

## Antibacterial effect of synthesized silicates for sewage treatment

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## ABSTRACT

This work is mating between a synthesis of kanemite and C<sub>16</sub>TMA-kanemite and a study of their anti-bacterial activity against *Staphylococcus aureus*. The kanemite polysilicate was synthesized by a hydrothermal medium. The kanemite obtained is then intercalated by organic molecules (alkyl tri methyl ammonium). The products obtained are illustrated by X-ray diffraction (XRD), infrared spectroscopy (FT-IR) and thermal Analysis TG-DSC, scanning electron microscopy (SEM). Their anti-bacterial activity is estimated by measuring the minimum inhibitory concentration (MIC) by exposing the bacteria to different concentrations of synthesized materials, then their efficiency is tested in water by bringing into contact; at different intervals of time; an initial bacterial density which is fixed in physiological water and the MCI of the synthesized materials. The results show a good antibacterial effect of the C<sub>16</sub>TMA-kanemite against *Staphylococcus aureus* with a minimum concentration of 0.05g per 5ml of physiological water during 15 minutes. So the C<sub>16</sub>TMA-kanemite is considered as an antibacterial agent against *Staphylococcus aureus* and can be used in the treatment of water.

**Keywords:** Lamellar polysilicate, Intercalation, Anti-bacterial agent, Pollution, Bacteria, Synthesis.

## 1. INTRODUCTION

The lamellar polysilicates form a family composed of magadiite, kenyaite, makatite, kanemite, silidrite and ilirite. These purely siliceous compounds are rarely found in nature because of the need for certain exceptional alkaline conditions. Since the first discovery by Eugster in 1967 in Lake Magadi in Kenya, hence the names of magadiite and kenyaite [1], Sliced silicates, having the general formula: Na<sub>2</sub>O (4-22) SiO<sub>2</sub> (5-10) H<sub>2</sub>O [1,3], are silicate sheet forms separated by sodium ions hydrates. The latter ensure the electrical neutrality of the anionic silicate framework and are easily exchangeable in solution, which gives them exchange, adsorption and intercalation properties [4-6]. Researchers have been interested in these materials mainly because of their intercalation and grafting properties. In fact, the existence of interfoliar compensation cations potentially exchangeable by organic or inorganic cations gives rise to various applications in fields as varied as catalysis [7,8], environment [9], health and nanotechnology. These natural compounds can also be synthesized in the laboratory [8,10]. However, the structures as well as the properties of most of these materials have remained poorly known for a long time because of the poor crystallinity of natural or synthetic compounds. As a result, researchers have been searching for new methods of synthesis with the aim of obtaining materials with larger properties [11]. The lamellar polysilicates are used in the preparation of microporous and / or mesoporous materials to

widen the field of catalysts as well as the preparation of new nanocomposite materials by intercalation of organic molecules [12-14]. However, these materials have been little observed in the field of the environment. Thus, one of the fixed objectives is to use these materials in water depollution reactions.

magadiite, included interlayer exchangeable cations which were often hydrated. Magadiite structure was composed of multiple negatively charged sheets of SiO<sub>4</sub> tetrahedra with abundant silanol-terminated surfaces. This type of reaction concerns the exchange of interlamellar cations of polysilicates in layers with other cations such as protons [15-17].

magadiite has an interesting cation exchange capacity (CEC) that has been applied to ion exchange, The exchange of sodium ions with protons to form silicic acid. On the other hand, different types of cations or large quaternary ammonium ions, therefore it was proven to be a good candidate for fabrication of organic-inorganic composites [17-23]. In recent years, the synthesis of materials with antibacterial activity has brought great interest to researchers because of the global concern over public health [24]. Clays are generally the most used materials in the preparation of antibacterial composite materials. [25]. Indeed, the compensation cations present in the interfoliar space can be exchanged by ions that can generate antibacterial activity. This results in a family of antibacterial composite materials [26].

