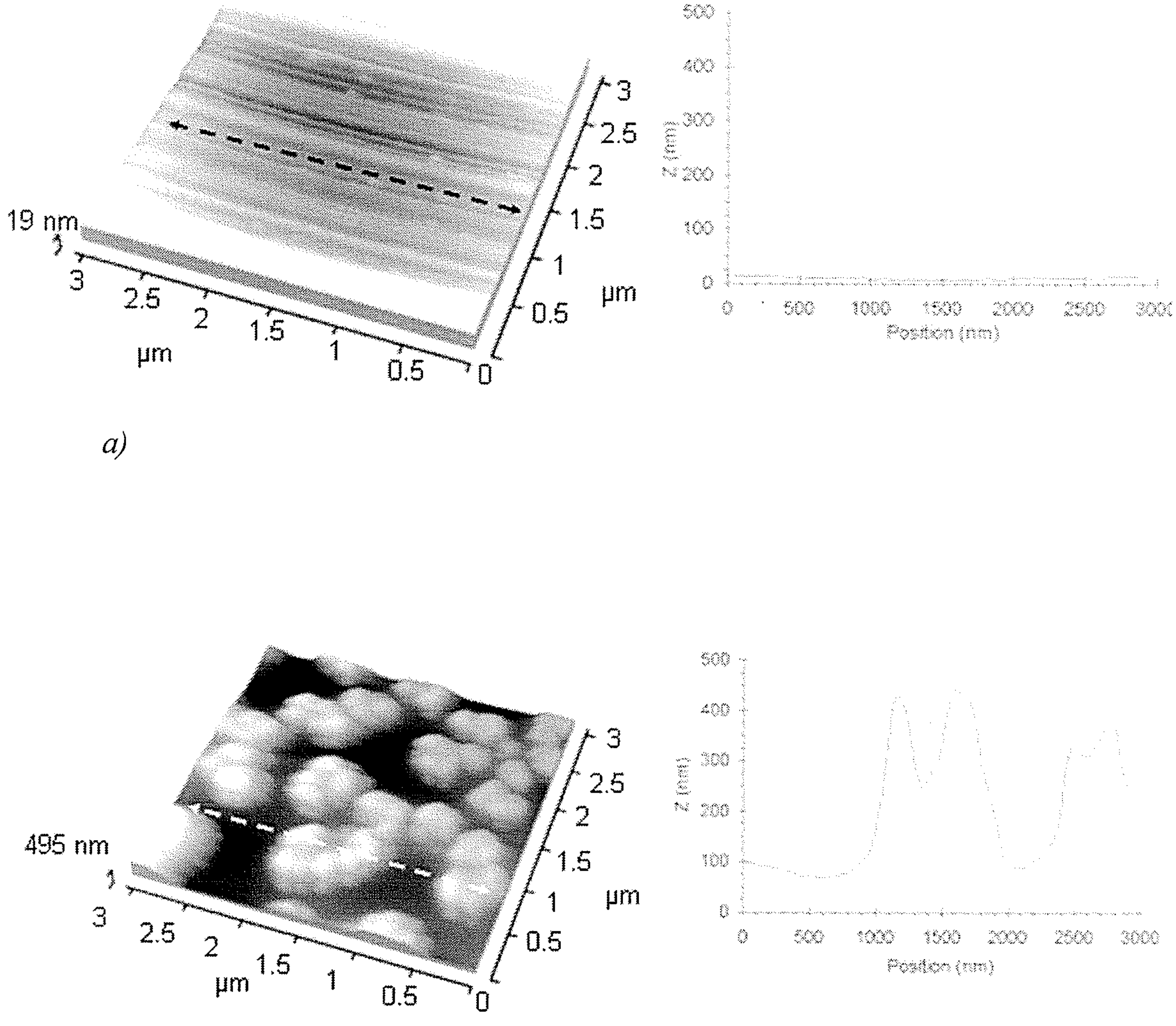


Figure 4.2**.** *3D images and line profiles of mica surface. (a) After 24 h immersion in water at pH 1.5, (b) After 24 h immersion in water at pH 7, (c) After 24 h immersion in water at pH 10.5*

4.3.2. Soil HA/FA sorption at the mica surface as a function of concentration

Sorption experiments were done using different humic-fraction concentrations (0.4, 4, 40 mg/L and 100 mg/L). At low concentrations (0.4 mg/L), a thin layer of organic material appears to be sorbed on the mica surface, especially in the experiments using 0.01 M NaCl as background electrolyte (Fig.4.3). At higher concentrations of the humic fraction in solution, a thick organic layer was formed on the mica surface, preventing proper imaging of the surface, at least by contact-mode AFM. This effect was more notable for the fulvic acid solutions at concentrations of 40 mg/L and higher (Fig. 4.4). In view of these results, a humic-acid solution with a concentration of 4 mg/L and a fulvic-acid solution with a concentration of 0.4 mg/L were used in subsequent experiments.



a)

b)

Figure 4.3. *3D images and line profiles of Soil HA on mica surface at pH 6.1, 0.01 M NaCl. (a) HA conc. 0.4 mg/L, (b) HA conc. 4 mg/L*

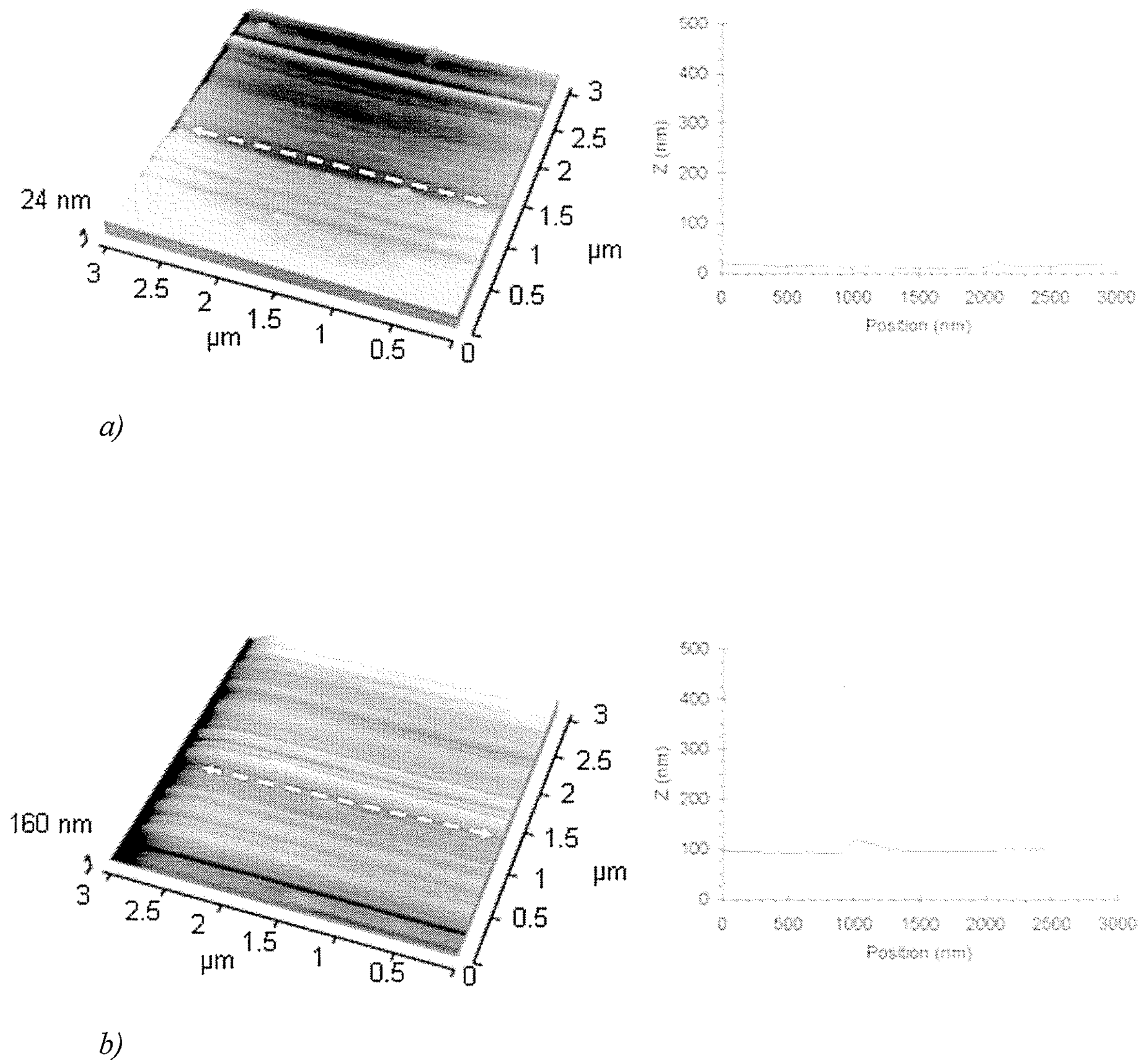


Figure 4.4. *3D images and line profiles of Soil FA on mica surface at pH 4.5. (a) FA conc. 4 mg/L, (b) FA conc. 40 mg/L*

4.3.3. Mica-plate position in solution

A number of samples obtained by placing mica plates vertically and horizontally in the same humic solution were imaged. The differences between the vertical samples and the horizontal samples were more marked for experiments in which 0.01 M NaCl was used as background electrolyte (Fig. 4.5). The subsequent experiments were done with the mica plate immersed vertically in the humic solution in order to avoid deposition of humic aggregates on the mica surface by sedimentation and to study only the humic material which truly sorbs on the mica surface.

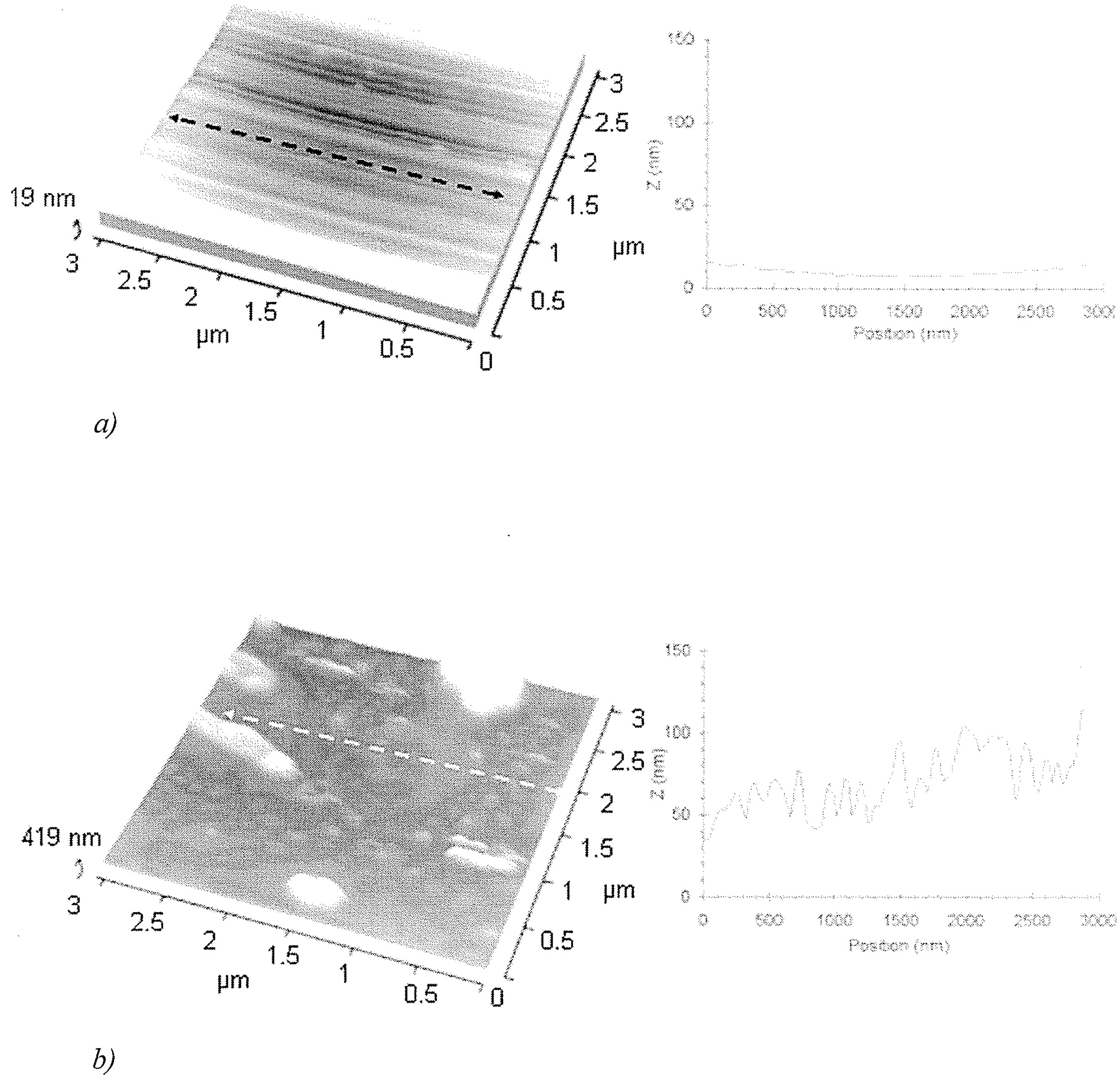


Figure 4.5. *3D images and line profiles of Soil HA on mica surface at pH 6, NaCl 0.01 M, HA conc. 0.4 mg/L. (a) Mica plate placed in solution vertically, (b) Mica plate placed in solution horizontally.*

4.3.4. Soil HA sorption at the mica surface as a function of pH

Fig. 4.6. summarizes the results for the study of Soil HA (4 mg/L) on mica. As predicted theoretically, the amount of humic acid sorbed on the mica surface decreases with increasing pH. At pH = 1.5, large disc-shaped humic-acid aggregates (120 x 250 x 250 nm) cover a significant proportion of the mineral surface. At pH = 7, the humic-acid aggregates have a similar discoid shape, but smaller dimensions (25-40 x 60 x 60 nm), whereas at pH = 10.5 just a few large aggregates (150 x 250 x 300 nm) are found. The presence of 0.01M NaCl as background electrolyte dramatically increases the amount of humic material on the mica surface: very large, composite aggregates (200-500 x 500 x 500 nm) cover most of the surface.

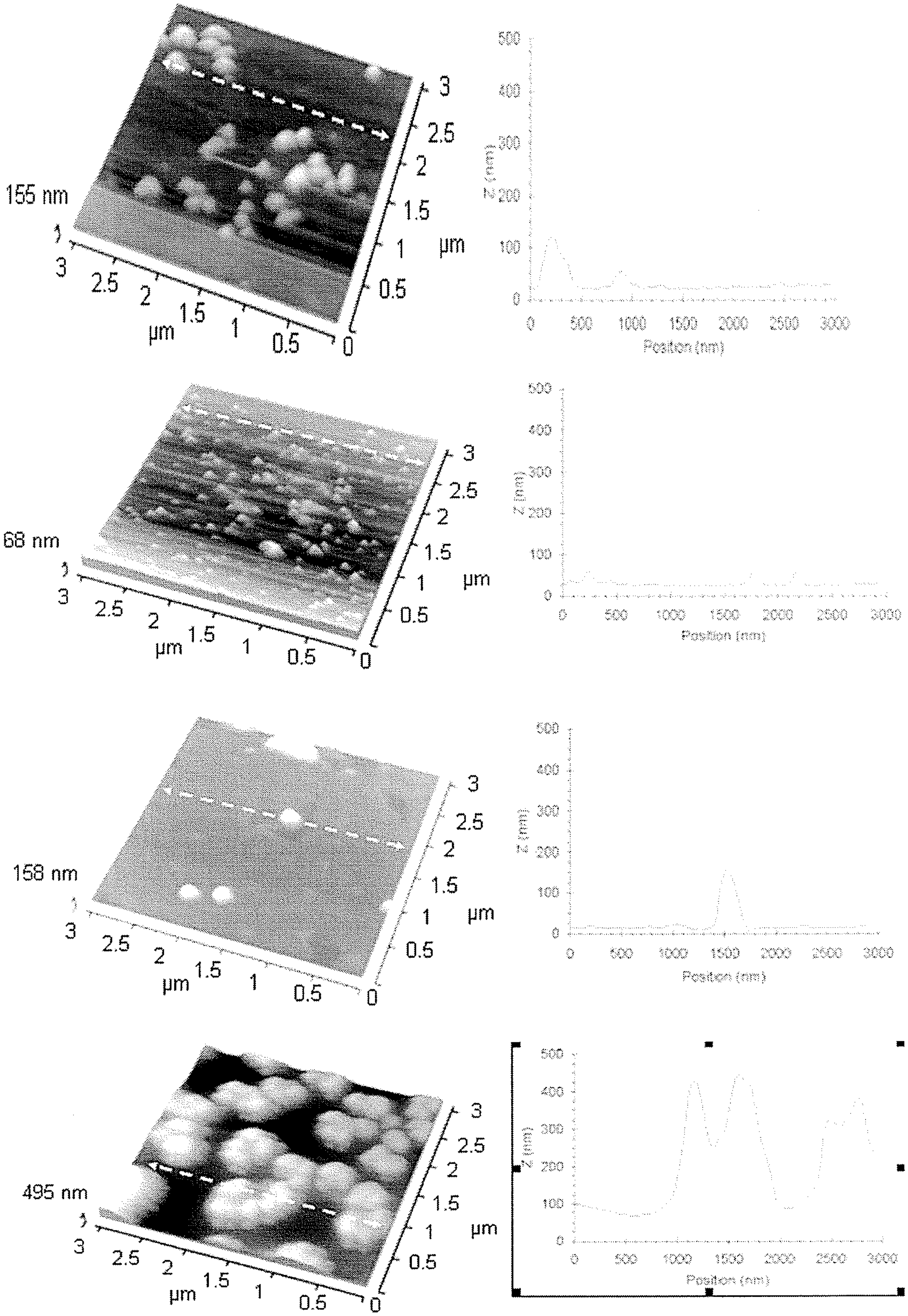


Figure 4.6. *3D images and line profiles of Soil HA on mica surfaces, HA conc. 4 mg/L. (a) at pH 1.5, (b) at pH 7, (c) at pH 10.5, (d) at pH 6.1, NaCl 0.01 M*

4.3.5. Soil FA sorption at the mica surface as a function of pH

Results of the study of Soil FA (0.4 mg/L) sorption on mica as a function of pH are presented in Fig. 4.7. Fulvic-acid aggregates appear smaller, under similar sorption conditions, than humic-acid aggregates. As in the case of humic acid, the dimensions of the fulvic-acid aggregates decrease with increasing pH (80-100 x 250 x 250 nm aggregates seem to be typical at pH = 1.5 and 40-60 x 150 x 150 nm aggregates at pH = 7). At pH = 10.5, there is almost no organic material present on the mineral surface, as predicted theoretically.

The size of fulvic acid molecules in hydrated state is somewhere between 1-1.2 nm (for Swanee River FA, Goldberg and Weiner, 1994). We also know that FA tend to be relatively monodisperse (Aiken et al., 1994) with respect to size and weight and they have a very high tendency to aggregate in aqueous solution (Wang, 1989; Tombacz and Rice, 1999). The aggregates we observed are typically larger than 4nm, often 40 nm in diameter and were formed in aqueous solution, so we asume they are aggregates rather then individual FA molecules, even at low FA concentration.

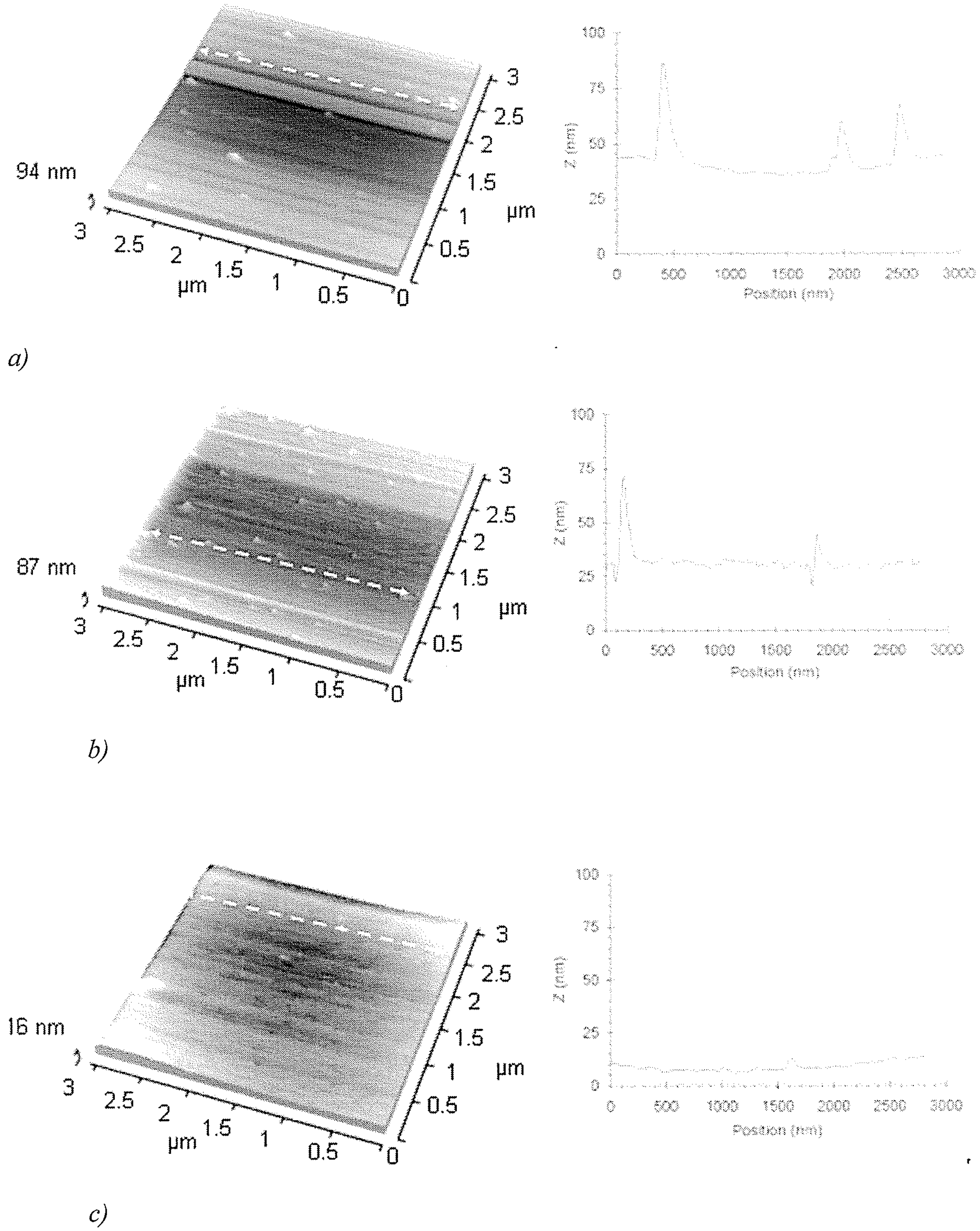


Figure 4.7. *3D images and line profiles of Soil FA on mica surface, FA conc. 0.4 mg/L/. (a) at pH 1.5, (b) at pH 7, (c) at pH 10.5.*

4.4. Discussion

4.4.1. Effect of humic/fulvic-acid concentration in solution on sample imaging

The capillary process does not draw all the solution away from the mica surface, so it is theoretically possible that the solution left would result in some deposits on the surface when the solution evaporates. To account for this, blank samples were imaged. An increased variability of the mica surface in the blanks compared with the freshly cleaved surface was found. The pattern of pH-dependence of this increased variability (highest at acid pH, lower at neutral pH and higher in alkaline solution) and the irregular appearance of the deposit suggest the sorption of organic material from the solutions as the cause of the unusual appearance of the blanks. Plaschke et al. (1999) noted a similar behaviour for their blanks.

Even at the lowest humic concentration tested (0.4 mg/L), the coverage of the mica surface appeared complete, but this could be an artifact because some of the "roughness" observed at very low humic concentration is apparent also on mica-water blanks. In addition to the main organic layer deposited on the mica surface, individual aggregates of different dimensions occur. An interesting observation refers to the shape of the humic aggregates: both humic-acid and fulvic-acid aggregates appear as disc-shaped individual aggregates at low humic concentration in solution. This is in marked contrast to observations of humic aggregates by electron microscopy: humic acid in suspension at pH = 7 appeared as elongated sheets or fibres, and at pH = 2 as massive short rods (Gu and Donner, 1992), as diffuse filamentous films associated with surfaces of 2:1 phyllosilicates in the fine- and medium-clay fractions of a soil, and as discrete particles of high-density metal-humic complexes in the coarse-clay fraction (Laird, 2001). It has been argued that the shape of humic aggregates may be an artifact due to drying on the mica surface, but the images obtained in solution (Plaschke et al., 1999) are very similar to images of dried samples: they show the same small regular disk-shaped aggregates. The difference between these observations may be due to the documented fractionation of humic fractions on the mica surface (Zhou et al., 2001, Zavarzina et al., 2001, see 4.2): at low humic concentration, a more hydrophobic fraction is sorbed preferentially, a fraction which is more likely to form rounded disk-shaped aggregates in water than the more hydrophilic components which constitute the bulk of humic materials. This hypothesis correlates with the observation that the fraction sorbed at low humic concentration on clay surfaces has lower ^{13}C abundance than fractions sorbed at higher humic concentrations (see 4.2.2.3). A lower ^{13}C abundance for more hydrophobic (acetone soluble) humic fractions was reported by Spaccini et al., 2000.

At higher concentrations of the humic fraction in solution, a thick organic layer formed on the mica surface, making proper imaging of the surface difficult and limiting the amount of information obtained from the images (very little topographic variation). In order to obtain better quality images, humic solutions of low concentration were used in subsequent experiments.

