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Adaptation and Conversion of an Algerian Bentonite for Specific Use

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ABSTRACT/RESUME

Abstract: *Bentonite is used in pharmacology and cosmetology for different applications, the chemical and mineralogical composition and characteristics of these materials are so important. Therefore, the aim of this work is to evaluate the suitability of application of Maghnia Bentonite (clay from Algeria) through different tests. Initially, it was performed the characterization by X-ray Diffraction (XRD), X-ray fluorescence spectroscopy (XRF), Swelling volum, Gel Formation , CEC and Surface area. The microbial content of natural bentonite and the activated one with 4% of Na_2CO_3 labelled (M4) are verified. The suspensions were characterized through tests of sedimentation rate, swelling volum and pH determination. The physical-chemical characterization of M4 indicated compatible characteristics with those of clean clay; it was observed smectite and quartz reflections as crystalline phases and in terms of chemical composition the major presence of Montmorillonite was verified. The microbiological evaluation showed that microbial content of M4 Bentonite presents acceptable limits, according to the Pharmacopeas.. The pH of the sample M0 was close to neutral (7.75 ± 0.05), while the pH of the sample M4 (9.2). The results showed a possible application of M4 in pharmaceutical products.*

I. Introduction

In the pharmaceutical field, bentonite is mainly used as an excipient or as an active ingredient at different forms ; solid (tablets, capsules and powders) or liquid (, emulsions or suspensions) for oral use and semi-solid (, ointments, creams) as topical application (Jamal Alyoussef A et al , 2017 ; Ju-Hwan Park et al, 2016; J.K. Park et al, 2008; Carretero & Pozo, 2009, 2010; Joshi et al, 2009; Silva et al, 2011; da Silva et al, 2019; Samanta et al, 2020). Bentonite for pharmaceutical use is a clay composed mainly of montmorillonite (US Pharmacopeia, 2015a), purified bentonite is a montmorillonite-rich colloidal clay treated to remove sand and the non-swelling component as quartz (US Pharmacopeia, 2015b). The use of bentonite as pharmaceutical product requires certain criterias such as purity, swelling volum, gel formation. Mineralogical inertia dictates that silica polymorphs (quartz and cristobalite) do not exceed 2% (Liaquat et al., 2013), as they are considered carcinogenic (IARC, 1997). In addition, the Pb and As contents must not exceed 15 and 3 ppm respectively in the purified bentonite (US Pharmacopeia, 2015a,b; European Pharmacopeia, 10th Edition). Pharmaceutical bentonite must not be contaminated with *Escherichia coli (E. coli)* and the total number of viable aerobic microorganisms must not exceed 10^3 microorganisms per gram (US Pharmacopeia, 2015a; European Pharmacopeia, 10th edition). Therefore, before use, bentonite must be purified, modified and evaluated to meet the requirements for pharmaceutical use. Important additional properties are required for the adaptation of bentonite for the pharmaceutical industry; specific surface, swelling volum, gel formation and cation exchange capacity (Liaquat et al, 2013;

Khiari et al, 2014; Mattioli et al, 2016; Gamoudi et al, 2017; da Silva et al, 2019; Pattaranun et al, 2020 ; Cochiran et al 2021). These properties depend directly on the type of interlayer cations of the bentonite

Montmorillonite is classified as either sodium montmorillonite (NaM) or calcium montmorillonite (CaM). Sodium bentonites have relatively high swelling and gelation properties (Abdel Mottalib et al, 2011), therefore they meet pharmacopoeial requirements (US Pharmacopeia, 2015a) and are also tolerated for pharmaceutical use such as suspension and tablet. disintegrants (Joshi et al., 2009; Fatma Dardir et al 2018). The objective of this contribution is to activate Maghnia bentonite with proportions of Na_2CO_3 followed by decantation to eliminate the non-swelling components, in particular quartz, and to evaluate the possibility of pharmaceutical and cosmetic use.