## 2. EXPERIMENTAL SECTION

**2.1. Materials.** The reagents used during this work are listed below:

- Colloidal silica ludox 40 % SiO<sub>2</sub>, 60% H<sub>2</sub>O Dupont
- Sodium hydroxide Sup 98 % Fluka
- Ethanol 96% Doks Alcohols
- Cetyl trimethylammonium chloride 25% solution CH<sub>3</sub>-(CH<sub>2</sub>)<sub>15</sub>-N (CH<sub>3</sub>)<sub>3</sub>CL Aldrich.

**2.2. Characterization.** X-ray diffraction was carried out with Cu K $\alpha$  monochromatic radiation using a diffractometer Philips PW1830.

The infrared spectra of our materials (KBr disc) were recorded on a spectrometer Perkin Elmer FT-IR in the region 400-4000cm<sup>-1</sup>.

The scanning microscopy analyses were performed on a model LEO. Sterioscan 440 electron microscope in the backscattered mode.

UV- VISIBLE spectrophotometer of UV-VIS 1202 SHIMADZU.

Thermogravimetric Analysis (ATG), The measurements were carried out on a SETARAM-LABSYS thermoanalyzer between 20°C and 1000°C under reconstituted air (O<sub>2</sub> / N<sub>2</sub> mixture), from a sample mass of the order of 20 mg and with a temperature rise of 5°C per minute. This makes it possible to determine the water content of the sample as well as that of the intercalated organic substances.

**2.3. Synthesis of kanemite.** The kanemite was prepared by the method described by Beneke and Lagaly [27]. A mixture of amorphous silica and NaOH (SiO<sub>2</sub>:NaOH =1:1) was dissolved in 100 mL of methanol with cooling; it was then dried at 100°C for 2 week after this dried material had then been calcined at 700°C for 5.5h, after cooling to room temperature put the product in the water, filtered, and air dried. The formation was checked by means of X-ray powder diffraction and FT-IR spectroscopy, and Scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) to confirm the structure of kanemite.

**2-4C<sub>16</sub>TMA-kanemite synthesis.** C<sub>16</sub>TMA-Kanemite was prepared by the ion-exchange of interlayer Na cations in Kanemite with C<sub>16</sub>TMA cations [28]. Kanemite (1.0 g) was added to an aqueous solution (200 ml) of 0.1 M C<sub>16</sub>TMACl and the mixture was stirred for 2 days at room temperature. The resulting solid products were separated by centrifugation and dried in air,

### 2.5. Antimicrobial Activity

**2.5.1 Measurement of the minimal inhibitory concentration.** To determine the minimal inhibitory concentration of C<sub>16</sub>TMA-kanemite, the following steps are followed:

- Various masses C<sub>16</sub>TMA-kanemite taken separately with 5 ml of nutrient agar contained in a sterile tube using a vortex are incorporated.
- The mixture is poured into a sterile petri dish, and strains of *Staphylococcus aureus*, also taken separately on the agar, are seeded with a non-incorporated nutrient agar seeded with the same strains as a control.
- The petri dishes are incubated at 37 ° C. for 24 hours [29].

We work under aseptic conditions (in front of the Bunsen burner) and we disinfect the bench before any manipulation. The first box or there is no growth is considered that of the minimum inhibitory concentration (MIC).

**2.5.2 Measurement of antibacterial activity in water.** To study their effectiveness of C<sub>16</sub>TMA-kanemite in water, they are tested in vitro in physiological water. As a next step we will study the behavior of these bacteria in physiological water vis-à-vis these macromolecules. In this part we will proceed as follows:

- A bacterial suspension of *Staphylococcus aureus* is prepared in a tube containing 5 ml of physiological saline having a fixed optical density of 0.78
- Take 0.1 ml of this tube and sow in a tube with 10 ml of nutrient broth.
- In the same first tube, the mass found when determining C<sub>16</sub>TMA-kanemite MIC is used
- The mixture is shaken for 35 minutes while 0.1 ml of the tube is withdrawn every 5 minutes using a sterile syringe and is seeded in a tube containing 10 ml of nutrient broth.
- The seed tubes are incubated at 37 ° C for 24 hours.
- After incubation, the optical density of each tube is read by means of a UV-VIS spectrophotometer of UV-VIS-1202SHIMADZU type at 600 nm for the bacteria [30].