The formation of a multiple layer is clearly visible in all sorption experiments. This observation has been also reported by other researchers (Zhou et al., 2001) and seems to contradict a number of studies in which adsorption isotherms conform to a Langmuir equation (Evans and Russel, 1959; Chassin et al., 1977; Ghabbour et al., 1998). Langmuir-type adsorption curves are derived based on a monolayer-sorption process, with a limited number of sorption sites available for the sorbate (Giles at al., 1974). It has been discussed before that an apparent Langmuir adsorption isotherm can also result from competition between two adsorbents for the same sorbate. In the system formed by the clay surface as sorbent and HA/ FA as sorbate the thickness of the organic layer has been found to increase with the increase in concentration by AFM studies. AFM imaging was always done at very low HA/FA concentrations in solution, due to experimental limitations discussed at the beginning of this chapter. At higher concentrations of HA/FA in solution a HA/FA colloid is formed, providing a second sorption surface (along with the initial clay surface now covered with an organic multilayer) in the aqueous system and the leveling of the HA/FA surface excess on the clay surface may be due to sorption on the second surface (Zhou et al., 2001).

4.4.2. Effect of mica-plate positioning in solution on the information provided by AFM images.

Under various pH and ionic-strength conditions in solution, both sedimentation and adsorption of humic aggregates on the mineral surface may occur (Tombacz et al., 1990). Positioning the mica surface vertically in the humic solution should eliminate the deposition of humic aggregates by sedimentation. The comparative study of samples obtained with vertically and horizontally positioned mica plates can provide information on the relative importance of precipitation-sedimentation processes versus adsorption processes on the humic colloid-mica surface interaction under various environmental conditions, a differentiation which is not otherwise easy to approach.

Many samples obtained by placing mica plates vertically and horizontally in the same humic solution were imaged. Differences between the vertical samples and the horizontal samples were apparent for most samples imaged, but were more marked for experiments in which 0.01 M NaCl was used as background electrolyte (Fig. 4.5.), suggesting that under higher ionic-strength conditions, precipitation and sedimentation of humic colloids predominate. The differences between vertical samples and horizontal samples were insignificant for experiments in which fulvic acid at pH 10.5 was used; under these conditions, the mica surface appears mainly uncovered and precipitation is not expected to occur (solubility of the hydrophilic components of the humic fractions is very high). The subsequent experiments were done with the mica plate immersed vertically in the humic solution in order to avoid deposition of humic aggregates on the mica surface by sedimentation, and to study only the humic material which adsorbs (as distinct from sediments) onto the mica surface.

4.4.3. Sorption of soil humic acid and soil fulvic acid at the mica-water interface as a function of pH

Sorption of Soil HA (4 mg/L) on mica was found to occur as predicted theorethically, the amount of humic acid covering the mica surface decreasing with increasing pH. The disc-shaped aggregates of humic acid also decrease in size with increasing pH.

At pH = 10.5, just a few large aggregates are found. The adsorption of even small amounts of humic acid at very high pH suggests the presence of a highly hydrophobic fraction in the humic acid and correlates well with the classical observation that no alkaline solution is able to extract all organic carbon present in a clay-humic complex.

The presence of 0.01M NaCl as background electrolyte dramatically increases the amount of humic material accumulated on the mica surface, and also the size of the aggregates. This suggests that ionic strength may play a more significant role than pH in determining both the amount of humic material associated with the clay fraction of soils and sediments, and the conformation of the organic interface, with important consequences for the hydrophobicity of this surface. The material present on a horizontal mica plate compared to a vertical mica plate in solutions containing 0.01 M NaCl is significantly more abundant and appears more heterogeneous in size and shape of aggregates. This strongly suggests a predominance of humic colloid coagulation versus sorption on the mica surface under conditions of higher ionic strength (the conditions predominating in soils).

FA aggregates appear smaller than HA aggregates, which correlates well with their average molecular weights. The dimensions of fulvic-acid aggregates seem to decrease with increasing pH, but the variation in size is less important than for humic-acid aggregates.

Even semi-quantitative estimation of the amount of material deposited on the mineral surface is a challenge, mainly due to difficulties in distinguishing between the intrinsic variability of the mica surface after treatment with electrolyte and flat layers of organic macromolecules (less than 4 nm high) which seem to be present and could reasonably be expected to be present on the mica surface. This is one important aspect of the morphology of clay-humic complexes and requires further work. Data about the thickness of natural organic matter layers sorbed on muscovite (0.6 nm) determined by X-ray reflectivity were published by Nagy et al., 2001, but the data are not directly comparable to the present experiment because the presence of Ca^{2+} ions in the Nagy experiment favours a cation-bridging mechanism for the binding of the humic fractions to the muscovite surface, a mechanism which is expected to be much stronger than forces implicated in the present experiment in which no Ca^{2+} were present.

The thickness of the main organic layer in the present experiment varies between 4 and 16 nm, depending on the solution parameters (concentration; pH; ionic strength).

In addition to the main organic layer deposited on the mica surface, individual aggregates of different dimensions occur. A quantitative evaluation of the amount of humic material adsorbed on the mica should take into account both the main organic layer, and the individual aggregates.

4.5. Conclusions

i) This experiment demonstrates that contact-mode AFM can be used for imaging the dried mica surface after HA and FA sorption, and provides useful information on the mechanism of the interaction and the morphology of the complexes formed between the organic fraction and the mica surface.

ii) Mica (muscovite) provides a reactive alumino-silicate surface and an appropriate support for AFM imaging. The higher variability of electrolyte-treated mica surface, as compared to the freshly cleaved mica surface, makes it difficult to distinguish small organic aggregates (height smaller than 4 nm) from the background and to estimate the degree of coverage of the mica surface. The more variable topography of the mica surface exposed to electrolyte could be due to chemical reactions involving the mineral surface or to deposition of small, undetectable amounts of colloids present in the purified water. In order to distinguish between adsorbed humic colloids and background each sample was compared to the appropriate blank.

iii) Humic materials were adsorbed on the mica surface (1) even at the lowest concentrations tested (0.4 mg/L), and (2) in the absence of any cations which did not originate from the mica structure. The coverage of the mica surface appeared extensive, even at the lowest concentrations of humic fractions used. In view of this observation, it seems very likely that the whole mineral surface is covered with a thin layer of organic material.

iv) The thickness of the main organic layer varies between 4-16 nm, and individual aggregates of different dimensions occur. Even semi-quantitative estimation of the amount of material deposited on the mineral surface is a challenge, mainly due to difficulties in distinguishing between the intrinsic variability of the mica surface after treatment with electrolyte and flat layers of organic macromolecules (less than 4 nm high) which seem to be present (and could reasonably be expected to be present) on the mica surface. Estimation of the degree of coverage and the amount of material deposited on the mineral surface is one important aspect of the morphology of clay-humic complexes, and requires further work.

v) Deposition from low-concentration humic solutions provides better images, from which information is easier to derive. The small aggregates observed at low concentrations of the humic solution are disk-shaped, suggesting a more hydrophobic nature than that normally attributed to humic compounds. A possible explanation could be preferential sorption of the most hydrophobic molecules at low concentrations, leading to fractionation of the humic material during sorption to the mica surface.

vi) Comparative study of samples obtained with vertically and horizontally positioned mica plates provides information on the relative importance of precipitation-sedimentation processes versus adsorption processes at the humic colloid-mica surface under various environmental conditions. This method differentiates between precipitation + sedimentation and true adsorption, a differentiation which was previously believed to be impossible to attain (Tombacz et al., 1990).

vii) Adsorption of soil HA and soil FA on mica occured as predicted theoretically, the amount of humic/fulvic acid adsorbed to the mica surface and also the size of the aggregates decreasing with increasing pH. Fulvic-acid aggregates are smaller than humic-acid aggregates. Small amounts of humic acid were adsorbed even at very high pH (10.5), which suggests the presence of a highly hydrophobic fraction in the heterogeneous mixture which composes the humic acid.

viii) The amount of humic material adsorbed on the mica surface in the presence of 0.01M NaCl as background electrolyte was significantly higher. My results suggests that ionic strength may play a more significant role than pH in determining the amount and the conformation of humic material associated with the clay fraction of soils and sediments. The concentration of salts in the soil solution may be the most important factor in determining the form of association of humic materials with the clay fraction of soils and the hydrophobic character of the clay/humic complexes formed. This is highly relevant information if one considers that most natural soil solutions have ionic strengths similar to those used in the present experiments and could explain observations that an important part of the humic material in soil occurs in the coarse clay fraction as large humic aggregates not bound to clay particles, with an important content of heavy metals (Laird, 2001).

CHAPTER 5. KINETIC STUDY OF SOIL HA/FA INTERACTION AT THE MICA-WATER INTERFACE

The purpose of this experiment was to improve our understanding of the mechanism and kinetics of soil HA/FA interaction at the mica-water interface and, if possible, to provide reliable information about the real time-scale for reaching equilibrium between sorption and desorption of humic molecules on the mica surface (a "steady-state"-Zhou et al., 2001).

Soil FA adsorption on mica over a 24-h period was done and samples were collected and imaged using AFM method (see Chapter 4) at 5 minutes, 20 minutes, 2 h, 6 h, 18 h, and 24 h. More frequent samples were collected at the beginning of the experiment, because there was some indication in the literature about rapid initial adsorption of fulvic acid on goethite, accompanied by enhanced fractionation (Zhou et al., 2001), and at the end of the experiment (near 24 h interaction time), in order to enable us to estimate a probable equilibration time for humic molecules in the solution/mica-surface system.

For each experiment, two samples were prepared under identical environment and time conditions in order to be able to distinguish between the adsorption process on the mica surface (reflected by the vertically-placed samples) and the adsorption + coagulation/sedimentation processes expected to occur on the horizontally-placed samples. Previous work (see 4.4.2.) had indicated that this procedure is applicable and provides reliable information.

The fulvic-acid/mica surface interaction experiment was repeated under identical conditions to account for the reproducibility of the observations.

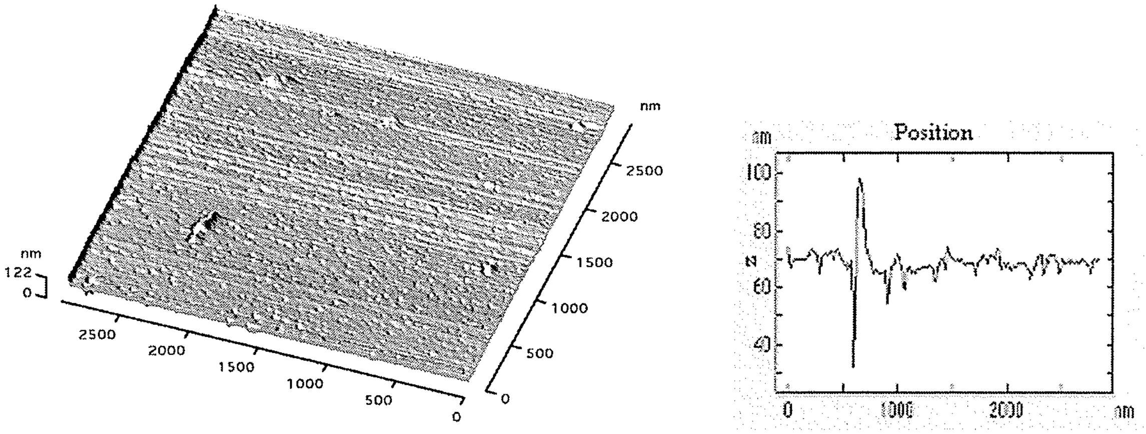
Soil HA adsorption on mica over a 24-h period was done and samples were collected and imaged at 5 minutes, 50 minutes, 3 h, 19 h and 24 h, using AFM. Vertically-placed and horizontally-placed samples were imaged for each interaction time.

5.1. Results

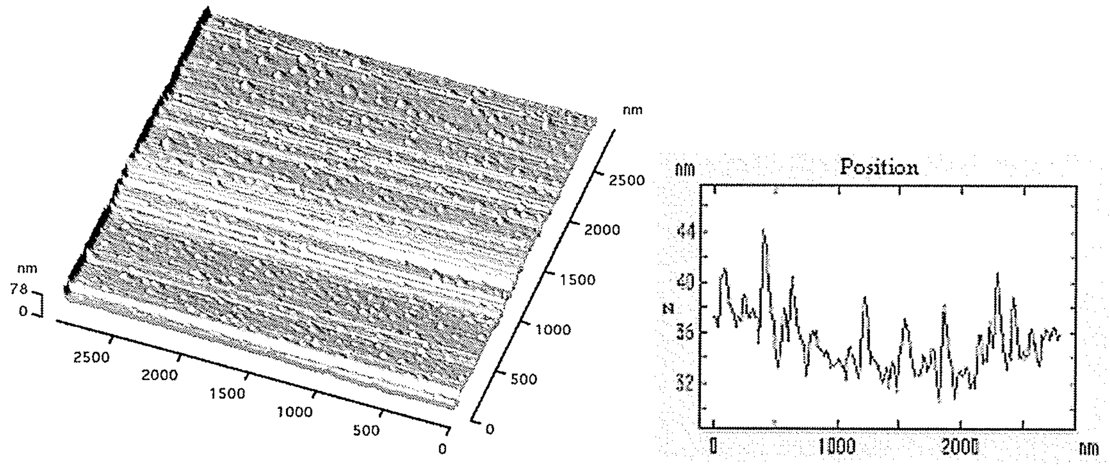
5.1.1. Study of Soil FA adsorption on mica over a 24-h period

Images of the fulvic-acid layer deposited on the muscovite surface from solution with a concentration of 0.4 mg/L are presented in Fig. 5.1 and Fig. 5.2. Fig. 5.1 shows samples obtained by immersing the mica plates vertically in solution and removing them one-by-one after 5 minutes, 20 minutes, 2 h, 6 h, 18 h and 24 h. Fig 5.2 presents the mica plates immersed horizontally in the same solutions and removed at the same intervals as for the vertical samples.

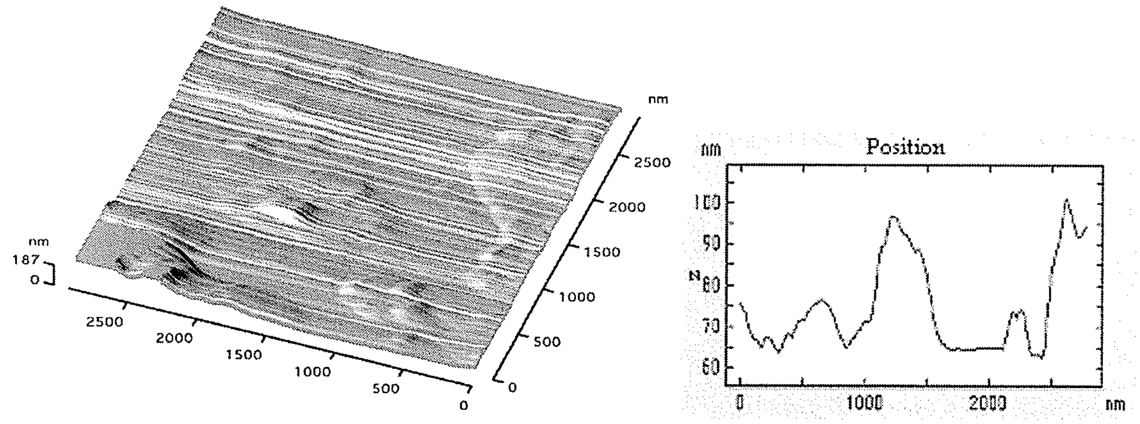
A. Vertical samples



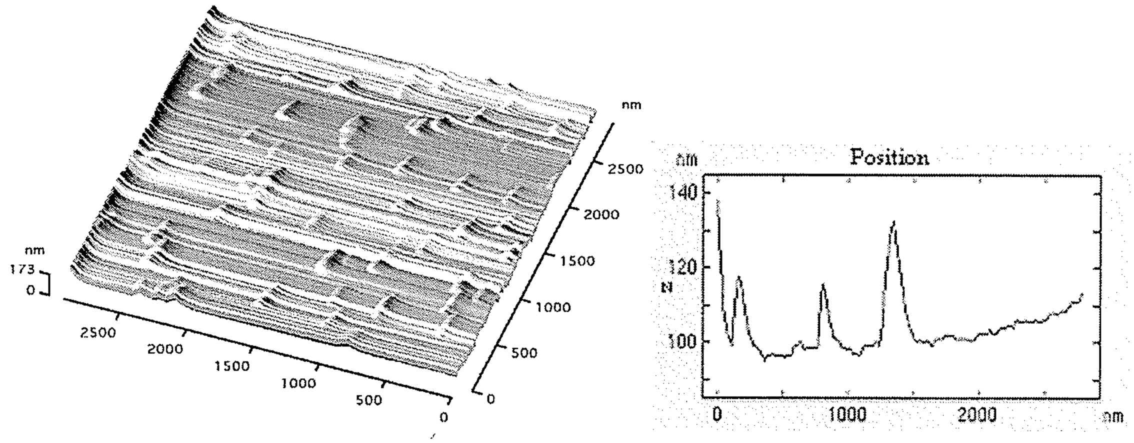
a) t = 5 minutes



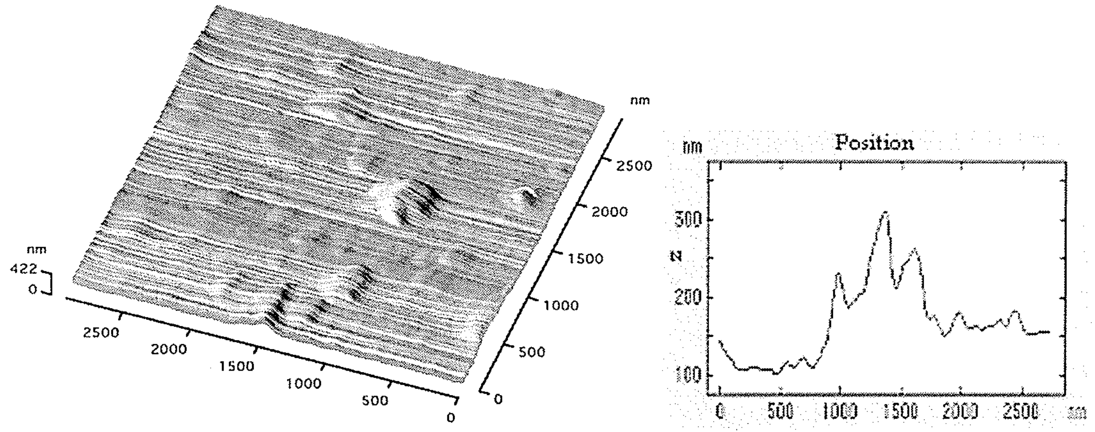
b) t = 20 minutes



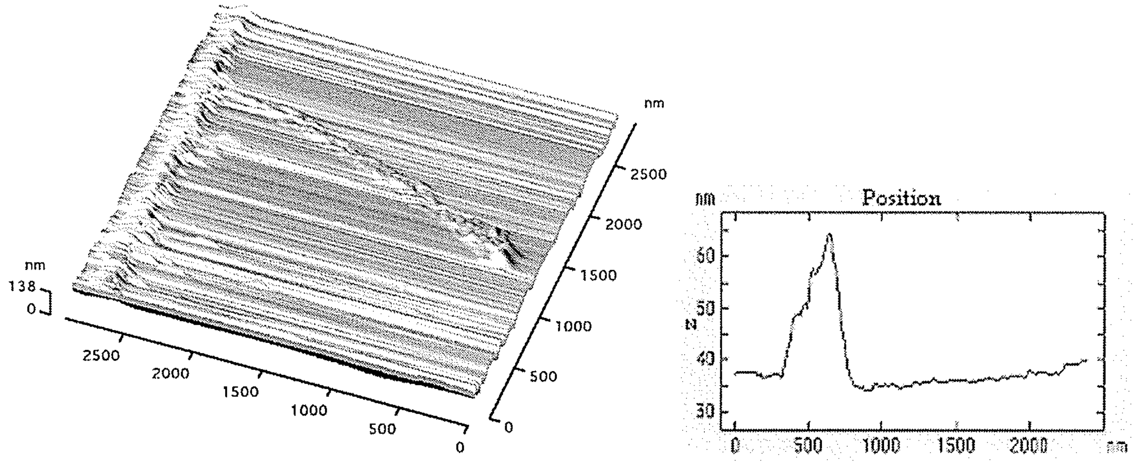
c) t = 2 h



d) t = 6 h



e) t = 18 h

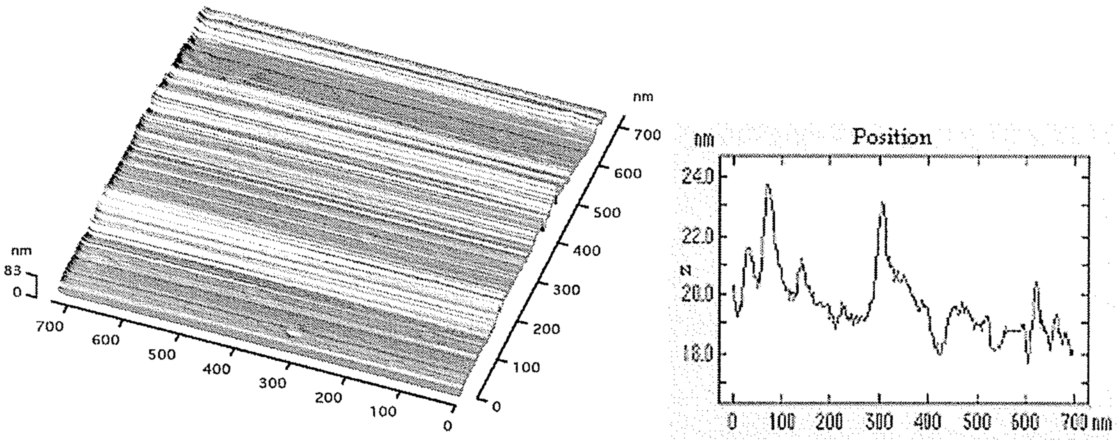


f) t = 24 h

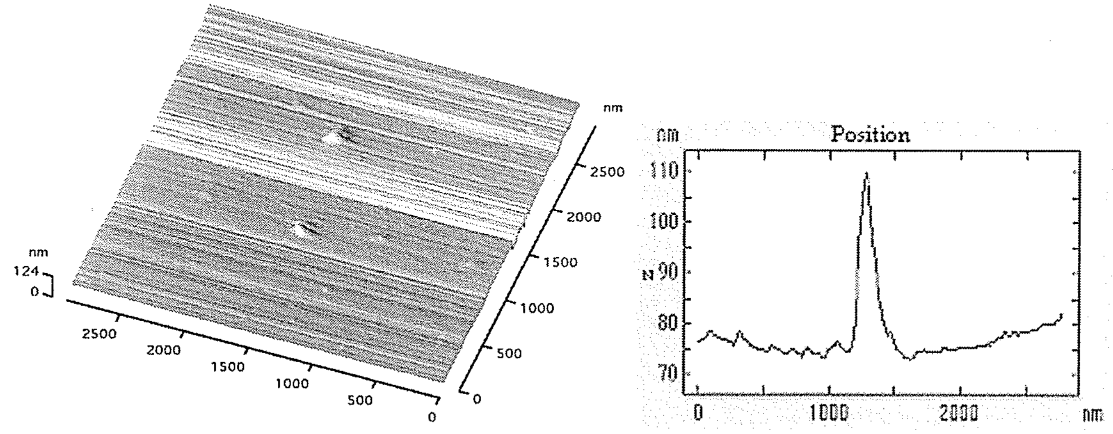
Figure 5.1. *3D images and line profiles of Soil FA on mica surface as a function of time: a) t = 5 minutes; b) t = 20 minutes; c) t = 2 h; d) t = 6 h; e) t = 18 h; f) t = 24 h. Samples placed vertically in solution.*

The vertically-placed mica/fulvic-acid sample removed after only 5 minutes interaction time shows few individual aggregates on the mica surface. The small, disk-shaped aggregates are 4-20 nm high and 40-100 nm in diameter. After 20 minutes, the whole surface is covered with disk-shaped aggregates similar to those in the previous image (4-12 nm high and 50-100 nm in diameter). After 2 h, the whole surface is covered with what seems to be a multilayered deposit with a much more variable profile, consisting of much larger aggregates (10-40 nm high, 100-500 nm diameter) in which the initial disk shape is no longer visible. Note that the measured high is the relative height of the aggregate in the profile, not the absolute height relative to the mica surface. After 6 h, the vertically-placed fulvic-acid/mica sample shows a smooth surface, well-covered by a multitude of regular "rod-shaped" (40 nm x 200 nm x 1000nm) aggregates. After 18 h, the whole surface is covered, the topography is highly variable, and large aggregates (40-100 nm x 300-1000 nm) seem to retain some of the elongated shape of the smaller aggregates visible in the previous sample. After 24 h, a thick, apparently smooth surface is observed, with a few features that seem to be clusters of disk-shaped aggregates (20 nm x 500 nm).