II. Materials and methods

The raw bentonite used in this research is calcium bentonite from Maghnia, a region located in the north west of Algeria. The color of the bentonite is light grey, mainly calcium montmorillonite with some illite, kaolinite and quartz.

II.1. Activation of Maghnia Bentonite :

The raw bentonite is soaked in distilled water for 48 h and stirred after each 24 h soaking time. The dispersed clay is sieved through a 45 µm sieve (ASTM) and allowed to settle The fine fraction extraction of fine fraction consists to centrifuging an homogeneous suspension of bentonite then pipetting the supernatant containing the fine fraction (infra 2 µm equivalent-sedimentation). The centrifugation - pipetting cycles are repeated until a clear supernatant is obtained, which allows quantification of the fine fraction. However, after 12 extraction cycles, the supernatant obtained is translucent but not limpid The dried sample is ground, and passed through a 45µm sieve, then the bentonite is activated by sodium carbonate (Na_2CO_3) according to Liaqat et al (2013). To determine the optimal activation dose, different mass ratios of Na_2CO_3 2, 4, 6 8 and 10 g per 100 g of clay sample, labeled M2, M4, M6… respectively added to 1000 ml of boiling water. The suspension is stirred and boiled for 1 hour with gentle stirring. After activation, the mixture is cooled to ambient temperature and diluted. The suspension is dispersed and left to stand for 24 h, the clay sample is dried at 60°C, then ground to obtain a fine powder for experimental use (. Zhijin Gong, et al, 2016)

The raw bentonite M0 and the activated bentonite M4 are selected and characterized by X-ray diffraction (XRD), fluorescence (XRF), Exchange capacity (CEC), Adsorption-desorption from N2 to T = 77.35 K, Microbial tests, and pharmaceutical tests (pH, swelling and gelation).

II.2. X-Ray Diffraction and Chemical Composition

The mineralogical compositions of samples M0 and M4 are determined by X-ray diffraction (XRD). XRD measurements are performed with an X'Pert Pro PANalytical diffractometer (CuKα radiation, accelerating voltage 40 kV, current 20 mA. The scan was recorded in the angular range of 2° to 30° (2θ) with a steps of 0.017° (2θ) and a scanning speed of 2° per minute. The chemical composition is carried out by X-ray fluorescence (XRF).Analysis on a Philips X-ray fluorescence spectrometer, model PW2400.the elements (Pb and Zn) are analyzed using a flame atomic absorption spectrometer (Model: Varian, AA 240FS).

The chemical compositions of the samples are obtained with a Philips PW2400 XRF spectrometer for major oxides and Arcos ICP-AES model spectrometer for trace elements.

II.3. Infrared Analysis and Scanning Microscope

The IR device is of the SHIMADZU model Fourier transform FTIR 8400 spectrophotometer operating in a wave number range from 400 to 4000 cm-1

The samples are analyzed on a microscope of the type: SEM ESEMXL 30 PHILIPS with tungsten filament.

II.4. Cation exchange capacity (CEC)

The cation exchange capacity (CEC) of the samples studied is determined by the ammonium exchange method. The powder, dried at 105°C (100mg), is saturated with a 1M NH4 ammonium solution at pH=7 by five successive contacts of 2 hours. The CEC is determined by Kjeldhal distillation followed by a 2M H_2SO_4 assay (AFNOR Standard X31, 130, Nov. 1985). The CEC is calculated from equation :

$$CEC = 100NV_{HCl}/m.$$

N = normality of standard acid (HCl),
V_{HCl} = volume of acid (HCl) used for titration
m = mass of the sample in gram

II.5. Specific surface and pore size

Specific surface and pore size analysis, N2 adsorption-desorption isotherms are obtained at T = 77.35K with a Micromeritics ASAP 2010 surface analyzer. The specific surfaces are calculated according to the Brunauer, Emmett and Teller (BET) method and the pore size distribution is determined by the Barrett Joyner-Halenda (BJH) model (Barrett et al., 1951). The t-plot method is used to calculate the volume/area of the micropores.