## 3. RESULTS SECTION

**3.1. Infrared absorption spectrum.** The IR measurements of product are in a good agreement with kanemite, IR spectrum in (Figure 1) shows absorption bands characteristic of the kanemite.

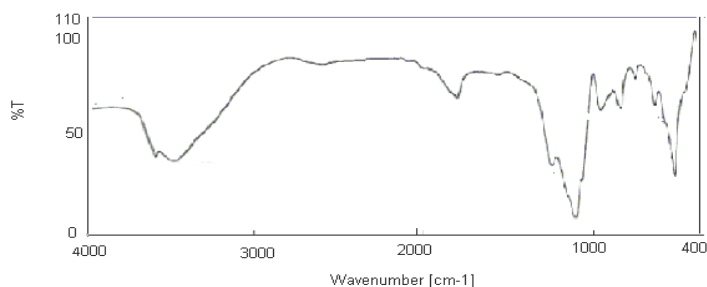


Figure 1. IR spectrum of kanemite.

The absorption bands are generally very wide and of low intensity. In addition to the vibration bands O-H and H-O-H at 3570, 3445 and 667 cm<sup>-1</sup>, the absorption bands corresponding to the vibrations of the Si-O bond at 1170, 1046 and 456 cm<sup>-1</sup> are observed. It should be noted a series of weak bands between 900 and 500 cm<sup>-1</sup>; these bands are detectable in the spectrum of

synthetic hydrated silica H<sub>2</sub>Si<sub>2</sub>O<sub>5</sub>, (2 SiO<sub>2</sub>.H<sub>2</sub>O), but are absent from the spectrum of amorphous silica.

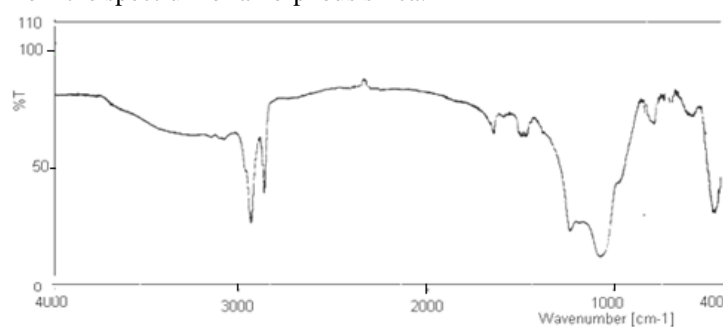


Figure 2. IR spectrum of the C<sub>16</sub>TMA- kanemite.

On the IR spectrum of the C<sub>16</sub>TMA-kanemite (Figure. 2) we recorded the absorption bands due to asymmetric methylene (CH<sub>2</sub>) vibration modes at 2917 cm<sup>-1</sup> and symmetric at 2850 cm<sup>-1</sup>, the band at 1471 cm<sup>-1</sup> is associated with shearing of CH<sub>2</sub> groups, whereas that at 1320 cm<sup>-1</sup> is characteristic of CH shear of a trans conformation. The band appearing between 900 and 950 cm<sup>-1</sup> arises from the C-N<sup>+</sup> stretching vibrations, the CH deformation

mode of the N<sup>+</sup>-CH<sub>3</sub> group can be seen at 1485cm<sup>-1</sup> (This band shows the expected decrease in intensity, relative to the alkyl CH<sub>2</sub> deformation vibration.

**3.2. X-ray diffraction.** The X-ray diffraction spectrum of kanemite (figure .3.) corresponded well to the diffraction of X-ray diffraction reported in the literature [27]. The peaks have a high intensity, which indicates a good crystallinity of the material. Thus, the peak at 2θ = 8.65° is attributed to the reflection (020) and indicates a base spacing of 1.02 nm.