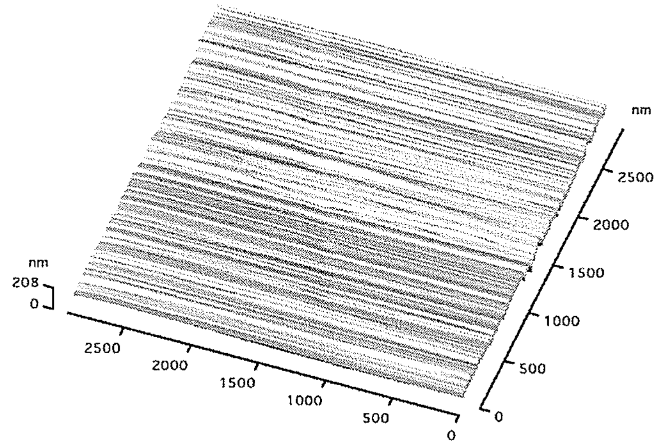
B. Horizontal samples



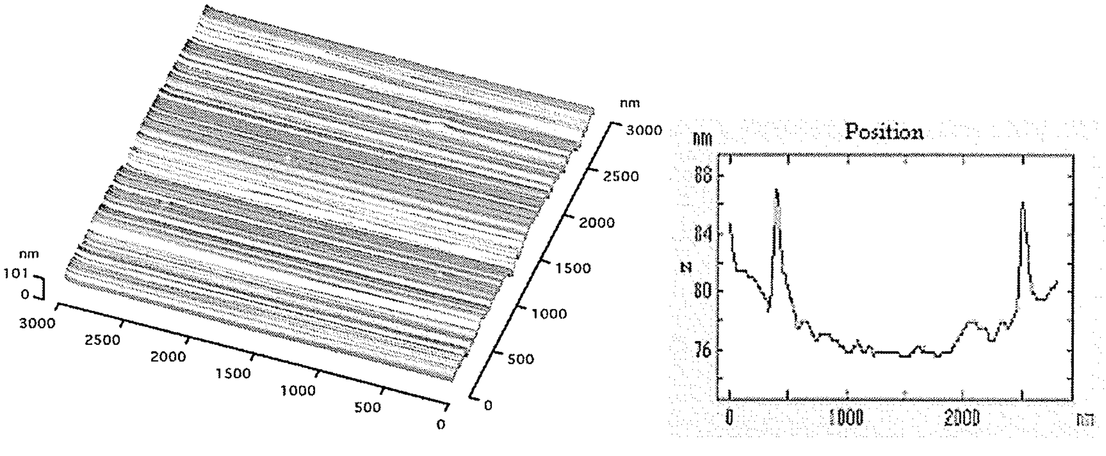
a) t = 5 minutes



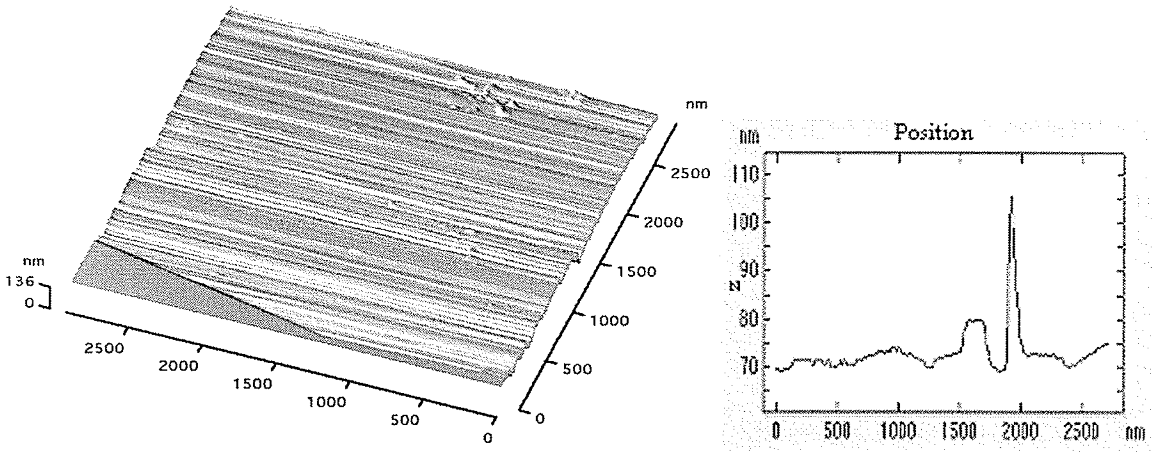
b) t = 20 minutes



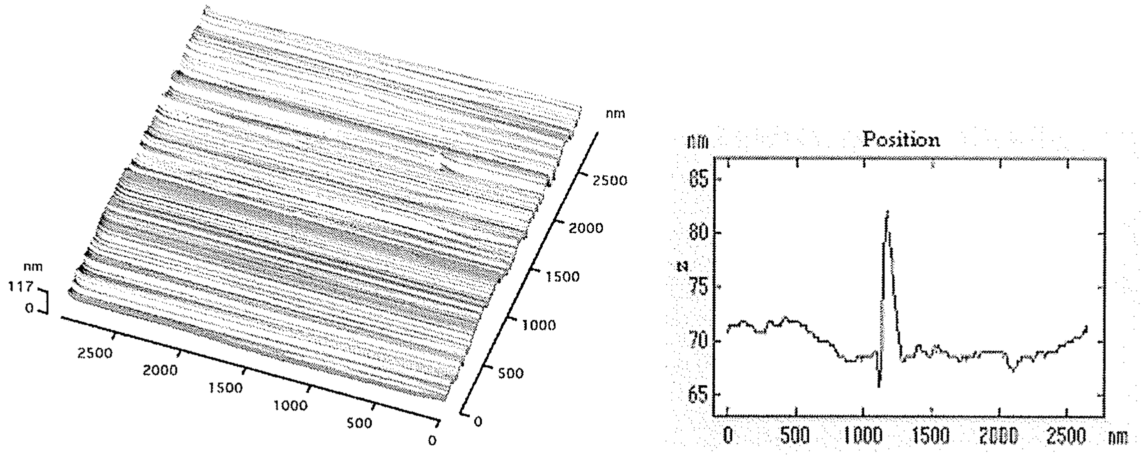
c) t = 2 h



d) t = 6 h



e) t = 18 h



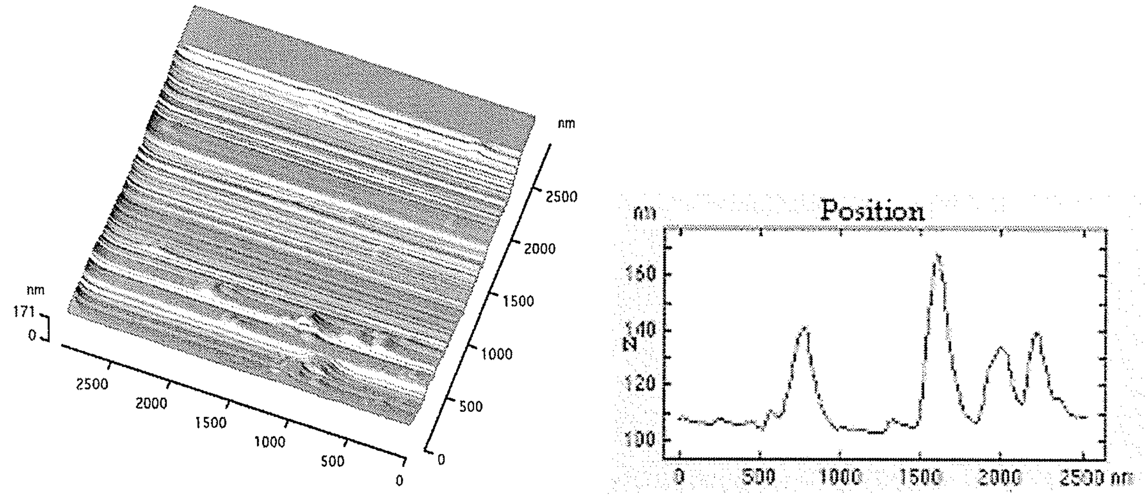
f) t = 24 h

Figure 5.2. *3D images and line profiles of Soil FA on mica surface as a function of time: a) t = 5 minutes; b) t = 20 minutes; c) t = 2 h; d) t = 6 h; e) t = 18 h; f) t = 24 h. Samples placed horizontally in solution.*

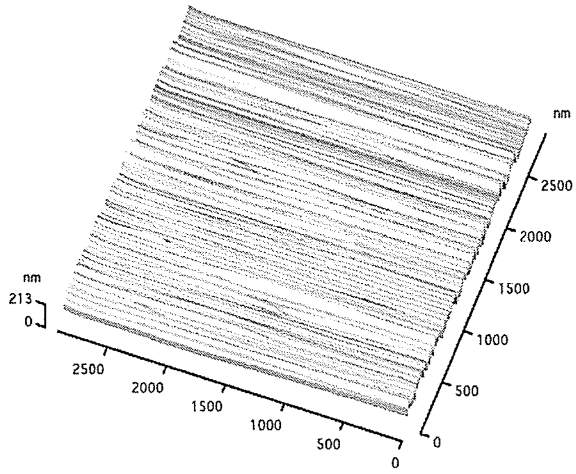
Horizontally-placed mica/fulvic-acid sample removed after only 5 minutes interaction time shows extensive coverage of the mica surface with small disk-shaped individual aggregates (4 nm high x 40 nm diameter). After 20 minutes, the whole surface is covered with a multiple layer of small aggregates, which gives the surface a smooth appearance, with occasional larger aggregates (20-40 nm high x 300 nm diameter). After 2 h, the whole surface appears smooth, covered by a thick featureless humic multilayer. Horizontal samples imaged after 6 h, 18 h and 24 h show a similar image to that found for the 2 h experiment. It is difficult to appreciate if any change in the thickness of the organic layer occurs, but no difference in the morphology was observed for interaction times longer than 2 h.

5.1.2. Experiment Reproducibility

Figures 5.3 and 5.4 show samples obtained in a second experiment reproducing the conditions of the first mica/fulvic-acid interaction experiment and using 5 minutes, 20 minutes, 3.5 h, 6 h, 18 h and 24 h interaction time.

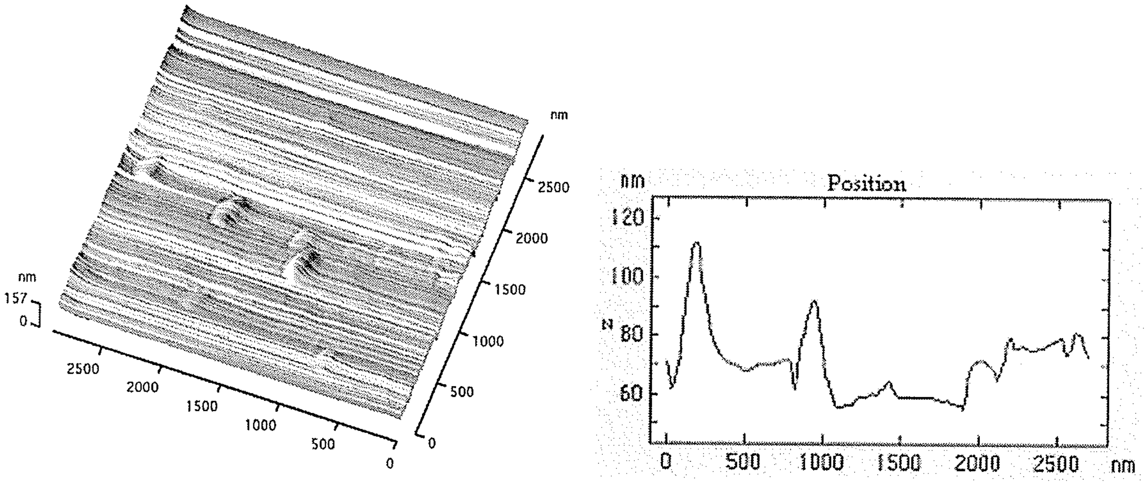


a) vertical

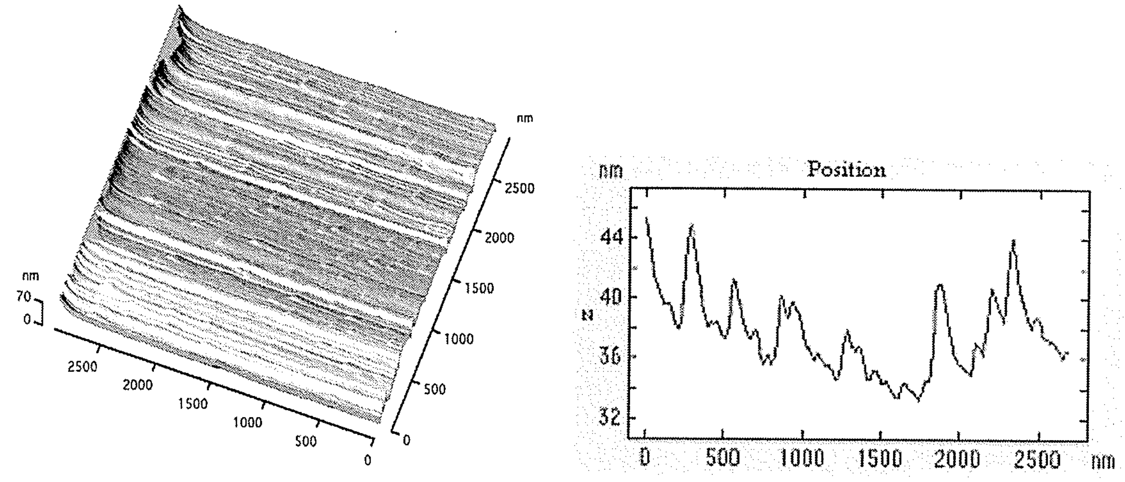


b) horizontal

Figure 5.3. *3D images and line profiles of Soil FA on mica after 6 h. Samples placed a) vertically and b) horizontally in solution.*



a) vertical



b) horizontal

Figure 5.4. *3D images and line profiles of Soil FA on mica after 3.5 h. Samples placed a) vertically and b) horizontally in solution.*

In a second experiment, the vertically-placed mica-fulvic acid samples after 6 h interaction-time show the same features as the equivalent sample from the first experiment: relatively large aggregates with an elongated shape ("rod shaped", aprox. 40

nm x 200 nm x 600). The abundance of the aggregates seems to be lower than for the equivalent sample in the first experiment.

The vertically-placed mica/fulvic-acid sample imaged after 3.5 h interaction-time in the second experiment shows the same large aggregates described for the 6 h experiment, but fewer aggregates seem to be present.

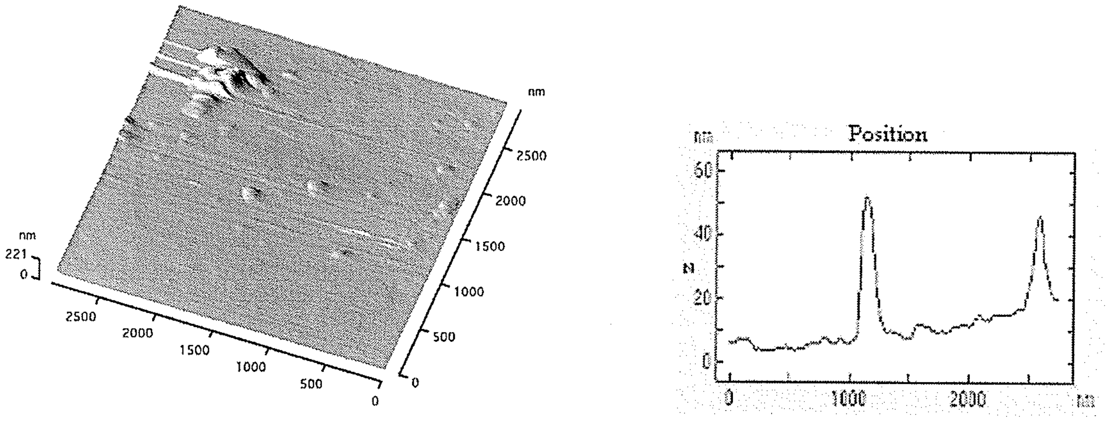
The horizontally-placed mica/fulvic-acid samples from the second experiment after 3.5 h and 6 h had the same aspect as the equivalent samples in the first experiment.

There were no significant morphological differences between equivalent samples imaged in the first and second experiments.

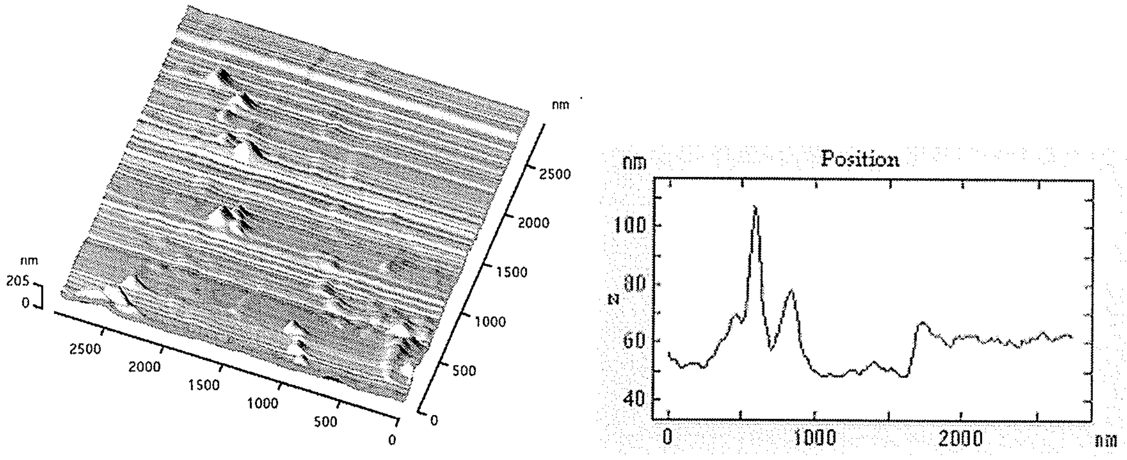
5.1.3. Study of Soil HA adsorption on mica over a 24 h period.

Images of the humic-acid layer deposited on the muscovite surface from solution with a concentration of 0.4 mg/L are presented in Figs. 5.5 and 5.6. Fig. 5.5 shows samples obtained by immersing the mica plates vertically in solution and removing them one by one after 5 minutes, 50 minutes, 3 h, 19 h and 24 h interaction time. Fig 5.6 presents the mica plates immersed horizontally in the same solutions and removed at the same intervals as the vertical samples.

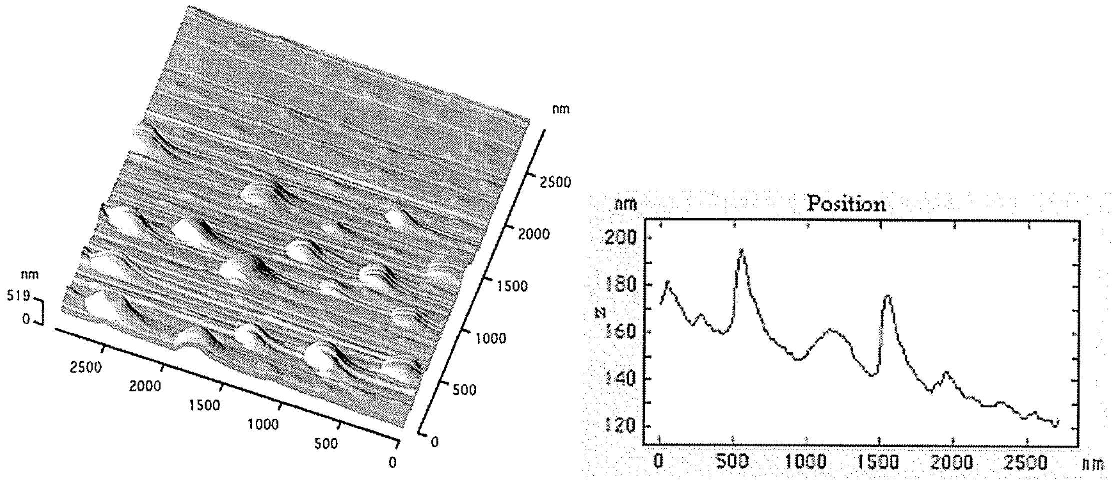
A. Vertical samples



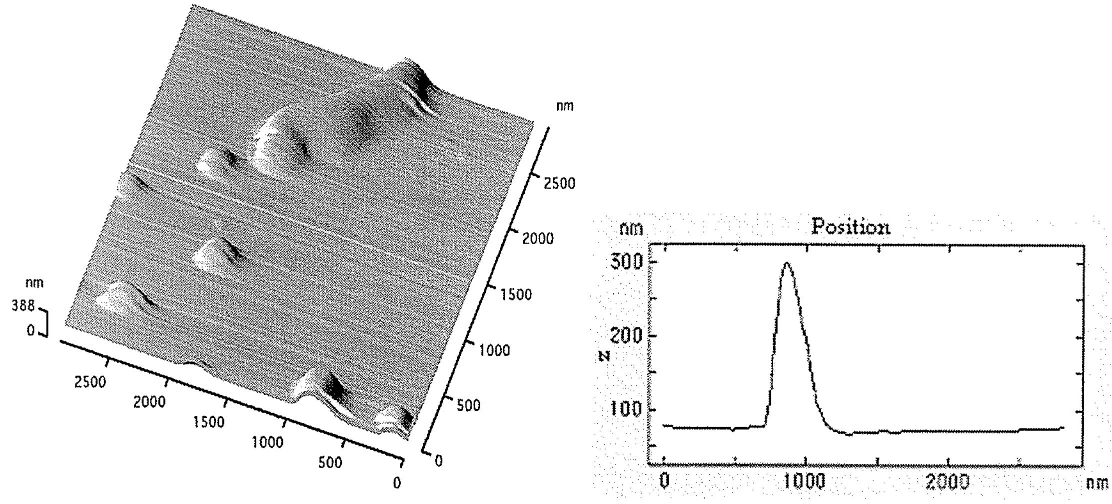
a) t = 5 minutes



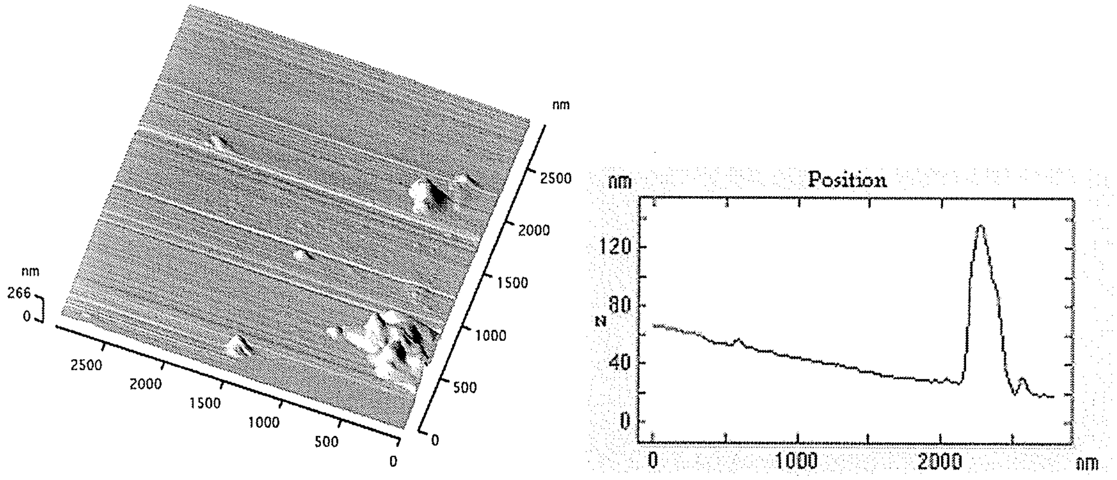
b) t = 50 minutes



c) t = 3 h



d) t = 19 h



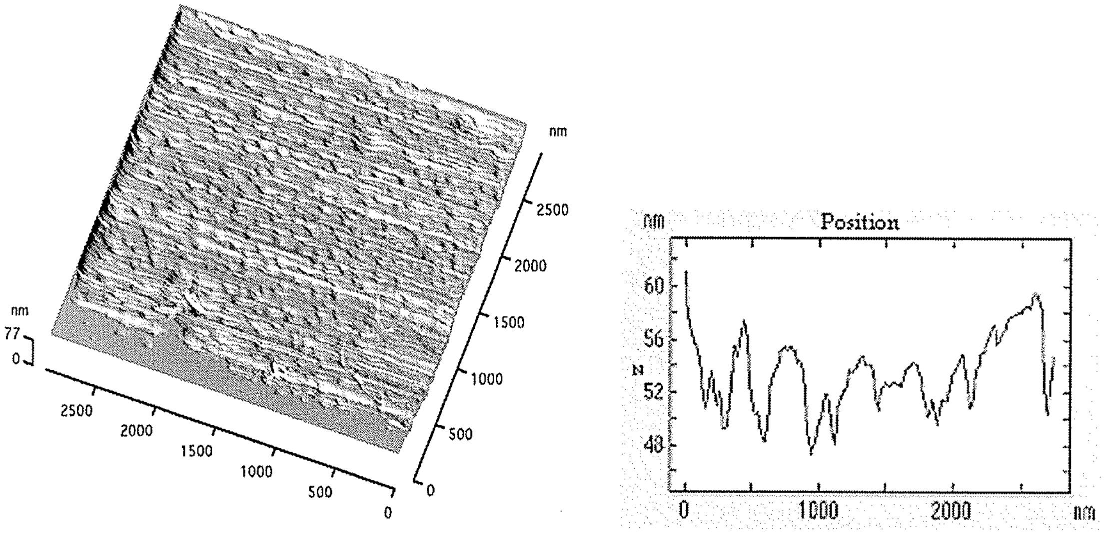
e) t = 24 h

Figure 5.5. *3D images and line profiles of Soil HA on mica surface as a function of time: a) t = 5 minutes; b) t = 50 minutes; c) t = 3 h; d) t = 19 h; e) t = 24 h. Samples placed vertically in solution.*

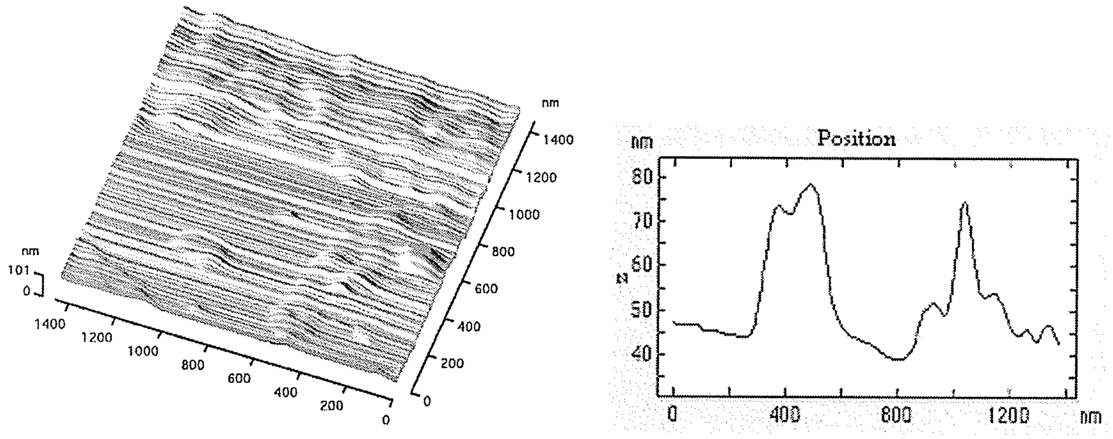
The vertically-placed mica/humic-acid sample removed after only 5 minutes interaction time shows extensive coverage of the mica surface by a thin organic layer with some individual disk-shaped aggregates (20-60 nm high x 300 nm diameter). Some large, amorphous agglomerations of these types of aggregates can also be observed. After

50 minutes, more material seems to be deposited on the surface; more agglomeration of aggregates is observed. After 3 h interaction time, two distinct types of aggregates are visible on the imaged surface: many larger aggregates, almost discoid in shape (40-60 nm high x 300 nm diameter) and also many smaller aggregates (about 5 nm high). After 19 h, the whole surface appears smooth, covered by a thick humic multilayer with fewer aggregates of a larger size: at 200 x 500 nm, the shape seems to be hemispherical. Sometimes elongated shapes (consisting of large aggregates) could be observed. The mica/humic-acid sample imaged after 24 h interaction time was smooth, with some agglomerations of large aggregates and some individual small particles visible.