In (Figure 4), the X-ray diffraction spectrum of C<sub>16</sub>TMA-kanemite is shown, it is clear that the intercalation of kanemite with C<sub>16</sub>TMA ions greatly affects its structural integrity, as long as the exchange reaction of kanemite ions with C<sub>16</sub>TMA cations led to the complete disappearance of characteristic kanemite peaks (Figure 3) and the appearance of new peaks at 2.92, 1.45 and 0.97 nm. This confirms that the exchange reaction has taken place.

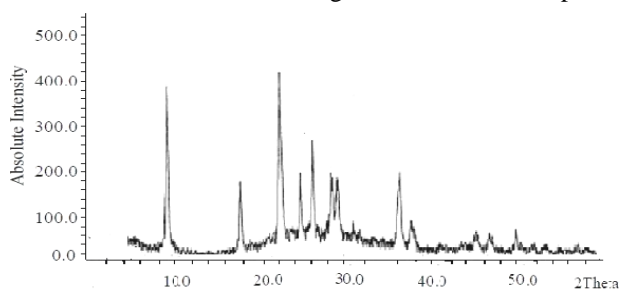


Figure 3. X-ray diffractogram of kanemite.

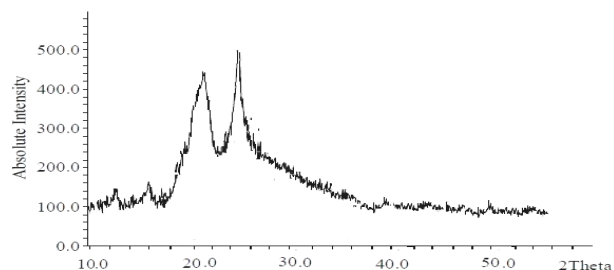


Figure 4. X-ray diffractogram of C<sub>16</sub>TMA-kanemite.

**3.3. Scanning electron microscopy (SEM).** (Figure-5) shows the images obtained by scanning electron microscopy analysis of kanemite and C<sub>16</sub>TMA-kanemite powders. It is well observed that these powders are composed of agglomerates, which are themselves composed of aggregates of heterogeneous sizes. The kanemite crystallites are large ranging from 2-5 μm.

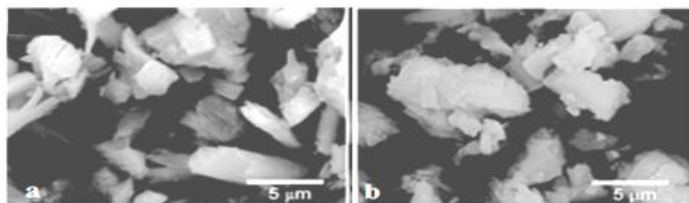


Figure 5. SEM image (a) of kanemite, (b) C<sub>16</sub>TMA-kanemite.

**3.4. Thermal Analysis.** The differential thermal analysis of kanemite, whose curve is shown in Figure 6, has three endothermic maximums at 120, 175 and 475°C.

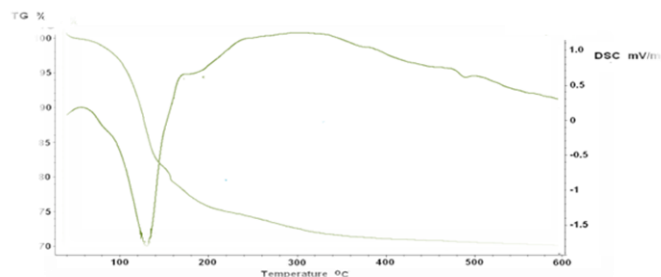


Figure 6. TG-DSC thermogram of synthetic kanemite.

**3.5. Determination of the MIC.** After incubation of 24 of the strain, the results are described in the following tables:

Table 1. Minimum Inhibitory Concentration of C<sub>16</sub>TMA-kanemite Versus Staphylococcus aureus.