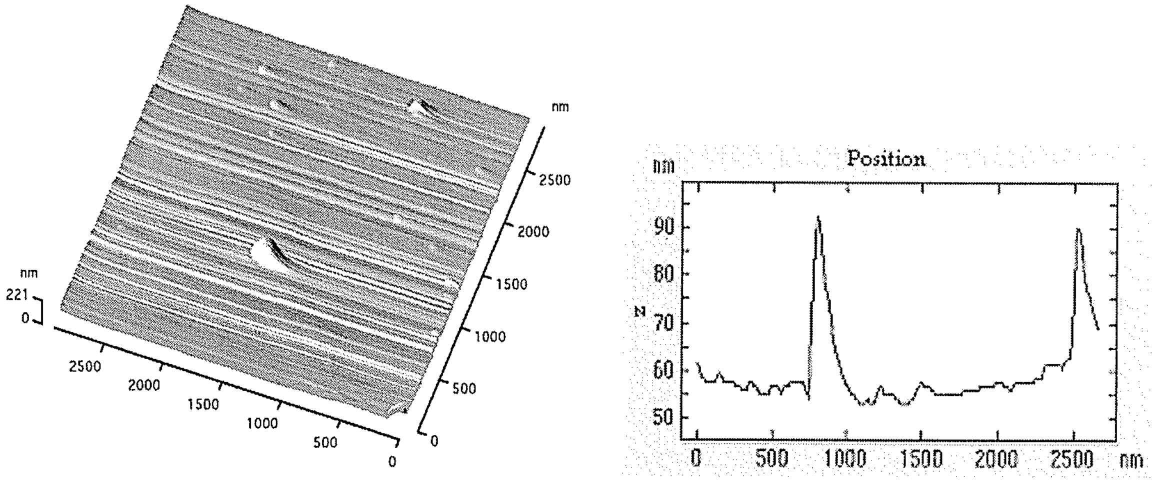
B. Horizontal samples



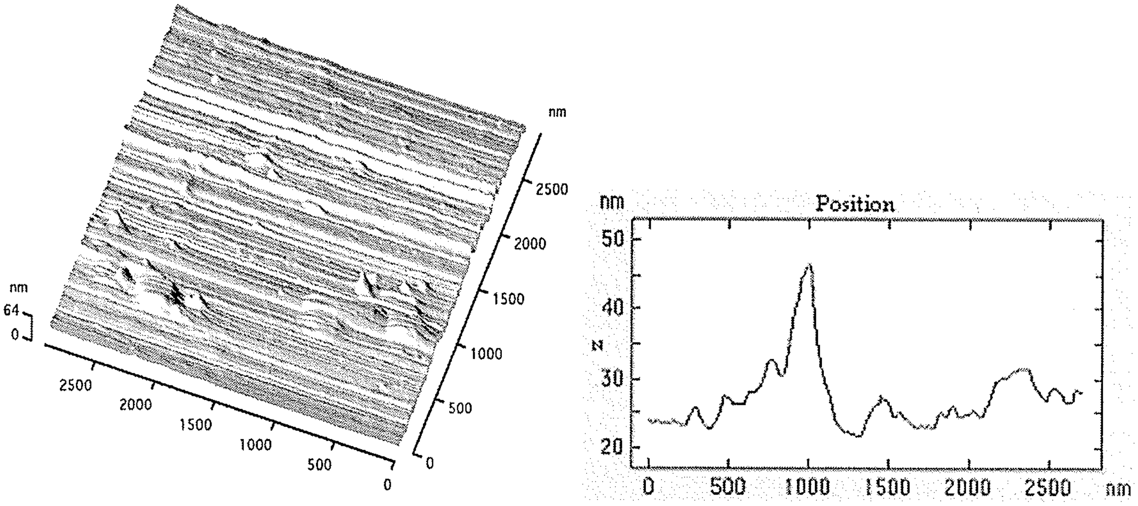
a) t = 20 minutes



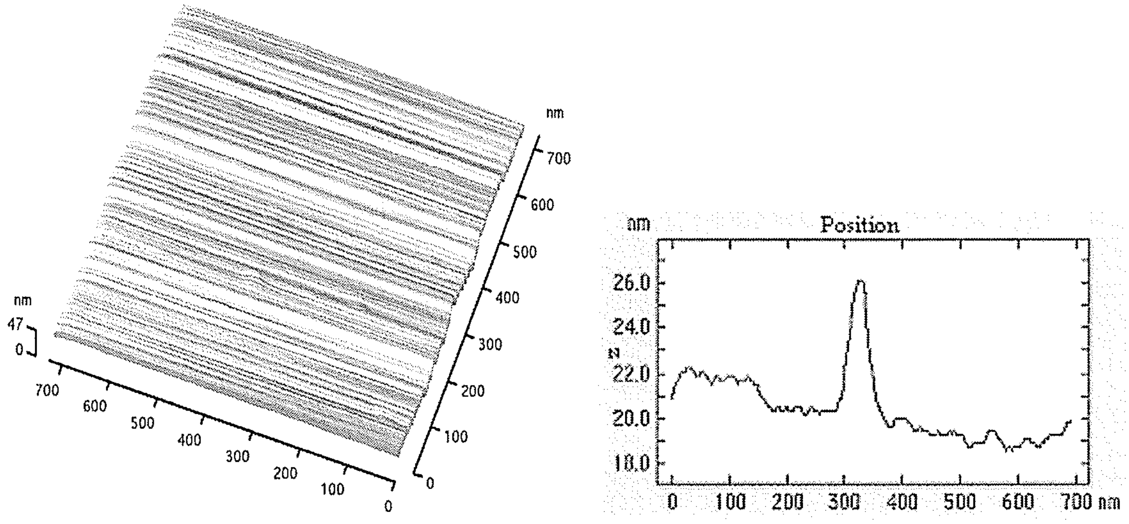
b) t = 50 minutes



c) t = 3 h



d) t = 19 h



e) t = 24 h

Figure 5.6. *3D images and line profiles of Soil HA on mica surface as a function of time: a) t = 20 minutes; b) t = 50 minutes; c) t = 3 h; d) t = 19 h; e) t = 24 h. Samples placed horizontally in solution.*

The horizontally-placed mica/humic-acid sample removed after 5 minutes interaction time was accidentally damaged during imaging and replaced with a sample with 20 minutes interaction time. The mica/humic-acid sample collected after 20 minutes interaction time shows extensive coverage of the mica surface with small disk-shaped individual aggregates (4-8 nm high x 50-200 nm diameter). After 50 minutes, multiple layers of the small aggregates form a veritable relief and the variability of the profile is considerably higher. After 3 h, the whole surface appears smooth, a thick humic multilayer with occasional large aggregates (40 x 200 nm). The image of the sample collected after 19 h interaction time is similar to the previous image: a smooth surface, which was considered to indicate a thick organic layer, with some small (4 nm high) and large (20-40 nm high) individual aggregates. The image of the surface appears essentially unchanged after 24 h interaction time. For the fulvic-acid experiment, it is difficult to appreciate if any change in the thickness of the organic layer occured, but no significant difference in the surface topography was observed for interaction times longer than 3 h.

5.2. Discussion.

5.2.1. Topographic observation of fulvic-acid/mica and humic-acid/mica surface-interaction, for a 24 h period.

After a short interaction time (5 minutes), deposition of fulvic acid occurs both on the vertically-placed mica surface and on the horizontally-placed mica plate. The amount of fulvic acid deposited on the horizontally-placed mica surface is larger than the amount of fulvic acid deposited on the vertically-placed mica plate (the abundance of the individual aggregates is significantly higher). The morphology of the fulvic-acid aggregates appears the same on both samples: small disk-shaped aggregates, 4-20 nm high and 40-100 nm diameter. After 20 minutes, the horizontally-placed mica surface appears completely covered with multiple layers of small aggregates, which give the surface a smooth appearance, with occasional larger aggregates (20-40 nm high x 300 nm diameter). After 2 h the whole surface of the horizontally-placed sample appears smooth, covered by a featureless humic multilayer whose thickness is difficult to evaluate. There is no significant change in the surface morphology up to 24 h. The appearance of the vertically-placed mica surface after 2 h interaction time is similar to the horizontally-placed sample, but the topography of the surface is more variable. At some point between 2 h and 3.5 h interaction time, regular "rod-shaped" (40 x 200 nm x 1000 nm) aggregates are sorbed on the vertically-placed mica surfaces. These large aggregates can also be observed in samples collected after 6 h interaction time, and in samples at 18 h, some of the large aggregates present seem to retain some of the elongated character of the "rod-shaped" form. No sign of the "rod-shaped" form was seen in the horizontal samples at any interaction time. After 24 h, a thick apparently smooth surface is observed, with a few features which seam to be clusters of disk-shaped aggregates in both vertically and horizontally placed samples.

Trends similar to those observed for fulvic-acid interaction with the mica surface were also observed for the humic-acid experiment. Extensive coverage of the mica surface with a thin organic layer, with some individual disk-shaped aggregates (20-60 nm high x 300 nm diameter) as well as some large agglomerations can be observed after 5 minutes interaction time on the vertically-placed sample. The similar horizontally placed sample was not available for imaging (it was damaged during manipulation), but the horizontally-placed sample removed after 20 minutes interaction time shows a similar image (with more humic material deposited), which can be due to both the horizontal positioning and the increased contact time. After 50 minutes, more material was deposited on the surface; more agglomeration of aggregates is observed, with images for the vertically-placed and horizontally-placed samples not significantly different. The amount of organic material deposited on the mica surface for both vertically- and horizontally-placed samples is higher than for the similar fulvic-acid samples and the humic aggregates are considerably larger (4-20 nm high for fulvic acid compared to 20-60 nm high for humic acid). The shape of the fulvic aggregates also differs slightly from the shape of the humic aggregates (although on the images, they appear deceivingly similar): the ratio between height and diameter is between 1: 10 and 1:5 for the fulvic-acid aggregates and about 1:5 for humic-acid aggregates, which makes the humic aggregates more rounded. This difference may be significant, reflecting a higher hydrophobicity of the humic aggregates.

After 3 h interaction time, the surface of horizontally-placed samples appears smooth, covered by a thick humic multilayer with occasional large aggregates. The image of the surface appears essentially unchanged in all samples collected up to 24 h interaction time. As in the fulvic-acid experiment, changes in the thickness of the organic layer are difficult to evaluate, but no significant difference in the surface topography is observable for interaction times longer than 3 h. For the vertically-placed samples a trend similar to that seen in the fulvic-acid experiment is observed: at 3 h interaction time, two types of aggregates are visible on the surface: many small discoid aggregates (about 5 nm high) and many larger disk-shaped aggregates (40-60 nm high x 300 nm diameter). Only one type of aggregate (the larger ones) was visible in the analogous fulvic-acid samples, and the shapes of the large aggregates were different (elongated for fulvic acid, as opposed to almost rounded thick disks for humic acid). The formation of two types of aggregates in the humic-acid samples suggests some type of fractionation of the humic fraction in solution, with different fractions forming aggregates of different sizes, and segregation of these fractions at micro-scale upon interaction with the surface. It is difficult to decide if the fractionation is due to differences in chemical character (hydrophobic/hydrophilic) or aggregation characteristics (ability to interact with other components of the humic-acid fraction in solution and form larger aggregates). The difference in shape between the large aggregates from the fulvic-acid experiment and the large aggregates in the humic-acid experiment could be due to a higher hydrophobic character for the large humic aggregates. After 19 h, the whole surface of the vertically-placed mica/humic- acid sample is covered by a thick humic multilayer with a few much rounder aggregates (200 x 500 nm) and some elongated form consisting of round large aggregates. At 24 h interaction time, a smooth surface with some agglomerations of large aggregates and some individual small particles was observed, very similar in topography to the corresponding, horizontally-placed sample.

5.2.2. Experimental Reproducibility

In order to evaluate the reproducibility of the data collected, the fulvic-acid/mica experiment was done in duplicate. There was good reproducibility of the results and relevant examples of the most interesting (and potentially controversial) images from the second experiment are presented in section 5.1.2.

The samples collected after 6 h in experiment # 2 (Fig. 5.3) show the same relation between the vertically-placed sample and the horizontally-placed sample as in the first experiment, and the unusual large elongated aggregates observed in the first experiment for the vertically-placed sample are present, although in the second experiment they are less abundant. It should be noted that perfect reproducibility is not expected, because of the intrinsic spatial variability of the samples, but the main features were observed at similar times and for similar positioning of the sample in the solution.

In the second experiment, an additional sample was collected after 3.5 h interaction time in an attempt to better approximate the moment of large-aggregates sorption on the vertically-placed mica surface. It is remarkable how well the 3.5 h sample from experiment # 2 fits in the sequence of events between the images for samples collected at 2 h and 6 h in experiment # 1 (see Fig. 5.4.). The observation of the 3.5 h sample from the second experiment allows us to place the first apparition of the large aggregates at somewhere between 2 h and 3.5 h, and to appreciate that the aggregates do not increase in size after sorption to the surface: they probably have the same size from the moment they are formed in solution and then become attached to the surface, up to the moment when they either desorb at a later moment or are "buried" by the additional later deposition of smaller organic aggregates. The large aggregates are very likely formed in solution and then adhere to the surface of the vertical samples. If they were formed by interaction of the humic/fulvic molecules with the interface their rounded shape would be very difficult to explain (a rounded particle in a water-based solution suggests "water exclusion" of the constituent material).

5.2.3. Difference in surface morphology of vertically-placed and horizontally-placed samples

There are significant differences between trends for vertically-placed and horizontally-placed samples: more organic material was deposited on the horizontal surfaces, at least at relatively short interaction times (under 2 h) and the organic layer formed was considerably smoother, with less features than the one formed on the vertical surfaces. These observations suggest that coagulation/sedimentation process probably predominates, at least in the early stages of the interaction process. The first layer(s) deposited on the mica surface appears to have similar features for both the vertically- and the horizontally-placed samples, differing only in the degree of coverage of the surface: small disk-shaped aggregates, about 4 nm high and 40 nm in diameter. The featureless appearance of the thick organic layer deposited on the horizontal mica surfaces, which does not seem to change in time after 2 h, may be due to sorption of more hydrophilic humic molecules on the first layer(s) of humic molecules in an uncoiled conformation, whereas the observation of large rounded aggregates on the vertical samples may be due to adsorption on the mica surface of aggregates of more hydrophobic humic fractions excluded from the water environment. This could explain the apparently paradoxic situation in which large particles seem to adhere to vertical surfaces while smaller ones sediment on the horizontal surface. Significant differences in density between humic aggregates formed in solution have never been reported and would not be easy to support theoretically in the absence of any chelating ions. An alternative explanation is that the large aggregates visible in the vertically-placed samples between 3 h and 18 h interaction-time were also deposited on the horizontal surfaces, but were not observable because of the much more significant deposition of smaller aggregates: the large aggregates were literally "buried" in the mass of smaller sedimented aggregates. This explanation seems less satisfactory because no trace of the larger aggregates was found on any of the horizontal samples imaged, at any of the locations. Differences between trends illustrated by vertically-placed samples and horizontally-placed samples are consistent throughout the two repetitions of the fulvic-acid/mica interaction experiment and the humic-acid/mica interaction experiment, and they must represent significant characteristics of the surface-interaction process.

5.2.4. Observed differences between fulvic-acid/mica and humic-acid/mica interaction

There are more similarities than differences between fulvic-acid and humic-acid behavior in their sorption on the mica surface, which is remarkable if one considers their differences in solubility, average molecular weight and molecular-weight distribution, and hydrophobic/hydrophilic-component content. Similar overall trends were observed for both fulvic-acid and humic-acid interactions with the mica surface:

i) in both cases, there is rapid sorption of organic material on the mineral surface;

ii) more accumulation of organic material can be observed for the horizontally-placed samples in both cases, at least for the first 2-3 h, suggesting a predominance of coagulation/sedimentation processes over sorption processes at the first stages of the interaction process;

iii) the organic aggregates have similar, discoid shape;

iv) for both fulvic acid and humic acid, there appears to be a time interval during which large aggregates tend to sorb to the mineral-organic surface previously created, probably by hydrophobic effects;

v) for both cases, the 24 h period seems to be a good approximation for the time interval in which a "steady state" is approached.

Compared with such similarities, the differences between fulvic acid and humic acid behavior during interaction with the mica surface seem more subtle, but none-the-less significant:

i) the amount of organic material deposited on the mica surface, in both vertically- and horizontally-placed samples, is higher for humic-acid than for fulvic-acid samples, and

ii) the humic aggregates appear considerably larger;

iii) the fulvic aggregates are flatter disks, whether the humic aggregates appear more convex (the ratio between height and diameter is between 1: 10 and 1:5 for fulvic-acid aggregates, and 1:2 to 1:5 for humic-acid aggregates), a difference which may reflect higher hydrophobicity of the humic aggregates. Also,

iv) there is no morphological evidence for fulvic-acid fractionation during sorption (which does not mean that fractionation does not occur, just that it is not reflected by the morphological evidence), but there seems to be morphological evidence for fractionation during sorption of humic acid (two types of aggregates, differentiated by size can be seen clearly at some stage during the sorption process in the images of vertically placed samples). This process may also be related to the environmental conditions: the fulvic-acid/mica interaction was studied in pure water, with no background electrolyte, whereas the humic-acid/mica experiments were done in the presence of 0.01 mol/L NaCl, which can be considered as stronger sorption conditions, as demonstrated in the first part of Chapter 4. Zhou et al., 2001, report fractionation for the sorption of fulvic acid on goethite and mention that fractionation was higher at strong sorption conditions.

5.3. Conclusions

i) Images of mica-humic surface complexes obtained by AFM can be used for qualitative study of the interaction mechanism and kinetic of humic- and fulvic-acid sorption on a mica surface, a simpler model for sorption of humic/fulvic-acid on clay particles.

ii) Differences in the surface topography of vertically-positioned and horizontally-positioned humic/fulvic-mica samples reflect the respective contributions of different sorption processes to the mineral and mineral-organic surface versus coagulation/sedimentation to the overall interaction between the mica surface and humic/fulvic-acid fractions.

iii) There is good experimental reproducibility.

iv) The main disadvantage of the method is that the thickness of the organic layer (and thus the absolute amount of humic material) deposited on the mica surface cannot be measured with any precision. As a consequence, AFM imaging could not be used for endpoint determination (as was hoped) and did not allow precise determination of the time interval necessary to attain the "steady-state" in the humic-mica system.

v) Fulvic acids and humic acids show some common characteristics in the interaction with mica surface: initial sorption was fast in both cases, and seemed to be dominated by coagulation/sedimentation processes, at least for the first 2 to 3 h. Large aggregates were sorbed to the mineral-organic surface, probably by hydrophobic forces, in an intermediate step of the interaction process, and were either desorbed at some later time or "buried" by deposition of smaller aggregates, for final formation of a thick organic layer, smooth in appearance which seems to be the normal appearance for the "steady-state" system attained at about 24 h interaction time.

vi) Differences between humic acid and fulvic acid were also evident during the interaction with the mica surface: fulvic acid tended to form smaller flatter disk-shaped aggregates, whereas the humic-acid aggregates were larger and more convex in shape. The organic material seemed to accumulate quicker on the mica surface, both by sorption as well as by coagulation/sedimentation, from the humic-acid solution. Fractionation was only apparent morphologically for humic-acid sorption on mica under stronger sorption conditions (0.01 mol/L NaCl used as background electrolyte).

AFM is a useful method in the study of humic/fulvic-acids interactions with different mineral surfaces and cations in solutions.

CHAPTER 6. MORPHOLOGICAL OBSERVATION OF KGa-1/HA COMPLEXES USING AFM AND SEM

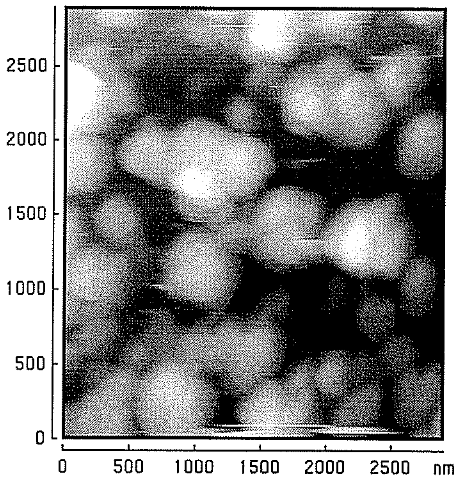
In this experiment, AFM images of well-ordered kaolinite (KGa-1) at different magnifications were obtained and compared with images of KGa-1/HA complexes deposited on a smooth glass surface (the glass surface was used as a flat mechanical support for AFM imaging). The complexes were formed in a 4 mg/L HA solution, in the presence of 0.01mol/L NaCl as background electrolyte, at a KGa-1/HA ratio of 10/1 and then deposited and air-dried on the glass support. The purpose of the experiment was to describe the morphology of the KGa-1/HA complex and to get information about the degree of coverage of the clay surface, thickness of the organic layer, and surface topography of the complex.

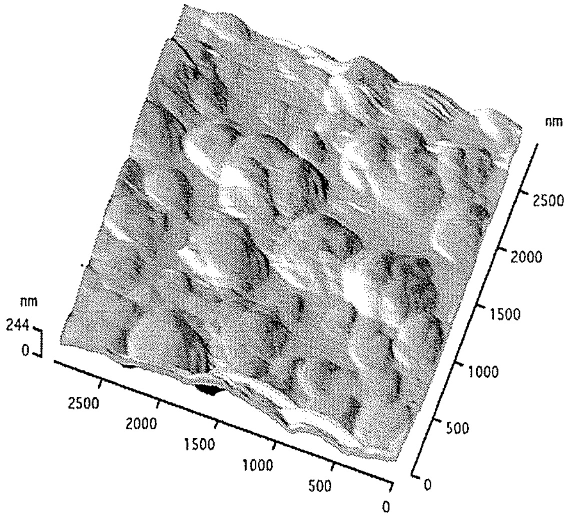
The well-ordered kaolinite from the Source Clay Collection of the Clay Minerals Society (KGa-1) was used in this experiment because it has large well-formed crystals with smooth surfaces, some of which should have similar chemistry to the mica (muscovite) surface. Images of crystals of the Source Clay KGa-1 by both SEM and AFM were published by Zbik and Smart, 1998. They show well defined plane surfaces which appear smooth across 200-500 nm regions, with cascade-like growth structures forming steps with 50-100 nm dimensions. In AFM images, the edges of the kaolinite sheets and steps appear bevelled and relatively smooth, rather than sharp and at right angles to the basal surface (as they appear in SEM images). This effect is an artifact in the AFM images caused by the tip dimensions (Zbik and Smart, 1998).

In view of previous work (see Chapter 4) involving AFM imaging of HA/FA sorbed onto mica surfaces we know that the sensitivity of the method is high enough to allow visualization of humic or fulvic aggregates attached to the clay surface. The surfaces of kaolinite particles are similar in chemical structure and properties to the mica surface used in the experiments described in the first two parts of this chapter. Visualization of the increased roughness due to the deposition of an organic layer and/or attachment of individual aggregates to the clay surfaces and clay-sheet edges should be well in the resolution of the AFM. The KGa-1/HA complex obtained in this experiment is expected to have a significant humic-acid content, as proved by the C content determined for the KGA-1/HA (see Chapter 7): 7.54 % C translates to about 15% HA content of the complex.

6.1. AFM study

6.1.1. AFM images of well-ordered kaolinite (KGa-1) at two different magnifications





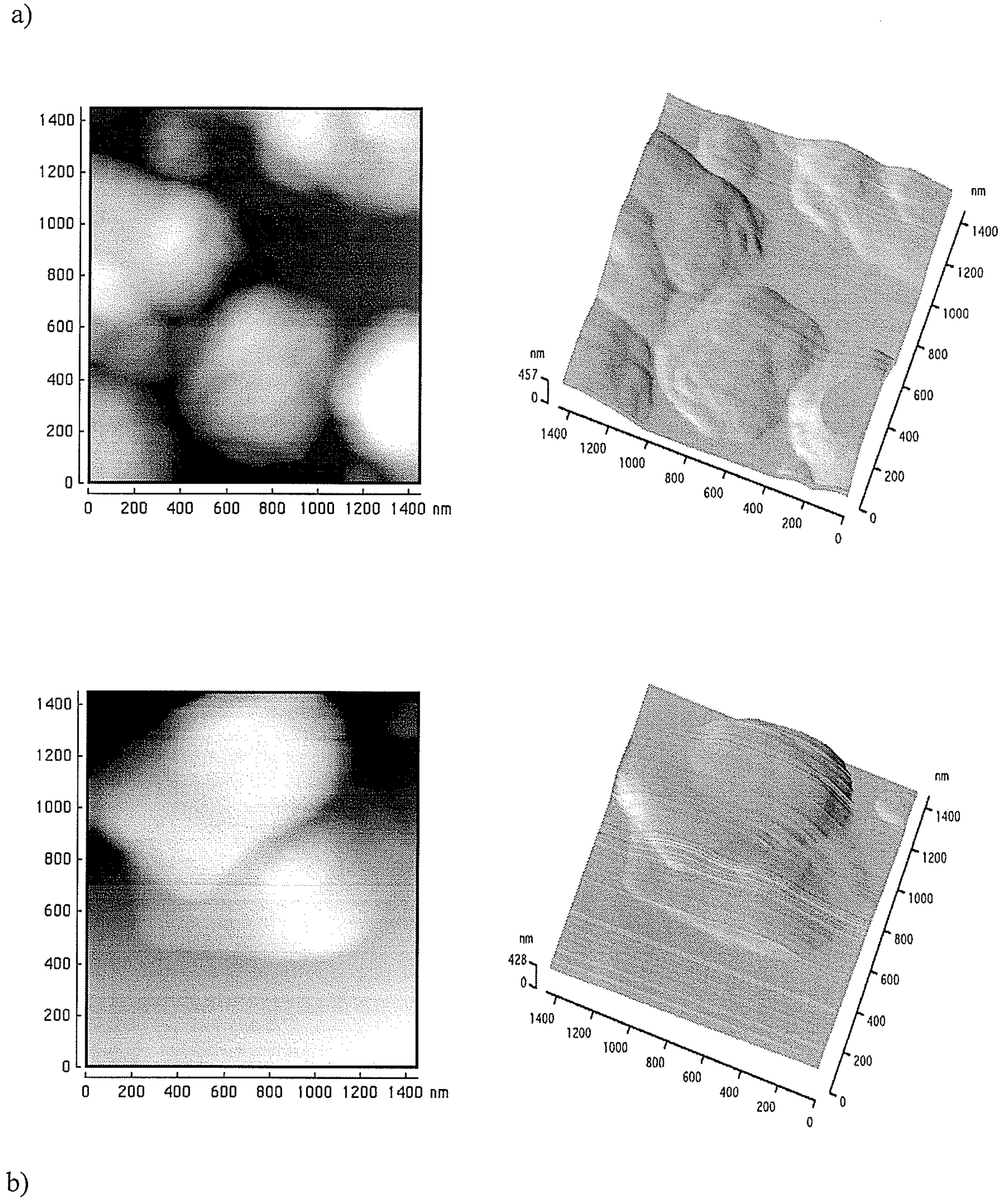
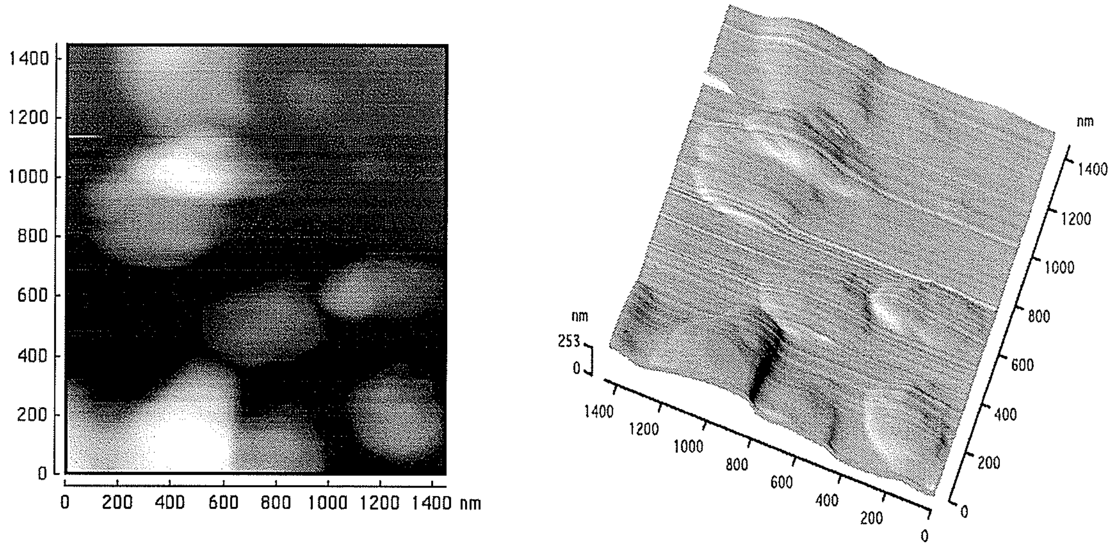
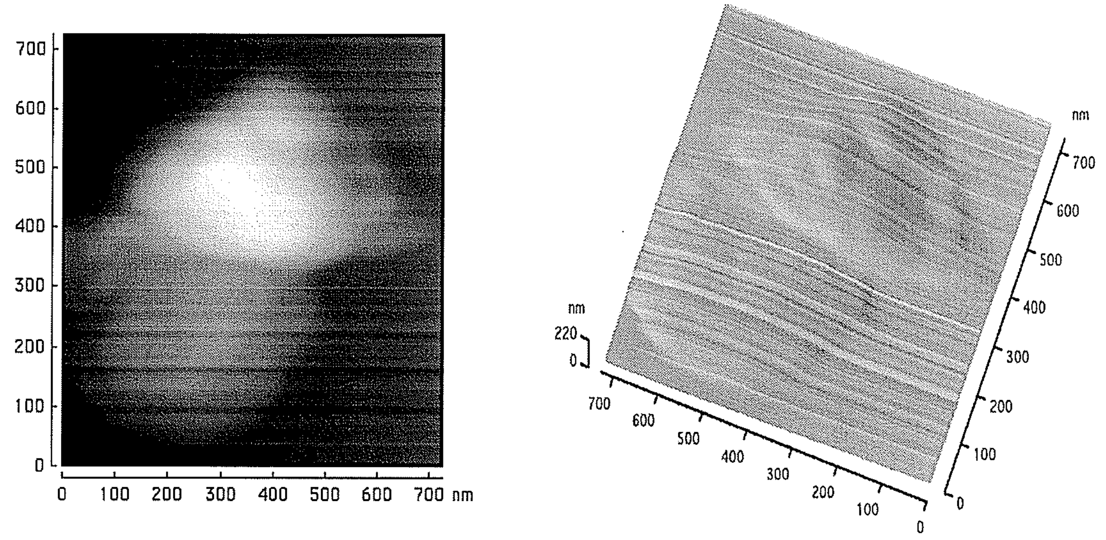


Figure 6.1. *2D and 3D images of well-ordered kaolinite (KGa-1) at two different magnifications: a) one location and b) two different locations*

6.1.2. AFM immages of kaolinite/humic-acid complexes (KGa-1/HA)



a)



b)

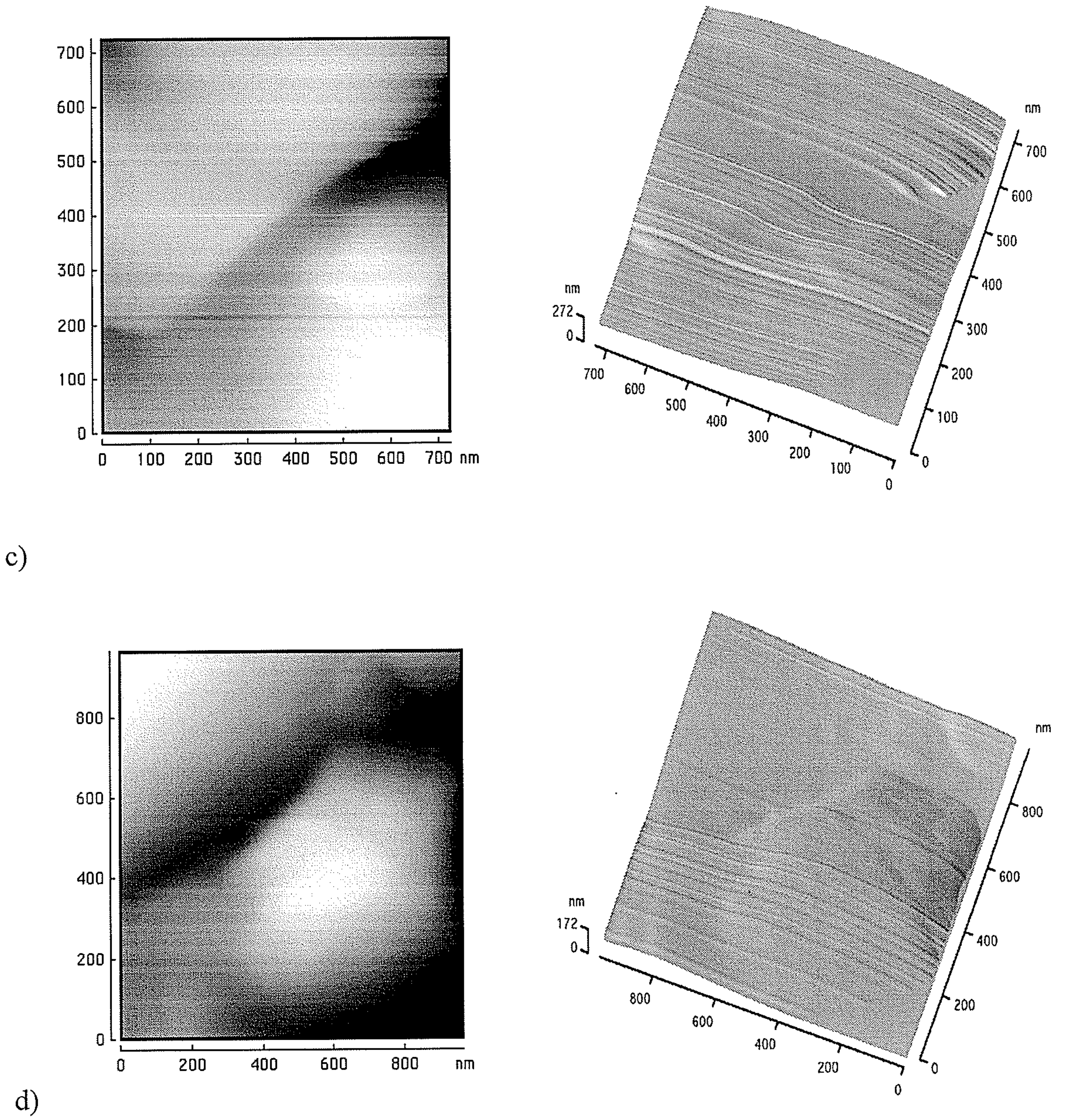


Figure 6.2. *2D and 3D images of well-ordered kaolinite (KGa-1)-humic acid complexes at three different magnifications. 3 different locations:a) and b) same location, c) and d) two additional locations.*

6.1.3. Discussion

The images of KGa-1 obtained in this experiment (Fig. 6.1.) are similar to those previously published by Zbik and Smart (1998). The images obtained for the KGa-1 complex do not differ in any discernable way from the images of the pure kaolinite crystals. No humic aggregate could be found on the clay surface and no increase in roughness of the clay surface was observed. The edges of the clay sheets appear as well defined as in the images of the initial clay samples, with no apparent proof of organic coating. No organic aggregates could be seen separate from the clay crystals (on the background glass surface used as support), although if the humic aggregates were too small (which is not expected because the humic acid aggregates sorbed on mica under similar conditions are in the order of hundred of nanometers, see Chapter 4), they may not be easily observable in samples dominated by large clay particles. The humic acid (representing at least 15% by weight of the sample) is invisible in AFM images. These observations are difficult to explain, but apparently similar observations result from the SEM study of the kaolinite-humic acid complexes (see 6.2.). No organic layer or organic aggregates were distinguishable in those studies either.

A very thin organic layer would not be visible under SEM conditions, but it should still modify the appearance of surfaces and edges in the AFM images. A very even distribution of organic material on a natural clay surface which is believed to have increased chemical reactivity at sheet edges and imperfections, is not easy to imagine. The freshly cleaved mica surface, which is theoretically much more homogenous in chemical character, does not induce formation of a thin even layer of organic matter, as proven by previous experiments (Chapters 4 and 5).

An alternative explanation could be that the bulk of the organic material is bound between (two) adjacent clay sheets (intercalated). This could explain why the organic layer is basically invisible to both SEM and AFM imaging methods, while the clay-humic complex has a relevant content of organic material and sorptive properties consistent with the presence of organic domains. The space between adjacent clay sheets could also be reasonably argued to be even more reactive than sheet edges toward humic molecules, especially if change in entropy is considered to be the main driving force in the interaction between the clay and the humic aggregates.

6.2. SEM study of KGa-1/HA complexes

6.2.1. Clay Standards for comparaison

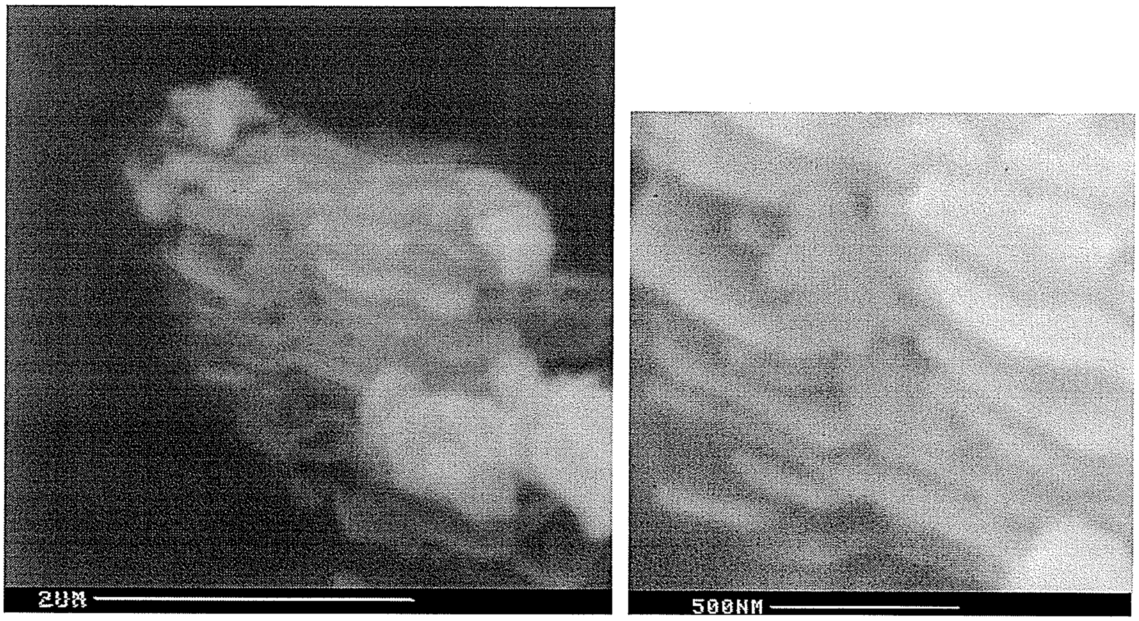


Fig. 6.3. *SEM images of well-ordered kaolinite (KGa-1)*

6.2.2. Clay-humic complexes (KGa-1/HA)

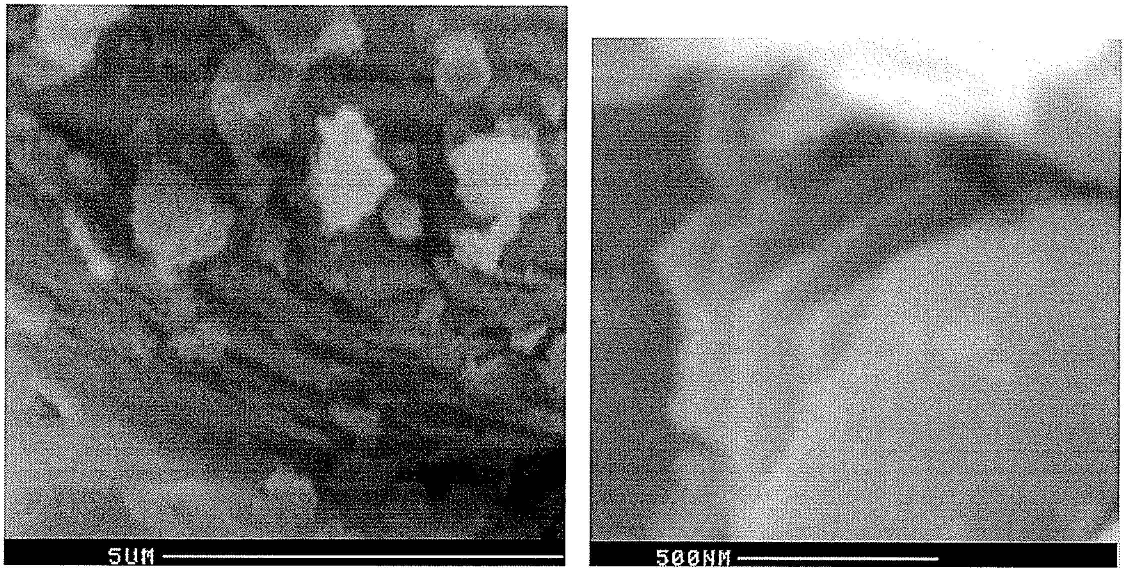


Fig. 6.4. *SEM images of well-ordered kaoliniet/humic-acid complex*

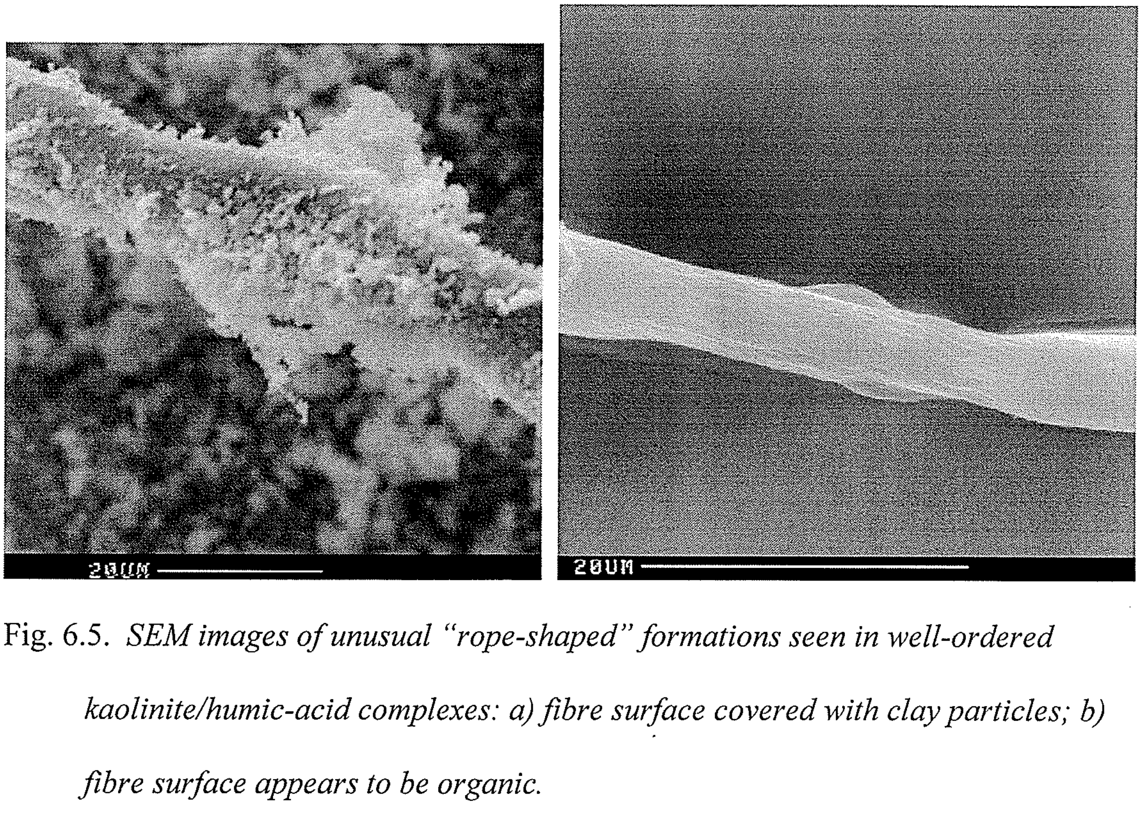


Fig. 6.5. *SEM images of unusual "rope-shaped" formations seen in well-ordered kaolinite/humic-acid complexes: a) fibre surface covered with clay particles; b) fibre surface appears to be organic.*

6.2.3. Discussion.

The morphology of individual clay minerals and humic fractions has been extensively studied using electron microscopy (TEM, SEM) (Stevenson and Schnitzer, 1981; Gu and Donner, 1992), but there are few studies of clay/humic complexes. The first image (to my knowledge) of a humic acid-montmorillonite complex is presented in a TEM clay-image atlas (Beutelspacher, 1975) and does not seem to differ significantly from other montmorillonite images in the same book. Synthetic clay/humic-acid complexes (Ohashi and Nakazava, 1996; Rothe et al., 2000), and natural clay/humic-acid complexes (Margesson et al., 1989; Laird, 2001) have since been studied. In most cases the morphology of the complexes was dictated by the morphology of the clay and the humic material was believed to be evenly distributed, as a thin layer on the clay surface, but there was little direct evidence of the humic layer conformation.

A complex of humic-acid/well ordered kaolinite (KGa-1/HA) was imaged using SEM in the present study and compared with the SEM image of the pure clay. This experiment was done in order to relate morphological features of the complex with the conditions under which the complex was synthesized, and to compare the SEM images with the images obtained by AFM. AFM and SEM observations for well-ordered kaolinite (KGa-1) were compared previously by Zbik and Smart (1998).

Well-ordered kaolinite was chosen for the present study for two reasons: i) it provides a very simple consistent image (which was published before), which allows one to distinguish any potential changes with a great degree of accuracy and ii) it has large smooth crystals (hexagonal sheets with edges of 200-1000 nm) appropriate for AFM imaging (see Chapter 4), which permits comparison of images obtained by SEM and AFM on similar samples.

SEM images obtained for the pure well-ordered kaolinite are similar to those published by Zbik and Smart, 1998 (Fig. 6.3). The SEM images of the complex of humic acid and well- ordered kaolinite are similar to images obtained for the pure clay, despite an approximately 7.5 % C content of the sample (see Chapter 7). No coating of the clay sheets and no agglomeration of humic material was observed. The results are similar to TEM results reported by Ohashi and Nakazava (1996) for a montmorillonite/humic-acid complex. The images are easier to interpret for a kaolinite complex than for a montmorillonite complex because the kaolinite crystals are larger and of more regular shape, with smooth surfaces and sharp edges. A thin layer of organic coating on the clay crystals would be transparent for the electron beam, but a certain new "roughness" of the clay surface (Margesson et al., 1989), a rounding of the clay crystal edges or accumulation of globular material should be observable, and was reported in a study of natural clay-humic complexes (Laird, 2001). Even if a thin organic layer on the clay surfaces is not visible by SEM, the organic coating is expected to decrease the count frequency for elements such as Si and Al, which are specific to the clay surface (Margesson et al., 1989). No such effect was found in this study.

Some unusual "rope-shaped" structures (Fig. 6.5) were observed in the humic-clay complex; these were not seen in the pure clay. Such "fibres" (Fig. 6.5. *a)*) have different dimensions (2-20 μm diameter and 50-200 μm length), appear hollow (they frequently "explode" under a concentrated electron beam at high magnification), and their surfaces show the same count frequency for Al and Si as the clay surfaces. A small number of those "fibres", the ones which are really very small (Fig. 6.5. *b)*), show a very low frequency count for typical clay elements (Si, Al), suggesting an organic nature. No explanation of the nature or formation process of these fibres is proposed, but similar structures have been found in natural clay-humic complexes (Laird, 2001).

6.3. Comparison of SEM and AFM observations.

Images of well-ordered kaolinite/humic-acid complexes (KGa-1/HA) obtained by SEM and AFM are expected to be similar becuse they both describe the morphology of the same materials. The images of well-ordered kaolinite/humic-acid complexes obtained by AFM and SEM in this study were found to be similar (see Fig. 6.2. and Fig. 6.4.). Relatively ordered stacks of large kaolinite sheets (300-500 nm sides) are easy to observe in both AFM and SEM images. The higher magnification offered by AFM should allow observation of humic aggregates on the surface of the clay particles similar the observations illustrated in Chapter 4 for humic-acid/muscovite complexes. SEM and AFM both fail to visualize the humic-acid component of the complex, although elemental analysis of the complex attests to the relatively high C content of the samples (about 7.5 % by weight).

The apparent absence of the humic coating from both SEM and AFM images is not considered proof that no association between the clay and the humic fraction takes place: the high C content would be difficult to account for in the absence of any organic particles in both the SEM and the AFM images. The failure of AFM and SEM to visualize an organic layer on the exposed surfaces of clay particles must be due to morphological characteristics of the clay-humic complex synthesized under particular conditions. The most plausible hypothesis at this point seems to be that most of the humic material is sorbed between kaolinite sheets.

An interesting observation is that both AFM and SEM imaging was done on dried samples (as was the TEM imaging mentioned at the beginning of this discussion). In contrast with the observations on dried samples, Rothe et al. (2000) were able to detect a specific form of montmorillonite-HA particle agglomeration at near-neutral pH, in fully hydrated form, using Soft X-ray Spectromicroscopy (STXM). Changes in the morphology of the clay-humic complexes from the hydrated form to the dried form are expected due to the different conformations of the fully hydrated forms of humic molecules which are predicted by computer models (Bruccoleri et al., 2001).

CHAPTER 7. CARBON CONTENT OF THE CLAY-HUMIC COMPLEXES FORMED BY THE SORPTION OF IHSS STANDARD SOIL HA/FA ON FOUR SOURCE CLAYS

The interaction between clays and humic substances is the basis of soil structure and properties (Stevenson, 1982). Experimental data suggest that the structure of humic materials (which varies with their source) highly influences their adsorption on clay surfaces, and the nature of the clay surface itself is responsible for the structure and sorptive properties of the resultant humic-clay complex (Chester et al., 1989;Murphy et al., 1990; Di Toro et al., 1991; Celis et al., 1997). In this experiment, model clay-humic complexes were synthesized using a diversity of well-characterized precursors (reference humic fractions and source clays) under controlled pH, ionic strength and clay/humic ratio and subsequently analyzed for C content and $^{13}C/^{12}C$ ratio. Synthetic model clay-humic complexes were used because they were expected to give the most meaningful information in terms of complex characteristics (Theng, 1979).