Mass of C <sub>16</sub> TMA-kanemite g	4.10 <sup>-2</sup>	5.10 <sup>-2</sup>	6.10 <sup>-2</sup>
inhibition	no inhibition	inhibition	Inhibition

**3.6. Anti-bacterial activity of C<sub>16</sub>TMA-kanemite.** After incubation for 24 hours, the optical density (DO) is read using a UV-Vis spectrophotometer at 600 nm. We will express the antibacterial activity of C<sub>16</sub>TMA-Kanemite synthesized according to the number of bacteria (number expressed in Colony-Forming Unit per millimeter CFU/ml). **An optical density of 0.7 corresponds to 10<sup>8</sup>CFU / ml [31].** The bacterial reduction is observed only after 15 minutes of contact (the optical density from 0.6 to 0), because generally with the increase of the alkyl chain of the quaternary ammonium. Quaternary ammoniums are effective on bacteria; Cram positive, the effectiveness of quaternary ammonium bacteria is directly related to their contact surface with the medium of these microorganisms.

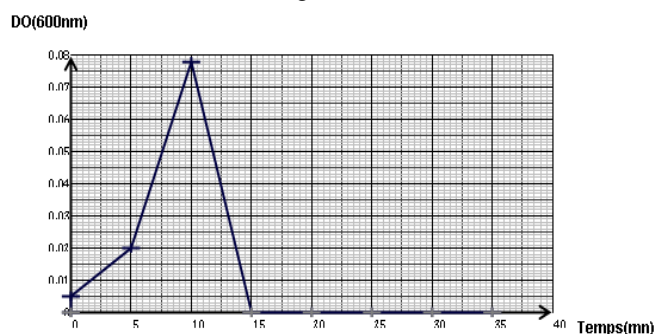


Figure 7. Antibacterial activity of C<sub>16</sub>TMA-kanemite against Staphylococcus aureus as a function of time.

Table 2. Antibacterial activity of C<sub>16</sub>TMA-kanemite against Staphylococcus aureus (Tears: DO = 0.60 = 8.57 10<sup>7</sup> CFU/ ml).

Time (min)	0	5	10	15	20	25	30	35
DO (600nm)	0.005	0.020	0.078	0	0	0	0	0
No (CFU/ml)	71.43. 10 <sup>6</sup>	2.85 .10 <sup>6</sup>	1.11 .10 <sup>6</sup>	0	0	0	0	0

**4. CONCLUSIONS**

The work done is mainly related to the synthesis of new intercalated nanocomposites. Nano composites have very

interesting properties. This study has therefore created new nanocomposites involving lamellar polysilicates while expanding

our knowledge of the chemistry that can be performed on this family of compounds. We first undertook the synthesis of a lamellar polysilicate, kanemite which was obtained by hydrothermal synthesis as used by several authors. The product obtained has been characterized by different methods of analysis. The latter confirmed the identity of the product obtained as kanemite. The kanemite obtained is then interposed with the alkyl tri methyl ammonium and then characterized. The material obtained is identified with C<sub>16</sub>TMA-kanemite. The bactericidal

effect of C<sub>16</sub>TMA-kanemite on staphylococcus is due to alkyl trimethylammonium. The antibacterial power of quaternary ammoniums results from their amphiphilic structure and their surfactant properties. The antimicrobial action is based on their activity of degradation of the cytoplasmic membrane of bacteria. The results of the bacterial reduction tests showed an effective activity of C<sub>16</sub>TMA-kanemite used on the bacterial strain (Cram +). It is concluded that C<sub>16</sub>TMA-kanemite can be adopted in water treatment to eliminate staphylococcal bacteria.