7.1. Choice of reaction parameters

One of the most challenging aspects of designing an experiment involving model clay-humic complexes involves choosing parameters for synthesis of the complexes which will reflect as closely as possible the prevailing environmental conditions and, at the same time, which will keep the reaction system uncomplicated enough to allow proper interpretation of the experimental data. The parameters which were considered in setting up the reaction system were: i) the nature of reactants (clay minerals and humic materials), ii) the concentration of the humic fraction, iii) the solution pH, iv) the presence of polyvalent cations and v) the ionic strength of the background electrolyte.

The diversity of humic materials and clay specimens used in clay-humic complex studies make experimental data difficult to compare and interpret. In this experiment, Standard Soil Humic Acid and Standard Soil Fulvic Acid from the IHSS collection (McCarthy et al., 1986) were used, toghether with four Source Clays from the Clay Minerals Society: well-ordered kaolinite (KGa-1, Georgia), poorly-ordered kaolinite (KGa-2), Na-montmorillonite (Swy-1), and Ca-montmorillonite (STx-1).

Experimental observations suggest that pre-treatment of clay specimens before the adsorption experiment may play an important role in its consequent surface reactivity toward humic mixtures (Evans and Russel, 1959; Schulthess and Huang, 1991). These observations are in accord with the prediction that the "real" surfaces of natural clays can be significantly modified from the ideal mineral surfaces (see section 2.1.6). In this experiment, the Source Clays were used as received from the Clay Minerals Society, without any pre-treatment.

The ratio (w/w) between the humic material and the clay was maintained at 1:10 throughout the experiment, in an attempt to reflect the composition of most mineral soils (The Canadian System of Soil Classification, 1987) and a high yield of the sorption process which was previously described in the literature (Filip and Alberts, 1994; Abu El-Fadl et al., 1994). The concentration of the humic fractions used varied from 400 mg/L to 2 mg/L. In most experiments, the solution pH was dictated by the weak acid character of the humic component. The pH was checked at the beginning and at the end of the reaction, but no significant differences between starting pH and the pH of the final solution were found. The pH was not corrected, in an attempt to limit the influence of ionic strength and added cations to the solution. It was previously observed that even small variations in the ionic strength of the solution, generated in an attempt to set the pH value, can significantly influence the sorption process (Tombacz et al., 1988;Tombacz et al., 1990). Polyvalent cations are known to play a major role in reactions between clay surfaces and humic fractions (Evans and Russel, 1959; Theng and Scharpenseel, 1975; Theng, 1976; Abu El-Fadl et al., 1994). In this experiment, no polyvalent cations were present, except the ones which originate from dissolution of the clay during interaction with the humic material. No background electrolyte was added in most reactions, in an attempt to minimize ionic-strength effects on the reaction system (see 2.4. 1.3.5. and results in Chapter 4). The effects of pH, polyvalent cations and background electrolyte were minimized in order to emphasize the role of the clay and humic structures and humic-fraction concentration on the sorption process.

7.2. Carbon content of Source Clays

Source Clay	%C
KGa-1	0.28
KGa-2	0.22
STx-1 (Ca-Mo)	0.17
Swy-1 (Na-Mo)	0.39

7.3. Carbon content of KGa-1/HA complexes

The experiment for well-ordered kaolinite and humic acid at all seven concentrations was done in triplicate in order to provide information on the reproducibility of the experiment.

HA conc. (mg/L)		400	200	100	40	10	4	2
%C in the solid phase	Exp.1	-	3.49	3.88	4.13	-	8.31	15.13
	Exp.2	4.45	4.16	4.00	6.53	9.29	9.12	8.89
	Exp.3	4.27	4.60	4.38	6.11	6.59	7.54	14.98

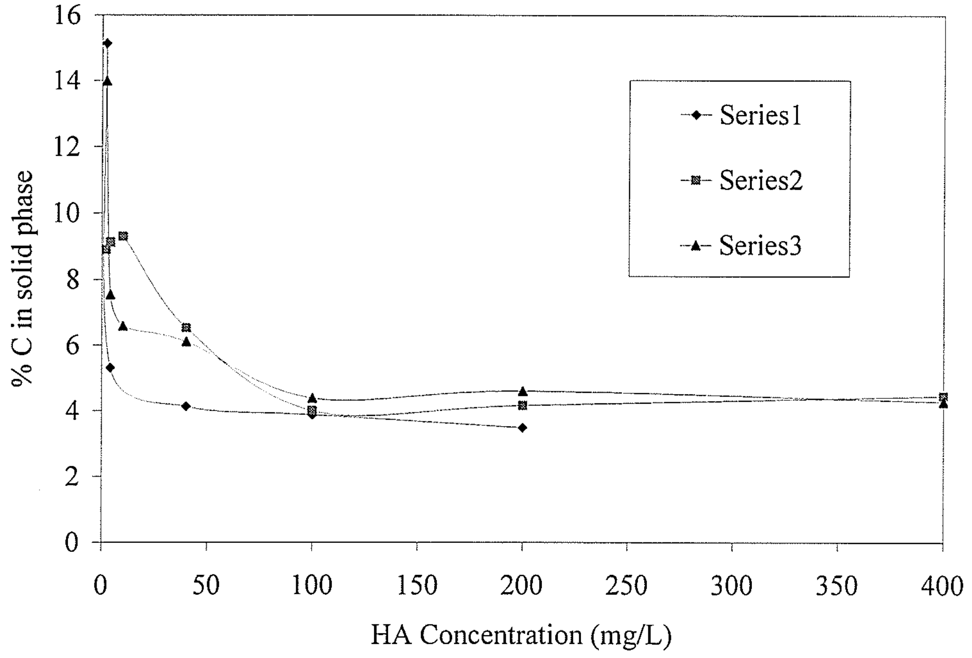


Fig 7.1. Soil humic-acid (HA) sorption on well-ordered kaolinite (K-Ga1).

Residual C concentration in the filtrate solution after the clay-humic reaction.

HA initial conc.(mg/L)	400	200	100	40
HA conc. after reaction (ppm C)	30.29	29.2	27.75	22.43

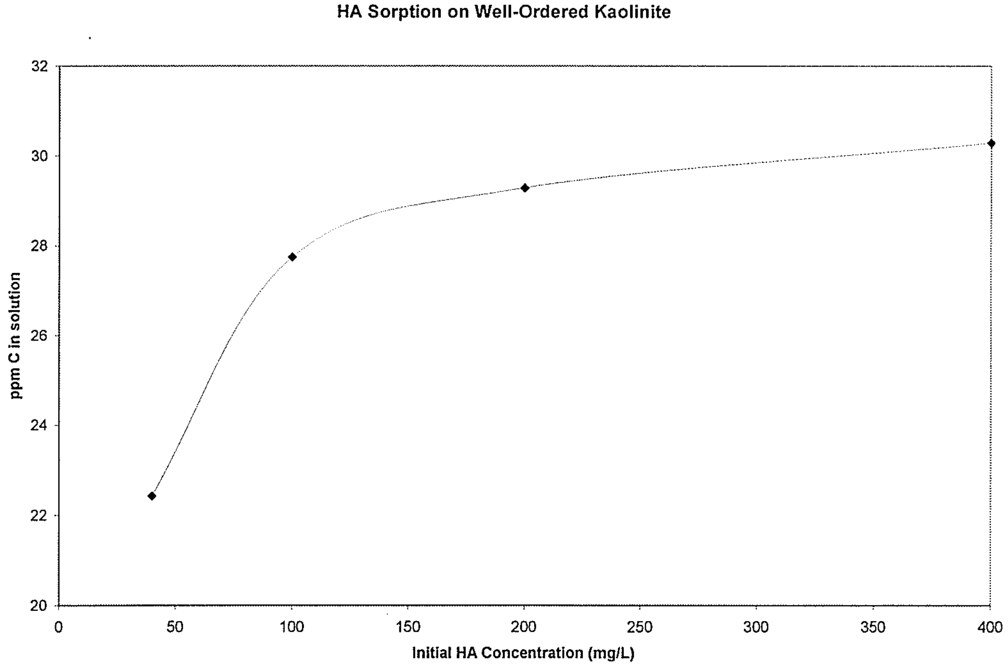


Fig. 7.2. C concentration in filtrate from HA sorption on KGa-1

7.4. HA complexes with different Source Clays

HA conc. (mg/L)		400	200	100	40	10	4	2
%C	KGa-1/HA	4.27	4.60	4.38	6.11	6.59	7.54	14.98
	STx-1/HA	4.03	4.34	4.47	4.29	7.99	11.92	24.34
	KGa-2/HA	5.14	4.42	5.21	4.64	5.63	5.58	14.98
	Swy-1/HA	4.85	4.74	4.84	5.08	5.04	6.94	-

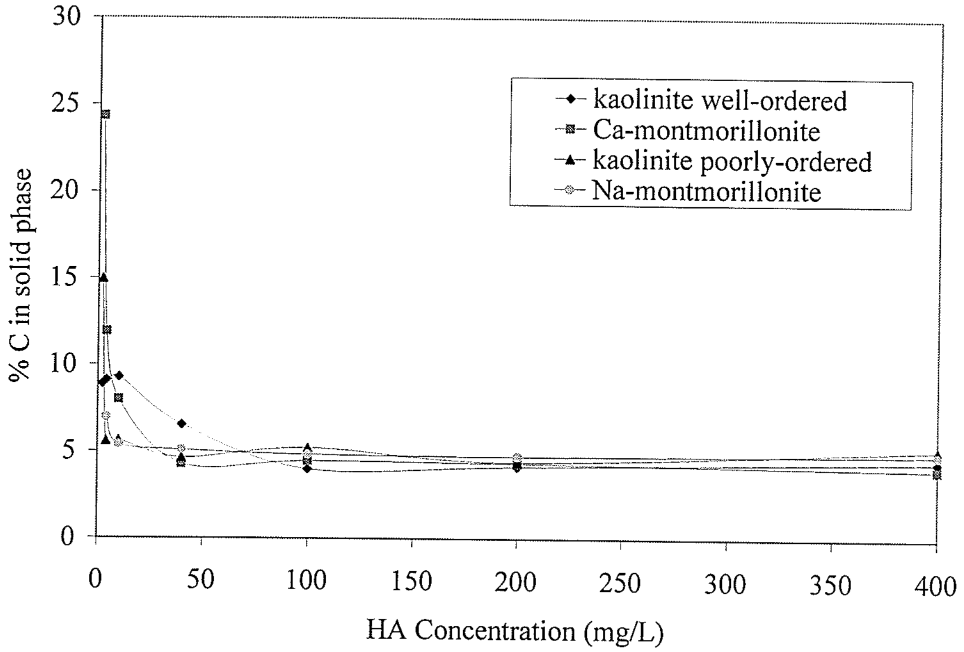


Fig. 7.3. Soil HA sorption on four Source Clays.

Residual C concentration in the filtrate solution after the clay-humic reaction (humic-acid concentration in the initial solution was 400 mg/L).

Clay-humic complex	C concentration in filtrate (ppm)
KGa-1/HA	30.29
STx-1/HA	42.04
KGa-2/HA	38.08
Swy-1/HA	31.24

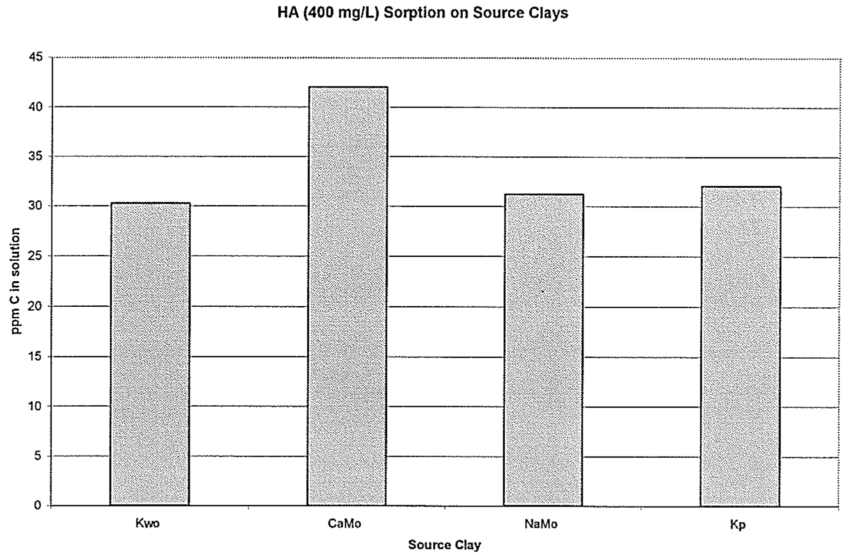
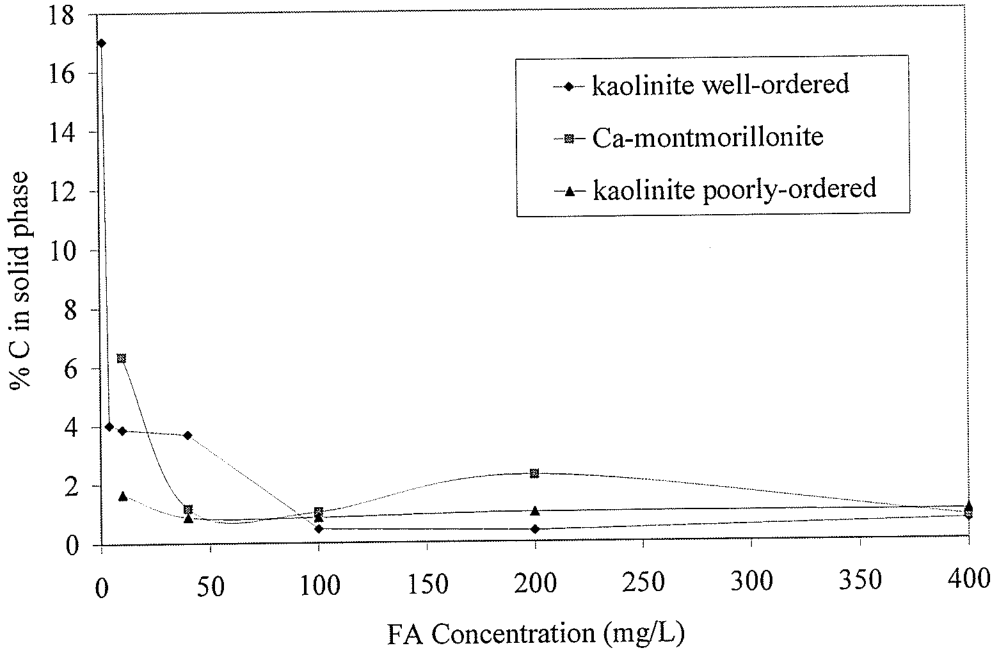


Fig. 7.4. C concentration in the solution from HA sorption on four Source Clays.

7.5. FA complexes with different Source Clays

FA conc. (mg/L)		400	200	100	40	10	4	2
%C	KGa-1/FA	0.68	0.37	0.46	3.68	3.86	4.01	17.02
	STx-1/FA	0.79	2.26	1.03	1.16	6.34	-	-
	KGa-2/FA	1.00	0.99	0.85	0.87	1.66	-	-



7.5. Soil FA sorption on three source clays.

Residual C concentration in the filtrate solution after the clay-humic reaction (fulvic acid concentration in the initial solution was 200 mg/L).

Clay-humic complex	C concentration in filtrate (ppm)
KGa-1/FA	79.23
STx-1/FA	57.37
KGa-2/FA	96.22

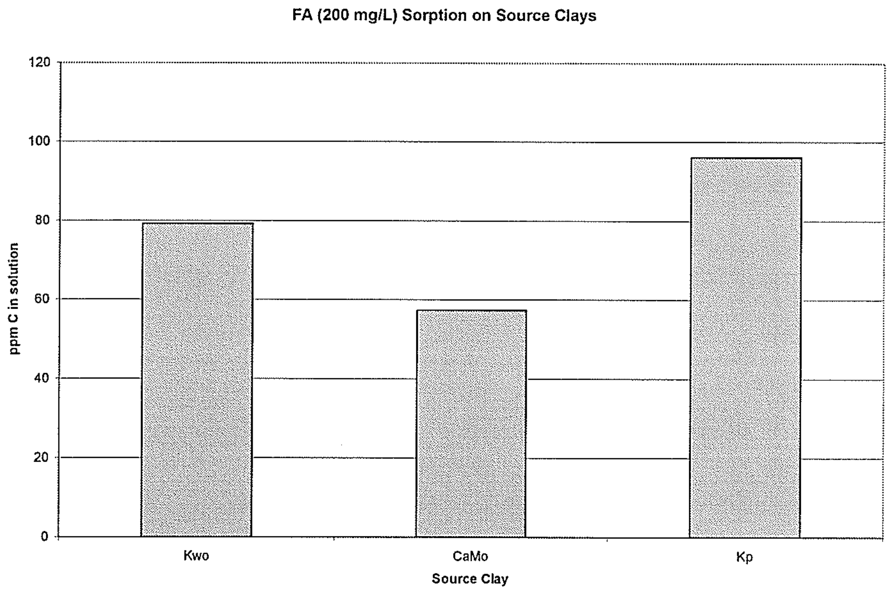


Fig. 7.6. C concentration in the solution from FA sorption on three Source Clays.

7.6. FA and HA complexes with well-ordered kaolinite (KGa-1).

FA/HA conc. (mg/L)		400	200	100	40	10	4	2
%C	KGa-1/FA	0.68	0.37	0.46	3.68	3.86	4.01	17.02
	KGa-1/HA	4.45	4.16	4.00	6.53	9.29	9.12	8.89

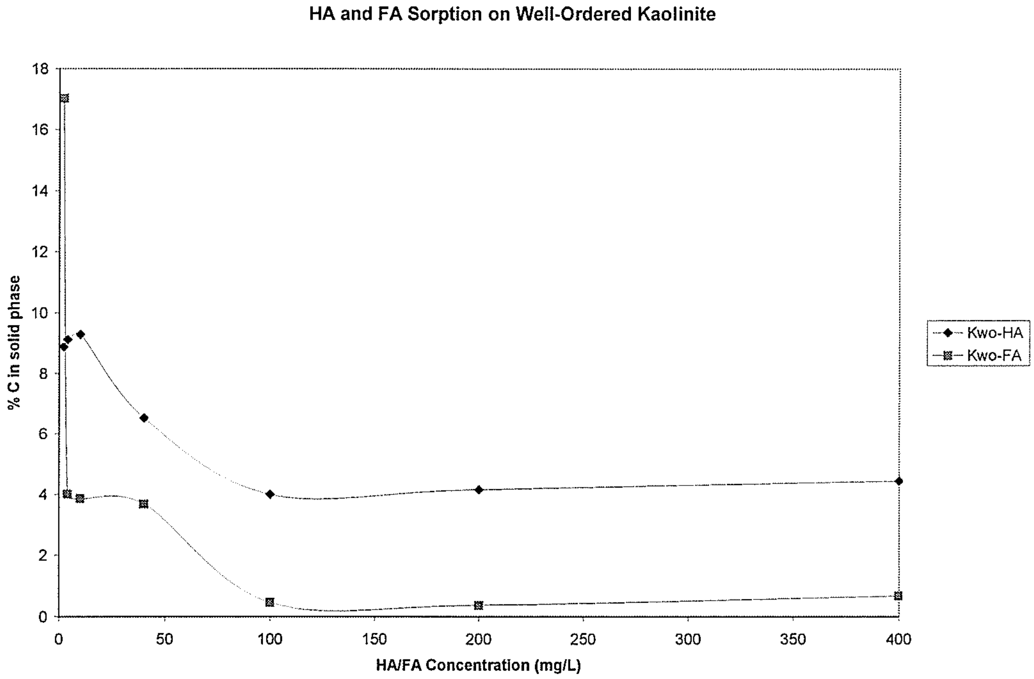


Fig. 7.7. Soil HA and Soil FA sorption on well-ordered kaolinite (KGa-1)

7.7. K-Ga1/HA complexes formed in different background electrolytes

	%C in solid phase	C in filtrate (ppm)
HA 200+NaCl (0.01M)	4.72	28.21
HA 200+NaOH (pH=10)	0.83	1.24
HA 200	4.16	29.2

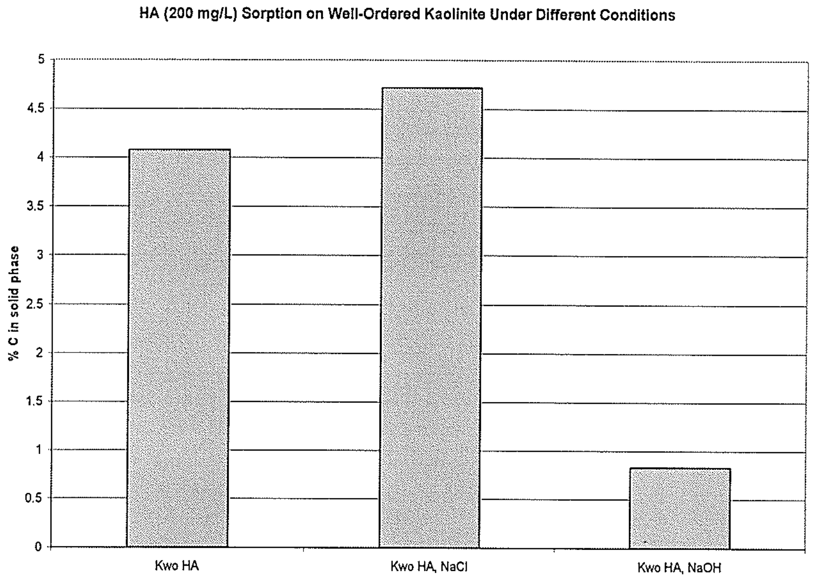


Fig. 7.8. Soil HA sorption on well-ordered kaolinite (KGa-1) under different conditions.

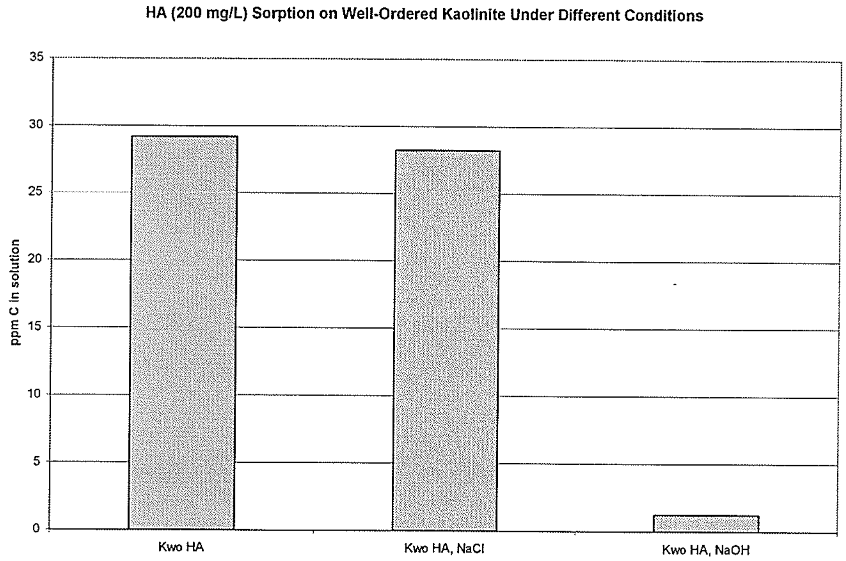


Fig. 7.9. C concentration in filtrates from Soil HA sorption on well-ordered kaolinite (KGa-1) under different conditions.

7.9. Discussion

7.9.1. Experiment reproducibility

The experiment using well-ordered kaolinite and humic acid at all seven concentrations was done in triplicate and proved the high reproducibility of the data for this reaction system (Fig. 7.1.). Due to limitations in the availability of the humic materials, not all experiments could be done in triplicate.

7.9.2. Influence of Source Clay on C content of clay-humic complexes

The C content of complexes between HA and all four Source Clays shows similar trends with respect to the humic-acid concentration in solution (Fig. 7.3). Moreover, the

C content of complexes obtained at high humic-acid concentrations in solution seems to be independent of clay type. Similar (very high, in excess of 80%) extents of adsorption for both kaolinite and Ca-montmorillonite were reported by Filip and Alberts (1994) in an experiment in which the ratio of humic acid to clay was very low (aprox. 1:1000, which explains the high adsorption ratio), but the concentration of humic acid in solution was 250mg/L (between the two highest concentrations used in the present experiment). This type of behavior suggests that, at high humic-acid concentration in solution, the extent of adsorption on the mineral surface is determined by a competition for the humic molecules in solution between the surface of the humic colloid and the mineral-organic surface.

For complexes synthesized from solutions with HA concentrations under 40 mg/L, significant differences in the C content as a function of the clay type are apparent. Ca-montmorillonite/HA complexes formed at concentrations lower than 40 mg/L exhibit a significantly higher C content than complexes of HA with the other three Source Clays. The higher affinity of humic acid for Ca-montmorillonite was expected in view of previously published data (Evans and Russel, 1959; Angle and Hamza, 1991), and is explained by the property of Ca^{2+} cations to form "bridges" between the clay surface and the humic molecules as well as the much higher surface area of the Ca-montmorillonite (see Appendix 1). More work would be needed in order to distinguish between the influence of these two factors.

7.9.3. Influence of HA/FA concentration in solution on the C content of clay-humic complexes.

The trend of increasing C content with decrease in humic-acid solution concentration is unusual and contradicts previously published data. Not only was this trend consistently found throughout the experiment, but it is confirmed by the decreasing C content of the mother solution (as seen in fig. 7.2.). Experimental data in adsorption studies of humic fractions on clays are difficult to compare rigorously, but increasing humic sorption with increasing humic concentration in solution is reported by most authors (Evans and Russel, 1959; Theng and Scharpenseel, 1975; Theng, 1976; Chassin et al., 1977; Ghabbour et al., 1998). Their data differ only with respect to the shape of the adsorption curve. All authors report small amounts of humic acid sorbed for clays saturated with monovalent cations (1-3 %).

A possible explanation for the unusual trend in the C content of the complexes formed in the present study could be related to the difference in the clay suspension concentration between the reaction systems. In order to keep the clay\humic ratio at 10:1 (w/w) and to vary the concentration of the humic-fraction solution 200 times, the volume of the solution was changed and, consequently, the concentration of the clay suspension also varied 200 times. The influence of the clay suspension concentration was not discussed in previous studies, but Angle and Hamza, 1991 observed that diffusion of the large humic molecules to the clay surface in concentrated clay suspensions was an unexpected factor in the formation of clay-humic complexes. The authors suggest that, depending on the nature of the clay and its concentration in the solution, a protective layer of gel may be formed, which acts as a protective barrier against the access of HA macromolecules. Clay was used in high excess in the reaction systems used in this study, and clay concentration was not supposed to play a major role in determining the C content of the resulting complex, but the particular nature of clay suspensions may limit the accessibility of the humic species to the clay surface, theoretical excess in solution notwithstanding. A drawback of this explanation is that if the dominant factor in the process was the clay concentration, a kaolinite suspension and a Na-montmorillonite or a Ca-montmorillonite suspension would be expected to have different diffusion properties and, by consequence, differences in the C contents of the complexes obtained should be expected. The results show a surprising similarity of the C content of all clay-humic complexes obtained at high humic-fraction concentrations. Future work should include the concentration of the clay suspension as an important parameter in clay-humic adsorption studies.

An alternative explanation for the unusual trend of humic sorption to clays reported here may involve competition between the humic-colloid surface and the clay surface for the dissolved humic components. A similar competition process was described by Zhou et al., 2001 for a FA/goethite system, and was considered to be the dominant factor in the fractionation of the fulvic fraction during sorption on the mineral surface. The relative importance of the humic-colloid surface in the process may increase with humic concentration in solution, becoming the dominant factor at concentrations higher than 40 mg/L humic material in solution. Experiments in which the clay/humic ratio and the clay concentration in solution will be varied, while the humic concentration will be kept constant could be used to prove this hypotheis.

The competition between the clay surface and a second surface - the humic colloid surface - could explain the similar C contents of the complexes obtained from the concentrated humic solutions, which does not seem to be influenced by the nature of the clay. Similar data were reported by Filip and Alberts, 1994 for a humic-acid solution with a concentration of 250 mg/L, as presented in the previous paragraph.

7.9.4. Differences in the adsorption of HA and FA fractions

Researchers have tried to answer the question of which fraction has a higher affinity for clay surfaces, HA or FA, since the beginning of systematic studies of humic adsorption on clay surfaces. The answer to this question was considered to provide some insight in the mechanism of clay-humic complex formation or even in the structure of the mysterious humin, the non-extractable fraction of humic material. Some researchers have questioned the relevance of comparing the affinity of the clay surface for fulvic and humic acid for understanding natural processes of soil and sediment formation, as fulvic and humic acids are operationally defined fractions of humic materials which do not occur separately in natural environments, and their components are known to interact with each other in a manner which will fundamentally change their properties in the sorption process (C. Langford, 1996, personal communication).

Available data on the comparative affinity of FA and HA seem contradictory: some authors report higher sorption of humic acids on clay surfaces (Evans and Russel, 1959; Theng an Scharpenseel, 1975 compared with Theng, 1976; Murphy et al., 1990), whereas others reported higher adsorption excesses for fulvic acids than for humic acids

(Chassin et al., 1977; Abo-El Fadl et al., 1994; Khalil et al., 1995). In many of those studies, various clays (kaolinite, montmorillonite, illite, vermiculite, natural soil clay fraction) were used and a variety of monovalent and polyvalent cations were present. Thus in most cases, data obtained for HA and FA are not directly comparable. The composition and properties of the humic fractions used vary greatly with the origin and extraction procedures used in their preparation (see 2.4.1.3.1.2)

In the present study, the fulvic and humic acids used were extracted from the same soil, and they are expected to represent typical behaviour for soil humic fractions, or at least for humic fractions extracted from black soils. For all Source Clays tested in this study, the affinity for humic acid was significantly higher than the affinity for fulvic acid at the same concentration of humic material in solution (Fig. 7.7). Interesting information could probably be obtained through competitive adsorption studies involving one clay and both a humic-acid and a fulvic-acid fraction extracted from the same soil, in variable ratios or sequence (first HA, then FA and in reverse sequence).

7.10. Conclusions

i) No influence of the clay type on C content was found for complexes obtained from solutions with a relatively high humic material concentration (400mg/L-40 mg/L). At these high concentrations of humic materials in solution the competitive affinity of the humic-colloid surface for the dissolved humic components seems to be the determining influence.

ii) The C content of complexes synthesized from humic solutions with less than 40 mg/L humic material seem to be influenced by the type of clay: Ca-montmorillonite has a significantly higher affinity for both HA and FA than both types of kaolinite and Na-montmorillonite, probably due to the significantly higher surface area and the presence of the Ca^{2+} ions which can form "bridges" between the humic molecules and the surface of the clay.

iii) The C content of clay-humic complexes declines with increasing humic-fraction concentration in solution, becoming almost constant at concentrations over 100mg/L HA/FA in solution for both humic fractions and all four clays used. This unusual behaviour may be explained by competition for the humic solute between the humic-colloid surface and the clay-mineral surface. The concentration of the clay suspension was also decreasing while the HA/FA concentration was increasing (the clay/humic ratio was constant at 10:1). Easier diffusion of humic aggregates to the clay surface in more diluted clay suspensions could have been a factor in the increase in C content of the complexes.The role of the concentration of the clay suspension in determining the C content of the complexes formed should be further investigated.

iv) All four clay types have higher affinity for HA than for FA fractions. For clay-humic complexes obtained from solutions with high humic-material concentration, the C content varied around 4% C for the HA complexes and 1% for the FA complexes. For clay-humic complexes obtained from dilute solutions, complexes with considerably higher C content (up to 17 % C) were obtained for both HA and FA.

CHAPTER 8. $^{13}C/^{12}C$ RATIO ($\delta\ ^{13}C$) IN CLAY-HUMIC COMPLEXES: ISOTOPIC FRACTIONATION IN HUMIC MATERIAL

8.1. Soil Organic Matter dynamics described by ^{13}C abundance data

^{13}C abundance in soils and soil-particle size fractions (sometimes in association with C/N ratio variations) has been used as a tracer in studies on the dynamics of organic matter in association with different mineral components of soils (Balesdent and Mariotti, 1996; Roscoe et al., 2000). $\delta^{13}C$ values are thought to describe the degree of transformation of vegetal material into humic compounds and also to reflect a number of climatic and vegetation conditions (Bird et al., 1996; Ford et al., 2001). Increase in ^{13}C abundance from litter to soil organic matter has been reported (Balesdent and Mariotti, 1996) and could be related to selective persistence of compounds rich in ^{13}C or to an isotopic fractionation produced during microbial respiration. At the same time, ^{13}C abundance was found to be significantly higher for the clay fraction of soils (Roscoe et al., 2000).

Isotopic fractionation and the formation and distribution of FA and HA among soil-particle size fractions are part of the same process of vegetal material transformation into chemically recalcitrant humic material found in soils, and must therefore be closely related. $\delta^{13}C$ data for various humic fractions were used to study the selective incorporation of fresh plant residue into soils (Spaccini et al, 2000). ^{13}C abundance variation in relation to HA/FA sorption on clay minerals could provide important information about some of the controversial issues in humic-substance chemistry: does fractionation of the FA/HA material occur during sorption to clay particles? What is the contribution of selective sorption on mineral surfaces to the ^{13}C distribution in soils and sediments ? Which is the "youngest" fraction in a soil, the HA or the FA?

8.2. ^{13}C abundance in natural clay-humic complexes

The ^{13}C abundance is significantly higher for the clay fraction compared to the other particle-size fractions of soil (Roscoe et al., 2000), a fact which could be related to selective persistence of compounds rich in ^{13}C or to isotopic fractionation during microbial respiration. C/N ratios also tend to decrease with particle size, suggesting that a more humified material is present in the finest fractions. These results support the hypothesis of Natelhoffer and Fry (1988) that isotope fractionation during decomposition results in accumulation of ^{13}C in more humified material. At the same time, selective sorption of more humified material on clay surfaces could contribute to enrichment of ^{13}C in the clay fraction of soils.

8.3. ^{13}C enrichment during HA/FA sorption on clays

In the present experiment, the ^{13}C abundance was determined for a series of complexes prepared under controlled conditions from four Source Clays and Standard Soil HA/FA (as described in Chapter 3). The ^{13}C abundance was also determined for some of the mother solutions from which the complexes were separated. The purpose of this experiment was: i) to determine if fractionation of humic material occurs during sorption on the clay surface, ii) if the sorbed material is enriched in ^{13}C, and iii) how the concentration of the humic material in solution or the nature of the clay surface influences isotopic fractionation.

8.4. Results

8.4.1. Carbon content and δ ^{13}C ratio in Source Clay samples

Source Clay	%C	δ ^{13}C
KGa-1	0.28	-22.59
KGa-2	0.22	-24.73
STx-1 (CaMo)	0.17	-24.91
Swy-1 (NaMo)	0.39	-18.38

8.4.2. δ ^{13}C ratio in KGa-1/HA complexes

HA conc. (mg/L)		400	200	100	40	10	4	2
δ ^{13}C	Exp.1	-	-22.27	-21.87	-22.02	-	-24.52	-25.24
	Exp.2	-22.14	-22.25	-22.29	-24.46	-26.75	-25.83	-26.14
	Exp.3	-22.17	-22.29	-22.01	-24.56	-23.33	-25.48	-26.76

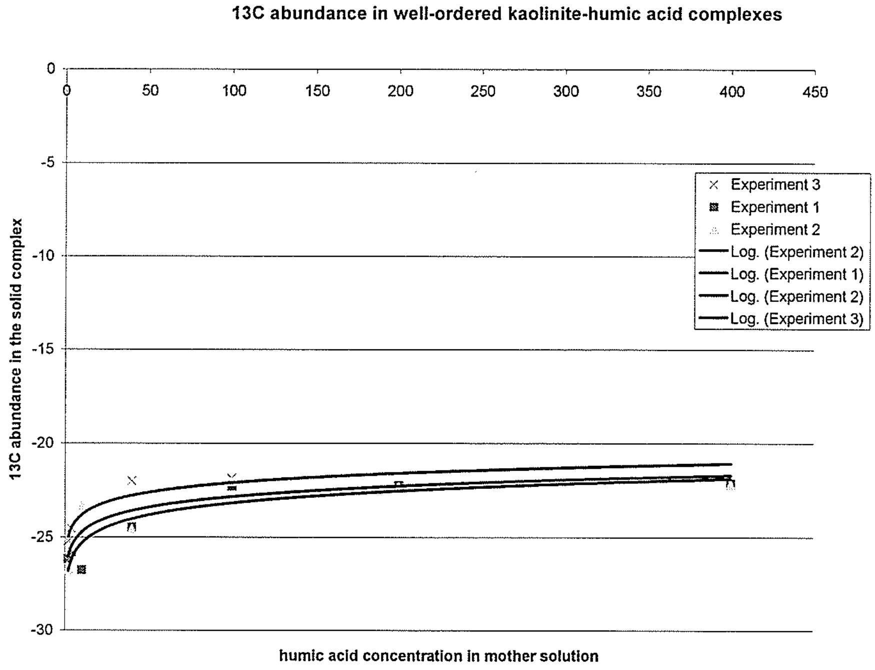


Fig. 8.1. δ ^{13}C ratio in KGa-1/HA complexes

8.4.3. δ ^{13}C ratio in KGa-1/FA complexes

FA conc. (mg/L)	400	200	100	40	10	4	2
δ ^{13}C	-25.42	-24.45	-25.8	-27.29	-28.83	-27.42	-28.95

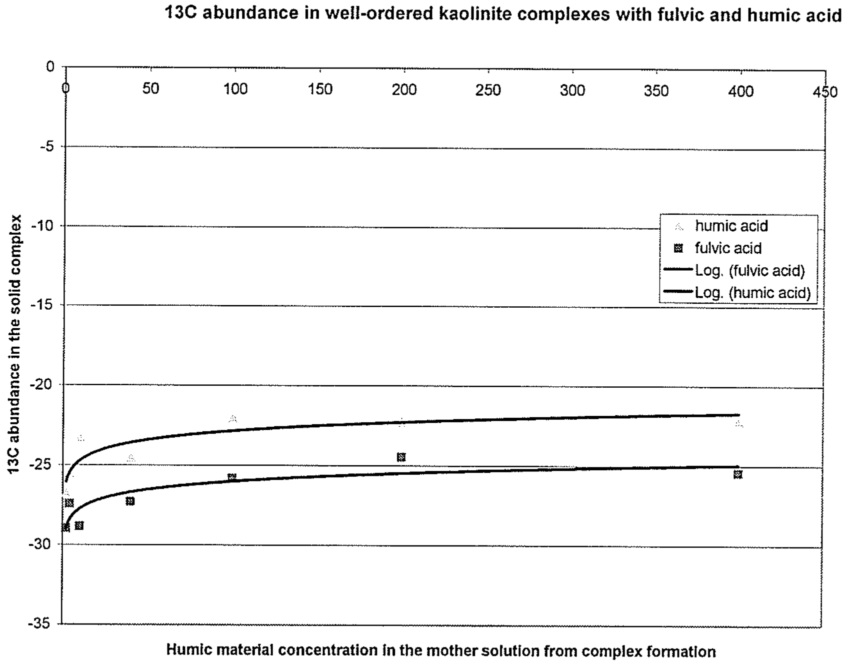


Fig. 8.2. δ ^{13}C ratio in KGa-1/FA and KGa-1/HA complexes

8.4.4. δ ^{13}C ratio in HA complexes with various Source Clays

HA conc. (mg/L)		400	200	100	40	10	4	2
δ ^{13}C	KGa-1/HA	-22.17	-22.29	-22.01	-24.56	-23.33	-25.48	-26.76
	STx-1/HA	-20.83	-22.56	-22.16	-22.26	-24.52	-27.19	-92.26
	KGa-2/HA	-22.25	-21.96	-21.99	-21.65	-23.67	-24.09	-26.5
	Swy-1/HA	-22.44	-22.03	-22.36	-21.47	-24.65	-25.83	-

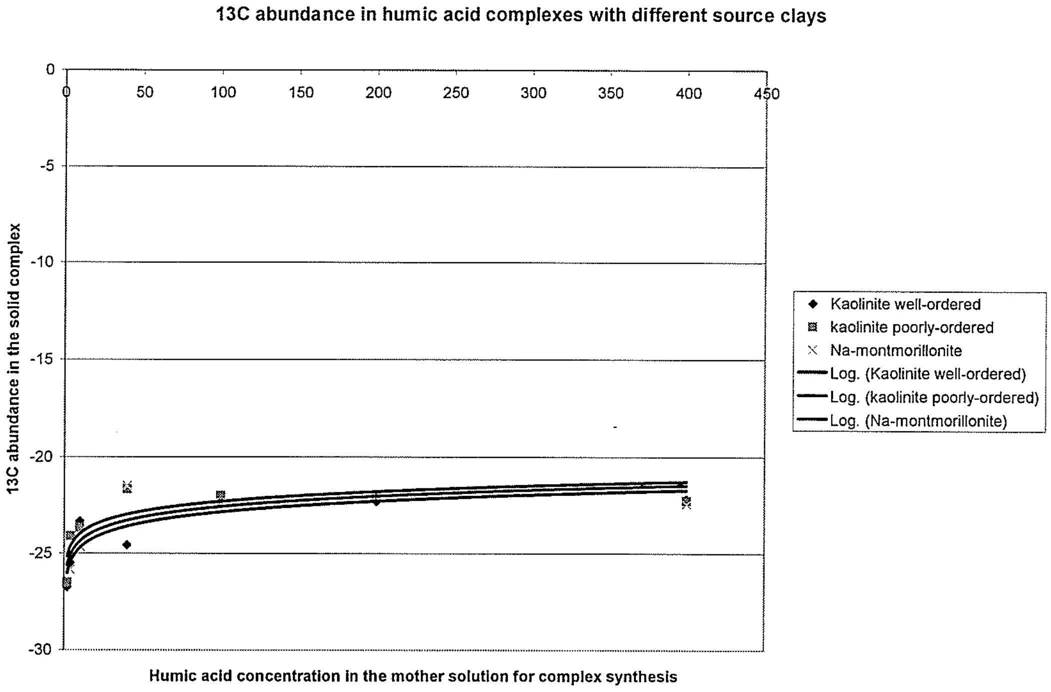


Fig. 8.3. δ ^{13}C ratio in HA complexes with four Source Clays

8.4.5. δ ^{13}C ratio in FA complexes with various Source Clays

FA conc. (mg/L)		400	200	100	40	10	4	2
δ ^{13}C	KGa-1/FA	-25.42	-24.45	-25.8	-27.29	-28.83	-27.42	-28.95
	KGa-2/FA	-23.65	-23.86	-23.69	-23.35	-27.59	-	-
	STx-1/FA	-25.02	-24.24	-24.97	-23.39	-29.46	-	-

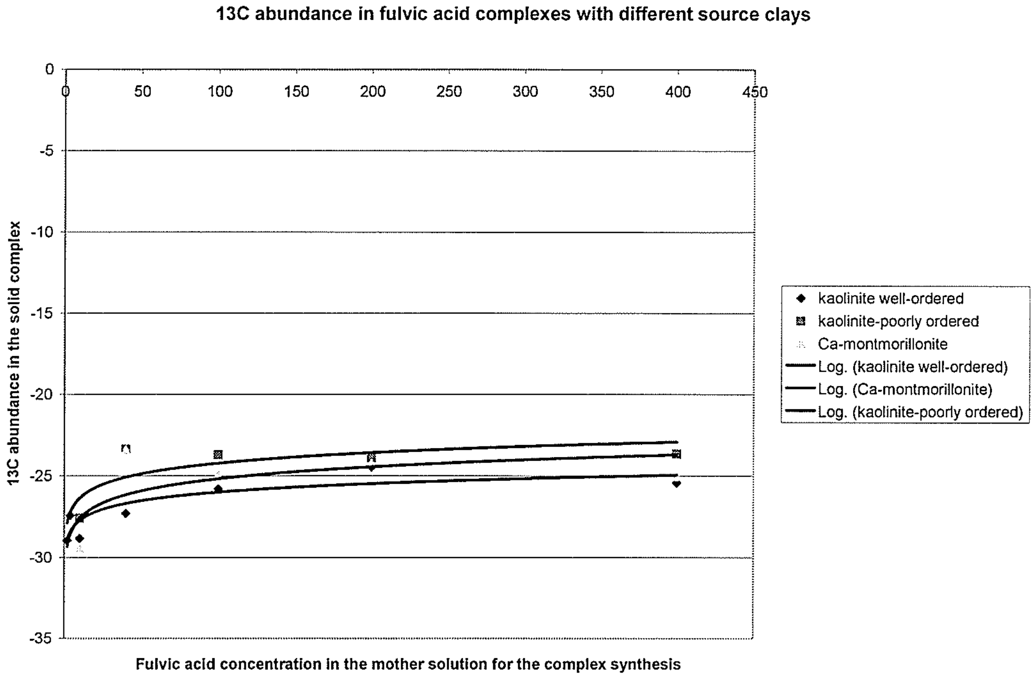


Fig. 8.4. δ ^{13}C ratio for FA complexes with three Source Clays

8.4.6. δ ^{13}C ratio in KGa-1/HA complexes formed in different background electrolytes

	δ ^{13}C
HA 200 + NaCl	-22.05
HA 200 + NaOH	-25.73
HA 200	-22.25

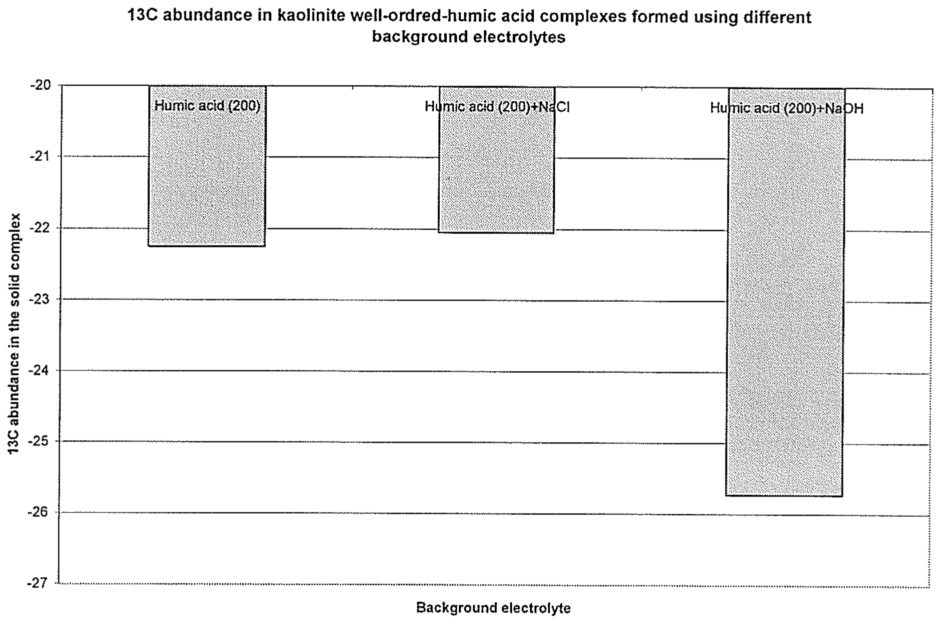


Fig. 8.5. δ ^{13}C ratio in KGa-1/HA complexes formed in various background electrolytes.

8.4.7. δ ^{13}C ratio in solutions from KGa-1/HA formation

HA conc.(mg/L)	400	200	100	40
δ ^{13}C	-24.7	-24.44	-24.38	-23.93

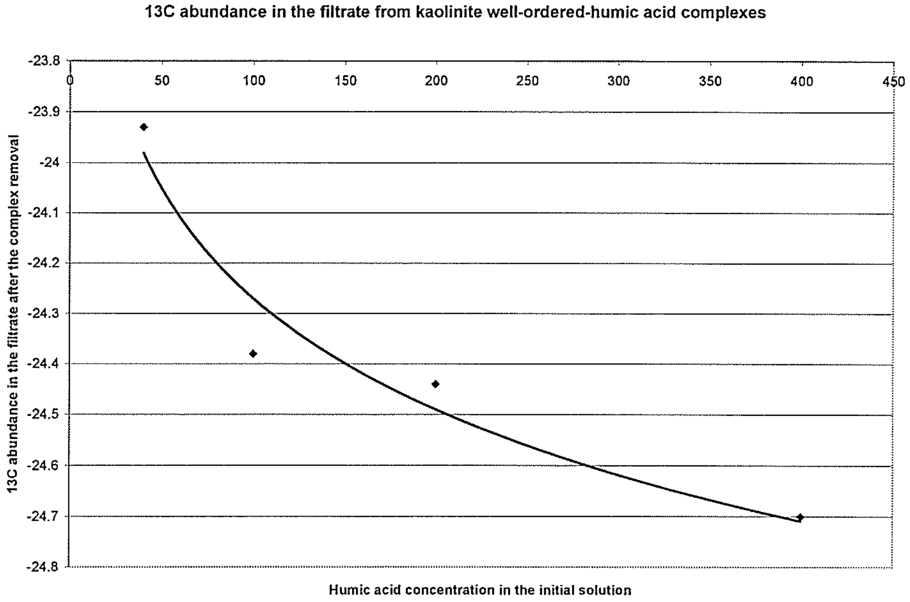


Fig. 8.6. δ ^{13}C ratio in solutions from the synthesis of KGa-1/HA complexes

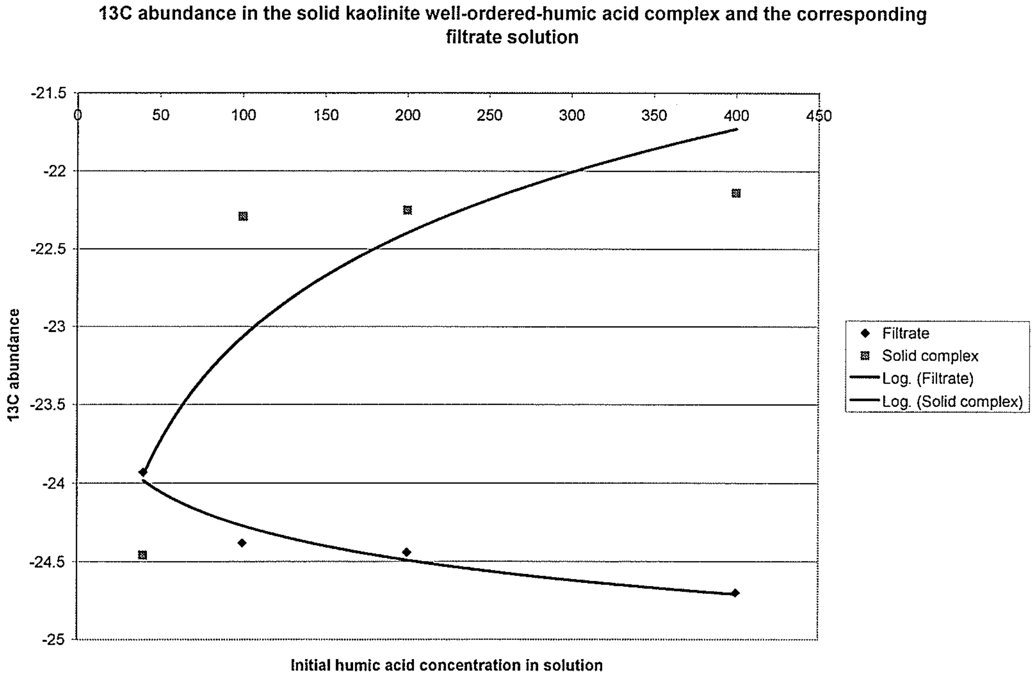


Fig. 8.7. δ ^{13}C ratio in KGa-1/HA complexes and residual solutions from the which they were filtered.