## 5. REFERENCES

- [1] Eugster H. P., Hydrous Sodium Silicates from Lake Magadi, Kenya: Precursors of Bedded Chert, *Science*, 157, 1177, **1967**.
- [2] Céline Eypert-Blaison, Emmanuel Sauzéat, Manuel Pelletier, Laurent J. Michot, Frédéric Villieras, and Bernard Humbert, Hydration Mechanisms and Swelling Behavior of Na-Magadiite, *Chem. Mater.*, 13 (5), pp 1480–1486, (doi: 10.1021/cm001130+), **2001**.
- [3] Heloise O. Pastore, Marcelo Munsignatti, Artur J. S. Mascarenhas, One -step synthesis of alkyltrimethylammonium-intercalated magadiite, *Clays and Clay Minerals*, 48(2), 224–229, **2000**.
- [4] Bi Y. F., Blanchard J., Lambert J.-F., Millot Y., Casale S., Zeng S., Nie H., Li D. D., Role of the Al source in the synthesis of aluminum magadiite, *Appl. Clay Sci.*, 57, 71–78, (doi:10.1016/j.clay.2012.02.001), **2012**.
- [5] Guerra D. L., Ferrreira J., Pereira M. J., Iana R., Airoidi C., Use of natural and modified magadiite as adsorbents to remove Th(IV), U(VI), and Eu(III) from aqueous media thermodynamic and equilibrium study, *Clay Clay Miner.*, 58 (3), 327–339, **2010**.
- [6] Benkhatou S., Djelad A., Sassi M., Boucekara M., Bengueddach A., Lead(II) removal from aqueous solutions by organic thiourea derivatives intercalated magadiite, *Desalin. Water Treat.*, 57(20), 1–13, (doi:10.1080/19443994.2015.1030701), **2015**.
- [7] Pal-Borbely G., Auroux A., Acidity of isomorphically substituted crystalline silicic acids with layer structure. I. H-magadiite, *Stud. Surf. Sci. Catal.*, 94, 55, **1995**.
- [8] Schwieger, W. ; Pohl, K. ; Brenn, U. ; Fyfe, C. A. ; Grondy, H. ; Fu, G. ; Kokotailo, G. T. Isomorphous Substitution Of Silicon By Boron Or Aluminum In Layered Silicates. In *Catalysis by Microporous Materials*; Beyer, H. K. ; Karge, H. G. ; Kiricsi, I. ; Nagy, J. B. ; Catalysis by Microporous Materials; Elsevier Science Publ B V: Amsterdam, Vol. 94, pp. 47–54, **1995**.
- [9] W. Schwieger, T. Selvam, O. Gravenhorst, N. Pfänder, R. Schlögl, G.T.P. Mabande, Intercalation of [Pt(NH<sub>3</sub>)<sub>4</sub>]<sup>2+</sup> ions into layered sodium silicate magadiite: A useful method to enhance their stabilisation in a highly dispersed state, *J. Phys. Chem. Solids* 65, 413–420, (doi:10.1016/j.jpcs.2003.08.032), **(2004)**
- [10] F. Feng, K.J. Balkus Jr., Direct synthesis of ZSM-5 and mordenite using poly(ethylene glycol) as a structure-directing agent, *J. Porous Mater.*, 10, 235–242, (doi:10.1023/B:JOPO.0000011384.86964.e5), **2003**.
- [11] Lagaly G., Beneke K., Weiss A., Magadiite and H-Magadiite: I. Sodium Magadiite and Some of Its Derivatives, *Am. Miner.*, 60, 642–649, **1975**.
- [12] Fathi Kooli, Yoshimichi Kiyozumi, Vicente Rives, Fujio Mizukami, Synthesis and textural characterization of a new microporous silica material, *Langmuir*, 18, 4103–4110, (doi: 10.1021/la0114124), **2002**.
- [13] Kunio Ohtsuka, Preparation and properties of two-dimensional microporous pillared interlayered solids, *Chem. Mater.*, 9, 2039–2050, **1997**.
- [14] Shuqing Song, Qiuming Gao, Jinhua Jiang, Lu Gao, Ling Yu, A novel kind of porous carbon nitride using H-magadiite as the template, *Materials Letters*, 62, 2520–2523, (doi:10.1016/j.matlet.2007.12.035), **2008**.
- [15] Heinrich Thiesen P, Beneke K, Lagaly G. Silylation of a crystalline silicic acid: an MAS NMR and porosity study. *J. Mater. Chem.* 12, 3010–3015. (doi:10.1039/B204314A). **2002**