8.4.8. δ ^{13}C ratio in filtrate from HA(400 mg/L)/FA (200 mg/L)+ Source clay reactions

Source Clay	HA(400mg/L)	FA(200/mg/L)
KGa-1	-24.7	-24.61
KGa-2	-25.68	-24.89
STx-1	-22.76	-24.84
Swy-1	-23.1	-

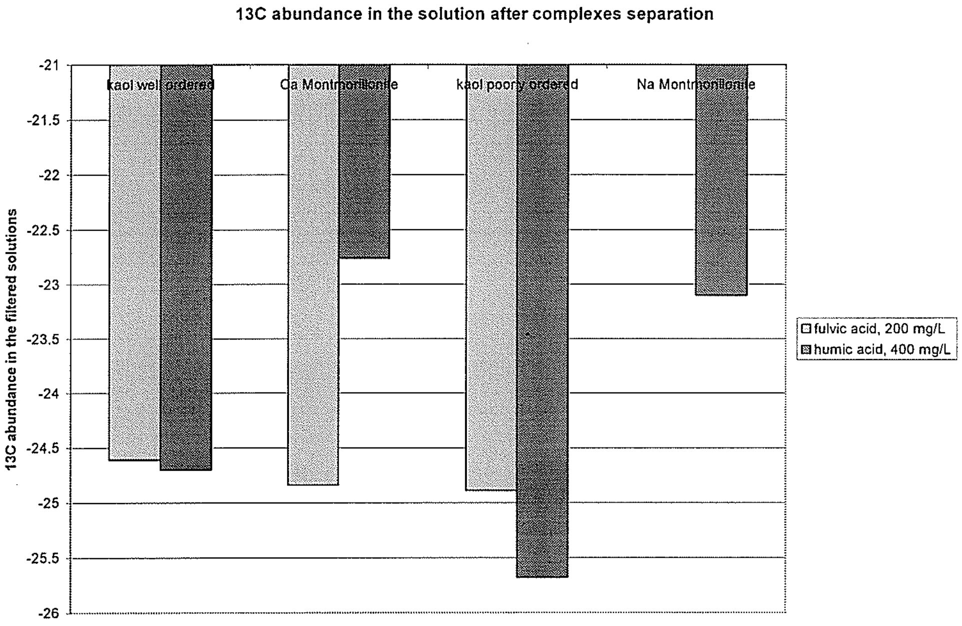


Fig. 8.8 δ ^{13}C ratio in residual solutions from the synthesis of complexes of HA/FA and Source Clays

8.4.9. δ ^{13}C ratio in filtrate from KGa-1/HA complexes formed in different background electrolytes

	δ ^{13}C
HA 200 + NaCl	-24.88
HA 200 + NaOH	-21.44
HA 200	-24.44

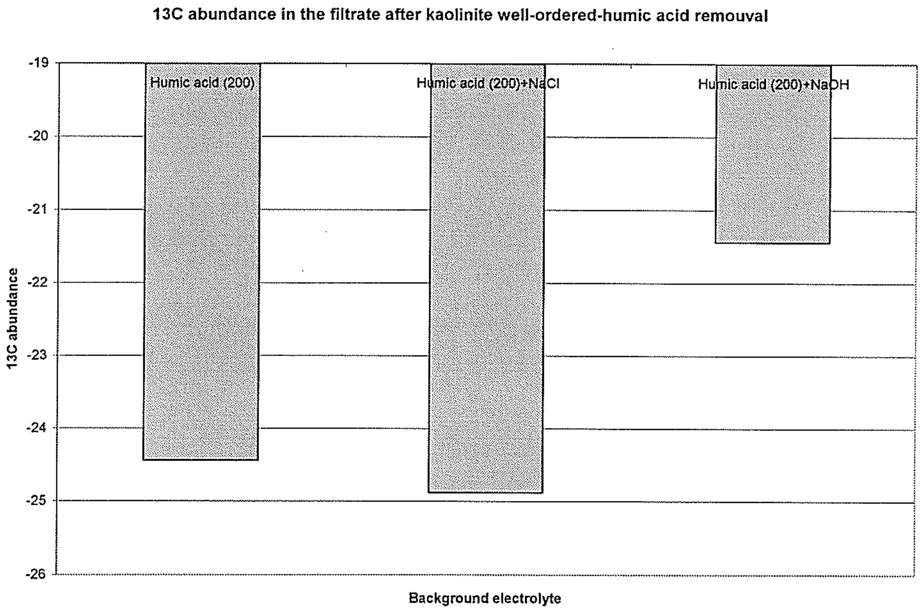


Fig. 8.9. δ ^{13}C ratio in residual solutions from the synthesis of KGa-1/HA complexes in various background electrolytes

8.5. Discussion

KGa-1/HA complexes were synthesized in three identical repetitions (see section 3.2.1.), and determination of $\delta\ ^{13}C$ data for the repetitions shows good reproducibility (Fig. 8.1). $\delta\ ^{13}C$ value for the Standard Soil HA is - 22.6 and for the Standard Soil FA the $\delta\ ^{13}C$ value is -25.6 (IHSS Collection data, see Appendix 2). The $\delta\ ^{13}C$ data for the Source Clays are in the same range (see 8.4.1.), which suggests a humic origin of the C in these samples. The $\delta\ ^{13}C$ value for Na-montmorillonite (Swy-1) is lower (-18.38), which suggests a contribution from a different C source. The C content of the Source Clays is low and it is not expected to significantly influence the $\delta\ ^{13}C$ value of the clay-humic complexes.

$\delta\ ^{13}C$ data for complexes of HA/FA and Source Clays show some significant trends: HA complexes have higher ^{13}C abundance than FA complexes for all clays and at all HA/FA concentrations used (Fig. 8.2). ^{13}C enrichment is larger for the complexes obtained from more concentrated solutions of both HA (Fig. 8.3) and FA (Fig. 8.4) and is less for complexes obtained from less concentrated solutions. The variation follows a logarithmic curve, with a significantly steeper decrease in $\delta^{13}C$ values at concentrations lower than 40mg/L humic-fraction in solution. It is remarkable that the C content of humic complexes seems to increase dramatically at concentrations lower than 40 mg/L humic fraction (see Chapter 7). The trends are similar for all four source clays used in the experiment. The increased ionic strength of the solution (obtained by the addition of solid NaCl to a concentration of 0.01 mol/L) did not significantly change the ^{13}C abundance of the KGa-1/HA complex formed, but the increase in pH of the mother solution to pH = 10 (using 0.1 mol/L NaOH) significantly decreased ^{13}C abundance in the KGa-1/HA complex (Fig. 8.5). The increase in pH also dramatically decreases the C content of the complex by enhancing the apparent solubility of the humic substances (see Chapter 7). The $\delta\ ^{13}C$ variations observed in the solid complexes are matched in all cases by inverse variations in $\delta\ ^{13}C$ values for the mother solutions (Fig. 8.6, Fig. 8.7, Fig. 8.8 and Fig. 8.9).

8.5.1. ^{13}C enrichment during HA/FA sorption on clays: proof of differential adsorption of humic components on the clay surface.

Differential sorption of components from a humic fraction was first investigated by Evans and Russel, 1959. They observed that only a small amount of the organic C in both the fulvic and the humic fractions (< 20 %) was adsorbed by the clay and that some of the darker components of the fulvic-acid fraction and the paler components of from the humic-acid fraction were preferentially adsorbed. Since then, many experimental observations suggested differential sorption of humic components occurs: Chassin et al. (1977) reported selective adsorption of lower-molecular mass-components of peat humic-fractions on Ca^{2+}- and Al^{3+} -saturated montmorillonite; Filip and Alberts (1994) observed preferential adsorption of aliphatic structures from a salted-marsh humic-acid on montmorillonite.

In this work, all experiments show increased ^{13}C abundance in clay-humic and clay-fulvic complexes, and ^{13}C depletion of the mother solutions from which the complexes were separated. These results are new and are direct proof of humic material fractionation during sorption on clay surfaces. What is more, if a certain ^{13}C abundance could be related to a certain chemically distinct fraction from the humic material (Spaccini et al., 2000), ^{13}C abundance data could provide valuable information about the driving forces of the fractionation process at the clay surface and improve our understanding of carbon-sequestration processes. This is an extremely promising direction for future work.

8.5.2. Conclusions

The δ ^{13}C data determined for the complexes of Standard Soil Humic Acid/Fulvic Acid with four Source Clays clearly demonstrate that:

i) fractionation of humic material occurs during sorption on clay minerals.This fractionation process results in increased ^{13}C abundance in the solid complex and ^{13}C depletion in the mother solution from which the complex is formed.

ii) This fractionation process does not seem to be influenced by the clay properties, but is dependent on the concentration of the humic fraction in solution. The complexes formed at lower humic concentration in solution, have lower δ ^{13}C values than complexes obtained at higher humic concentrations in solutions.

iii) A slight increase in ionic strength of the mother solution does not seem to influence ^{13}C abundance in the synthesized complexes, whereas an increase in pH seems to have a negative influence, however there are insufficient data to support a firm conclusion. Decrease in ^{13}C enrichment at high pH could be related to the very low C content of complexes formed under alkaline conditions, or it could be related to the high hydrophobicity of the humic material sorbed on the clay under these conditions.

The study of complexes formed under a variety of ionic strength and pH conditions could provide valuable information on the mechanism of humic material fractionation during sorption in view of the fact that the process seems to be influenced primarely by the solubility and aggregation properties of humic compounds. Correlation between $\delta\ ^{13}C$ data and C/N ratios could also provide insight in the degree of humification of the fractions which seem to be preferentially sorbed by clay minerals.

CHAPTER 9. CONCLUSIONS

The experiments described in this thesis have provided valuable information about the use of AFM methods in the study of humic/fulvic acid adsorption on clay, the morphology of the surface complexes formed, and the kinetics of the adsorption process. In a complementary experiment, the carbon content and the isotopic distribution of the stable isotopes of carbon in clay/humic complexes synthesized under various experimental conditions, illustrate the process of fractionation of the humic materials during adsorption on clay.

Our experiments prove that contact-mode AFM can be used for the study of the adsorption process of humic fractions to mineral surfaces. Some methodological observations will be useful for the development of future experiments:

1) Mica (muscovite) provides a reactive alumino-silicate surface and an appropriate support for the AFM imaging. Mica surface can be used as a simplified model for the clay surface.

2) A higher variability of electrolyte treated mica surface as compared to the freshly cleaved mica surface was observed. The extent of topographic variability was pH-dependent, which suggests that the cause of this phenomenon is the deposition of extremely small amounts of organic colloids present in the purified water. In order to distinguish between adsorbed humic colloids and background samples must be compared to the appropriate blank.

3) Deposition from low concentration humic solutions provides better images from which information is easier to derive.

4) Using the comparative study of vertically and horizontally positioned samples, this method differentiates between precipitation + sedimentation and true adsorption on the mineral surface, a differentiation which was previously believed to be impossible to attain (Tombacz et al., 1990).

5) Images of mica-humic surface complexes obtained by AFM can be used for the qualitative study of the interaction mechanism and kinetic of humic/fulvic acid sorption

6) Good reproducibility of the experiments was achieved.

7) The main limitation of the AFM method is that the thickness of the organic layer and, consequently, the absolute amount of humic material deposited on the mica surface cannot be estimated with precision. In future work, ellipsometry may be tried as an effective method to estimate the thickness of the organic layer (

The results of the AFM studies on the adsorption of humic/fulvic acid on mica give us some suggestions about the characteristics of humic materials adsorption on clays:

1) The coverage of the mica surface with humic aggregates was extensive even at the lowest concentrations of humic fractions used and in the absence of any cations which did not originate from the mica structure. In view of this observation it seems very likely that the whole mineral surface is covered with a thin layer of organic material (4-16 nm) even at very low organic matter concentration in water and under low ionic strength conditions.

2) The small aggregates observed at low concentrations of the humic solution are disk-shaped, suggesting a more hydrophobic nature than that normally attributed to humic compounds. This could be an indication of preferential sorption of the most hydrophobic molecules at low concentrations, leading to a fractionation of the humic material.

3) The amount of humic/fulvic acid covering the mica surface decreased with increasing pH, as predicted by theory. The organic aggregates also decrease in size with increasing pH, the variation being less important for the fulvic acid aggregates than for the humic acid. Fulvic acid aggregates are smaller than humic acid aggregates.

4) At pH=10.5 a few large humic acid aggregates were found. The adsorption of n small amounts of humic acid at very high pH suggests the presence of a highly hydrophobic fraction in the humic acid.

5) The amount of humic material adsorbed on the mica surface as well as the size of the aggregates increased dramatically in the presence of 0.01M NaCl as background electrolyte, which suggests that ionic strength may play a more significant role than pH in determining the amount of humic material associated to the clay fraction of soils and sediments.

6) Initial sorption of humic/fulvic acid was fast (of the order of minutes) and the interaction process seemed to be dominated by coagulation/sedimentation processes. Final formation of a thick organic layer, smooth in appearance, which seems to be the normal appearance for the "steady-state", was attained after about 24 h interaction time.

7) The organic material seemed to accumulate quicker on the mica surface from the humic acid solution.

Images of well-ordered kaolinite/humic acid complexes obtained by SEM and AFM are similar, as expected. What was not expected is the fact that the humic acid component of the complex is not visible in either AFM or SEM images, although the elemental analysis of the complex attests to the relatively high carbon content of the samples (about 15 %). A possible explanation of these observations may be due to a particular morphology of the clay-humic complex: most of the humic material may be sorbed between the opposite faces of kaolinite sheets.

The carbon content of clay-humic complexes obtained from humic/fulvic acid and four different clays at various concentrations of the humic fraction provides information about the influence of individual factors on the characteristics of the resulting complexes:

1) No influence of the clay type was found for complexes obtained from solutions with a high humic material concentration (400mg/L-40 mg/L). At high concentrations of humic materials in solution the competitive affinity of the humic colloid surface for the dissolved humic components appears to be the determining influence.

2) Complexes synthesized from humic solutions with less than 40 mg/L humic material appeared to be influenced by the clay type: Ca-montmorillonite was found to have a significantly higher affinity for both humic and fulvic acid than both types of kaolinite and the Na-montmorillonite, probably due to the presence of the Ca^{2+} ions.

3) Unexpectedly, the C content of clay-humic complexes declined with increasing humic fraction concentration in solution, becoming almost constant at concentrations

over 100mg/L humic/fulvic acid in solution. This unusual behavior was explained by the competition between the humic colloid surface and the clay-mineral surface for the humic solute. The decreasing concentration of the clay suspension while the humic /fulvic acid concentration was increasing (the clay/humic ratio was constant at 10:1) could have been a factor in the increase in C content of the complexes.

4) All four clay types had a higher affinity for humic acid than for fulvic acid fractions, especially in solutions with high humic material concentration.

The $\delta\ ^{13}C$ data determined for the complexes of Standard Soil Humic Acid/Fulvic Acid with four Source Clays clearly demonstrate that a fractionation of the humic material occurs during sorption on the clay minerals. Some of the characteristics of this process are:

1) The fractionation process results in increased ^{13}C abundance in the solid complex and 13C depletion in the mother solution from which the complex is formed.

2) The fractionation process does not appear to be influenced by the clay type

3) The fractionation process is dependent on the concentration of the humic fraction in solution. The complexes formed at lower humic concentration in solution, which have a higher C content, have lower $\delta\ ^{13}C$ values than complexes obtained at higher humic concentrations in solutions.

4) The 13C abundance in the synthesized complexes does not seem influenced by an increase in ionic strength of the mother solution, whether an increase in pH seems to decrease the ^{13}C abundance. The decrease in 13C abundance at high pH vales could be

related to the very low C content of the complexes formed and to the high hydrophobicity of the humic material sorbed on the clay under alkaline conditions.

AFM images and 13C abundance offer interesting new approaches to the study of humic material adsorption on clays. For the first time a method (AFM) that can distinguish between precipitation-sedimentation and true adsorption is available. Future work should be dedicated to improving the capacity to estimate the degree of coverage and the amount of material deposited on the mineral surface from AFM images. That will allow the progress from qualitative observation to quantitative estimation and provide a valuable tool for the study of the formation of clay/humic complexes. The study of ^{13}C abundance data for complexes formed under a variety of ionic strength and pH conditions could provide valuable information on the mechanism of humic material fractionation during sorption.

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APPENDIX 1

SOURCE CLAY PHYSICAL/CHEMICAL DATA

Listed below are data from Data Handbook for Clay Minerals and OtherNon-metallic Minerals, edited by H.Van Olphen and J.J. Fripiat, published by Pergamon Press.

SOURCE CLAY MINERALS

Kaolin KGa-1, (low-defect)

ORIGIN: Tuscaloosa formation? (Cretaceous?) (stratigraphy uncertain)

County of Washington, State of Georgia, USA

LOCATION: 32o58' N-82o53' W approximately, topographic map Tabernacle, GeorgiaN 3252.5-W 8252.5/7.5, Collected from face of Coss-Hodges pit, October 3,1972.

CHEMICAL COMPOSITION (%): SiO2: 44.2, Al2O3: 39.7, TiO2: 1.39, Fe2O3: 0.13,FeO: 0.08, MnO: 0.002, MgO: 0.03, CaO: n.d., Na2O: 0.013, K2O: 0.05, F:0.013,P2O5: 0.034, Loss on heating: -550oC: 12.6; 550-1000oC: 1.18.

CATION EXCHANGE CAPACITY (CEC): 2.0 meq/100g

SURFACE AREA: N2 area: 10.05 +/- 0.02 m2/g

THERMAL ANALYSIS: DTA: endotherm at 630oC, exotherm at 1015oC, TG: dehydroxylationweight loss 13.11% (theory 14%) indicating less than 7% impurities.

INFRARED SPECTROSCOPY: Typical spectrum for well crystallized kaolinite,however not as well crystallized as a typical China clay from Cornwall,as judged from the intensity of the 3669 cm-1 band. Splitting of the 1100cm-1 band is due to the presence of coarse crystals.

STRUCTURE:(Mg.02 Ca.01 Na.01 K.01)[Al3.86 Fe(III).02 Mntr Ti.11][Si3.83Al.17]O10(OH)8, Octahedral charge:.11, Tetrahedral charge:-.17,Interlayer charge:-.06, Unbalanced charge:0.00

Kaolin KGa-2, (high-defect)

ORIGIN: Probably lower tertiary (stratigraphic sequence uncertain)

County of Warren, State of Georgia, USA

LOCATION: 33o19' N-82o28' W approximately, topographic map Bowdens Pond,Georgia N 3315-W 8222.5/7.5, Collected from face of Purvis pit, October4, 1972.

CHEMICAL COMPOSITION (%): SiO2: 43.9, Al2O3: 38.5, TiO2: 2.08, Fe2O3: 0.98,FeO: 0.15, MnO: n.d., MgO: 0.03, CaO: n.d., Na2O: <0.005, K2O: 0.065,P2O5: 0.045, S: 0.02, Loss on heating: -550oC: 12.6; 550-1000oC: 1.17, F:0.02.

CATION EXCHANGE CAPACITY (CEC): 3.3 meq/100g

SURFACE AREA: N2 area: 23.50 +/- 0.06 m2/g

THERMAL ANALYSIS: DTA: endotherm at 625oC, exotherm at 1005oC, TG: dehydroxylationweight loss 13.14% (theory 14%) indicating less than 7% impurities.

INFRARED SPECTROSCOPY: Typical spectrum for less crystallized kaolinite,however the mineral is not extremely disordered since the band at 3669 cm-1is still present in the spectrum.

STRUCTURE:(Catr Ktr)[Al3.66 Fe(III).07 Mntr Mgtr Ti.16][Si4.00]O10(OH)8, Octahedral charge:.16, Tetrahedral charge:0.00,Interlayer charge:.16, Unbalanced charge:.15, Extra Si:.04

Texas Montmorillonite STx-1

ORIGIN: Manning formation, Jackson group (eocene)

County of Gonzales, State of Texas, USA

LOCATION: 29o30' N, 97o22' W approximately, topographic map: Hamon, Texas,N 2922.5-W 9715/7.5, Collected from face of pit, October 17, 1972.

CHEMICAL COMPOSITION (%): SiO2: 70.1, Al2O3: 16.0, TiO2: 0.22, Fe2O3: 0.65,FeO: 0.15, MnO: 0.009, MgO: 3.69, CaO: 1.59, Na2O: 0.27, K2O: 0.078, F:0.084, P2O5: 0.026, S: 0.04, Loss on heating: -550oC: 3.32; 550-1000oC:3.22, CO2: 0.16.

CATION EXCHANGE CAPACITY (CEC): 84.4 meq/100g, major exchange cation Ca.

SURFACE AREA: N2 area: 83.79 +/- 0.22 m2/g

THERMAL ANALYSIS: DTA: endotherms at 185oC (shoulder at 235oC), desorptionof water: 725oC, dehydroxylation; shoulder at 920oC, exotherms at 1055oC,1065oC, 1135oC. TG: Loss in dehydroxylation range: 3.88% (theory: 5%).

INFRARED SPECTROSCOPY: The spectrum indicates a low iron content. Quartz(697 cm-1), a silica phase (797 cm-1), and a trace of carbonate (1400 cm-1)are detectable.

STRUCTURE:(Ca.27 Na.04 K.01)[Al2.41 Fe(III).09 Mntr Mg.71Ti..03][Si8.00]O20(OH)4,Octahedral charge:-.68, Tetrahedral charge:0.00,Interlayer charge:-.68, Unbalanced charge:-.08,Extra Si:.59

Na-Montmorillonite (Wyoming) SWy-1(SWy-2)

ORIGIN: Newcastle formation, (cretaceous)

County of Crook, State of Wyoming, USA

LOCATION: NE 1/4 SE 1/4 Sec.18, T 57 N, R 65 W; 8, Topographic map: Seeley(15'), The upper 63 of recently stripped area was removed to expose clean,green upper Newcastle, Collected from which samples was taken, October 3,1972.

CHEMICAL COMPOSITION (%): SiO2: 62.9, Al2O3: 19.6, TiO2: 0.090, Fe2O3:3.35, FeO: 0.32, MnO: 0.006, MgO: 3.05, CaO: 1.68, Na2O: 1.53, K2O: 0.53,F: 0.111, P2O5: 0.049, S: 0.05, Loss on heating: -550oC: 1.59; 550-1000oC:4.47, CO2: 1.33.

CATION EXCHANGE CAPACITY (CEC): 76.4 meq/100g, principal exchange cationsNa and Ca.

SURFACE AREA: N2 area: 31.82 +/- 0.22 m2/g

THERMAL ANALYSIS: DTA: endotherms at 185oC (shoulder at 235oC), desorptionof water: 755oC, dehydroxylation; shoulder at 810oC, exotherms at 980oC. TG: Loss in dehydroxylation range: 5.53% (theory: 5%).

INFRARED SPECTROSCOPY: Typical spectrum for Wyoming bentonite with a moderateFe+3 content (band at 885 cm-1). Quartz is detectable (band at 780, 800,698, 400, and 373 cm-1), a trace of carbonate (band at 1425 cm-1).

STRUCTURE:(Ca.12 Na.32 K.05)[Al3.01 Fe(III).41 Mn.01 Mg.54Ti.02][Si7.98 Al.02]O20(OH)4,Octahedral charge:-.53, Tetrahedral charge:-.02,Interlayer charge:-.55, Unbalanced charge:.05,

APPENDIX 2

Elemental Compositions and Stable Isotopic Ratios of IHSS Samples

Sample	**H_2O**	**Ash**	**C**	**H**	**O**	**N**	**S**	**P**	**$\delta^{13}C$**	**$\delta^{15}N$**
Standard HA										
Suwannee River	10.0	3.10	52.55	4.40	42.53	1.19	0.58	<0.01	-27.7	-1.41
Elliott Soil	***8.2***	***0.88***	***58.13***	***3.68***	***34.08***	***4.14***	***0.44***	***0.24***	***-22.6***	***5.34***
Pahokee Peat	11.1	1.12	56.37	3.82	37.34	3.69	0.71	0.03	-26.0	1.29
Leonardite	7.2	2.58	63.81	3.70	31.27	1.23	0.76	<0.01	-23.8	2.13
Standard FA										
Suwannee River	8.8	0.46	52.44	4.31	42.20	0.72	0.44	<0.01	-27.6	-1.85
Elliott Soil I *	8.8	0.86	50.57	3.77	43.70	2.72	0.56	0.03	-25.4	3.89
Elliott Soil II	***11.2***	***1.00***	***50.12***	***4.28***	***42.61***	***3.75***	***0.89***	***0.12***	***-25.6***	***5.40***
Pahokee Peat I *	11.7	4.61	50.45	3.52	45.47	2.56	0.73	0.02	-25.8	1.42
Pahokee Peat II	9.3	0.90	51.31	3.53	43.32	2.34	0.76	<0.01	nd	nd
Reference HA										
Suwannee River	9.8	3.46	52.89	4.14	43.40	1.17	0.58	<0.01	-28.2	-2.42

*

Pahokee Peat	10.4	1.72	56.84	3.60	36.62	3.74	0.70	0.03	-26.3	1.43
Nordic Lake	9.1	0.31	53.33	3.97	43.09	1.16	0.58	0.01	-27.8	-1.55
Summit Hill Soil	8.1	1.41	54.00	4.84	37.90	5.13	0.64	0.40	-26.3	2.99
Waskish Peat	6.93	1.60	54.72	4.04	38.54	1.47	0.36	0.31	nd	nd
Reference FA										
Suwannee River	8.9	0.98	53.04	4.36	43.91	0.75	0.46	<0.01	-27.9	-2.76
Pahokee Peat *	11.8	1.58	52.12	3.23	43.93	2.43	0.53	0.01	-26.1	0.98
Nordic Lake	9.2	0.45	52.31	3.98	45.12	0.68	0.46	<0.01	-27.8	-3.19
Waskish Peat	8.29	0.16	53.63	4.24	41.81	1.07	0.29	0.12	nd	nd
Aquatic NOM										
Suwannee River	8.15	7.0	52.47	4.19	42.69	1.10	0.65	0.02	nd	nd
Nordic Lake	nd	41.4	53.17	5.67	nd	1.10	nd	nd	nd	nd
Bulk Materials										
Elliott Soil	***1.52***	***nd***	***2.9***	***nd***	***nd***	***0.25***	***nd***	***nd***	***nd***	***nd***
Pahokee Peat	7.1	15	45.70	4.74	nd	3.13	nd	nd	nd	nd
Leonardite	10.9	13	49.2	4.52	nd	0.9	nd	nd	nd	nd