- [16] Rojo JM, Ruiz-Hitzky E, Sanz J Proton-sodium exchange in magadiite: spectroscopic study (NMR, IR) of the evolution of interlayer OH groups. *Inorg. Chem.* 27, 2785–2790. (doi:10.1021/ic00289a009). **1988**
- [17] Wang Q., Zhang Y., Zheng J., Wang Y., Hu T., Meng C., Metal oxide decorated layered silicate magadiite for enhanced properties: insight from ZnO and CuO decoration, *Dalton Trans*, 46, 4303–4316, (doi:10.1039/c7dt00228a), **2017**.
- [18] Ogawa M., Maeda N., Intercalation of tris (2, 2 - bipyridine) ruthenium (II) into magadiite, *Clay Miner.*, 33, 643–650, **1998**.
- [19] Ruiz-Hitzky E, Aranda P, Darder M, Ogawa M. Hybrid and biohybrid silicate based materials: molecular vs. block-assembling bottom-up processes. *Chem. Soc. Rev.* 40, 801–828. (doi:10.1039/C0CS00052C) **2011**.
- [20] Ren Z., Zhang F., Yue L., Li X., Tao Y., Zhang G., Wu K., Wang C., Li B., Nickel nanoparticles highly dispersed in silica pillared clay as an efficient catalyst for chlorobenzene dichlorination, *RSC Adv.*, 5, 52 658–52, 666, (doi:10.1039/C5RA05926G) **2015**.
- [21] Wang Q, Zhang Y, Zheng J, Hu T, Meng C Synthesis, structure, optical and magnetic properties of interlamellar decoration of magadiite using vanadium oxide species. *Micropor. Mesopor. Mater.* 244, 264–277. (doi:10.1016/j.micromeso.2016.10.046) **2017**.
- [22] Ogawa M., Takizawa Y., Intercalation of tris (2, 2 - bipyridine) ruthenium (II) into a layered silicate, magadiite, with the aid of a crown ether, *J. Phys. Chem B*, 103, 5005–5009, (doi:10.1039/C7DT00228A) **1999**.
- [23] S. Okutomo, K. Kuroda, M. Ogawa, Preparation and characterization of silylated magadiites, *Appl. Clay Sci.* 15, 253–264, (doi:10.1016/S0169-1317(99)00010-1), **1999**.
- [24] Y. L. Ma, Z. R. Xu, T. Guo and P. You, Adsorption of methylene blue on Cu (II)-exchanged montmorillonite, *J. Colloid Interf. Sci.*, 280–283, **2004**.
- [25] Yamada Z., Ohta K., Takauchi S., Preparation and properties of antibacterial clay interlayer compound, *Kagaku Kogaku Ronbun*, 17, 29, **1991**.
- [26] Herrera P., Burghardt R. C., Phillips T. D., Adsorption of Salmonella enteritidis by cetylpyridinium-exchanged montmorillonite clays, *Vet. Microbiol.*, 74, 259, **2000**.
- [27] Beneke, K. and Lagaly, G., 'Kanemite—innercrystalline reactivity and relations to other sodium silicates', *American Mineralogist* **62**, 763–771, **1977**.
- [28] Kimura T., Itoh D., Okazaki N., Kaneda M., Sakamoto Y., Terasaki O., Sugahara Y., Kuroda K., Lamellar

hexadecyltrimethylammonium silicates derived from kanemite, *Langmuir*, 16, 2000.

[29] Laid Boukraa, Hama Benbarek, Ahmed Moussa, Synergistic action of starch honey against *Candida albicans* correlation with diastase number, Departement of Veterinary Sciences Faculty of Agro-Veterinary Science Ibn-Khaldoun University of Tiaret

Algeria, January, 18, 2008.

[30] Jean pelmont, bacteries et environnement adaptation physiologique, presses universitaires de Grenoble, 1993.

[31] Fatima hamadi et al, Adhésion de *Staphylococcus aureus* au verre et au téflon, *Rev. Microbiol. Ind. San et environn*, 3, 01, 1-16, 2009.

## 6. ACKNOWLEDGEMENTS

All my gratitude to those who helped me to accomplish this work. I would like to thank the laboratory staff TA-biochemistry of the University of Tiaret.

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