II.6. Microbiological test

The microbial test is performed by the dilution method (US Pharmacopeia). Ten grams of each bentonite sample is suspended in 100 mL of phosphate buffer and adjusted to pH 7.2. A serie of dilutions 10^2, 10^3, 10^4, etc. is carried out for each sample in the same way. the dilution of the bentonite samples (in duplicate) is poured into sterilized Petri dishes. The agar medium for casein digestion (oxide) used as a culture The medium is sterilized and cooled to 45°C. 15 mL of the sterilized medium is added to each plate. The boxes are then shaken and left undisturbed for some time to solidify, then inverted and incubated for 48-72 h at 37°C. Only plates with 30 to 300 colonies are taken into account. The average of the colonies counted for the two dishes are calculated, multiplied by the dilution factor and are expressed in colony-forming units per gram (cfu/g) of sample

Test for *E. coli and Salmonella* species
A volume of Fluid Lactose medium is added to the bentonite sample in a container to reach 100 mL and then incubated at 37°C. One mL of this pre-enriched culture medium is transferred into two containers containing 10 mL of Fluid Selenite-Cystine and Fluid Tetrathionate respectively, mixed and incubated for 12 and 24 hours at 37ºC.
For the striated portions of the Salmonella species, Selenite-Cystine and Tetrathionate media are placed on the surface of bismuth sulphite agar medium and xylose-lysine-deoxycholate agar medium. For *E. coli*, part of the remaining lactose is streaked on the surface of agar medium in the Petri dish. All Petri dishes are incubated at 37°C for 24 hours. the plates are examined after incubation for the presence characteristic colonies of Salmonella and E. coli species (US. Pharmacopeia, 2015c).

II.7. Pharmaceutical trials

II.7.1. Swelling Volum
Swellability is determined according to the US Pharmacopeia(USPharmacopeia,2015a).Two grams of bentonite are gradually added to100ml of purified water in a 100ml test tube. The swelling is measured by determining the apparent volume after 2 hours.

II.8.2. Gel formation
From (Ph, European, 10th edition). Six grams of each bentonite are added to 200 ml of water, which is then centrifuged at 10,000 rpm. Then 100 ml of each suspension are transferred to a 100 ml graduated cylinder.the volume of sedimentation is then measured to determine the volume of clear supernatant after 24h.

II.7.3. pH measurement
According to the American Pharmacopoeia (US Pharmacopeia, 2015a), the pH of clay water suspensions (4 g/200 ml) is measured after 2 min of agitation

III. Results and discussion

III.1. Mineralogical composition
The bentonite obtained by activation with sodium carbonate at 4 g of Na_2CO_3/100 g of bentonite has a d001 at the order of 12 A˚ the raw bentonite 15 A˚

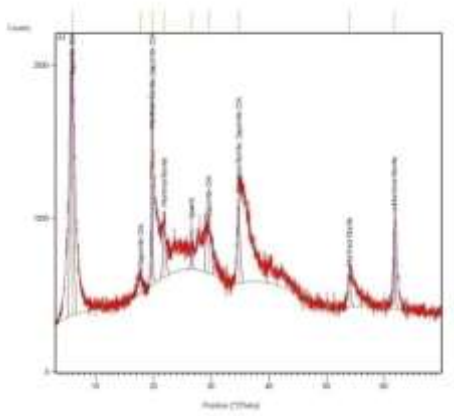

Figure1 . *XRD of raw bentonite M0*

(Fig. 2), suggesting that the Ca^{2+} ions in the montmorillonite spacer are replaced by Na^+ ions. Larger According to Abdelmoutalib et al. (2011), the quartz of activated bentonite decreased markedly by activation with soda after sedimentation.

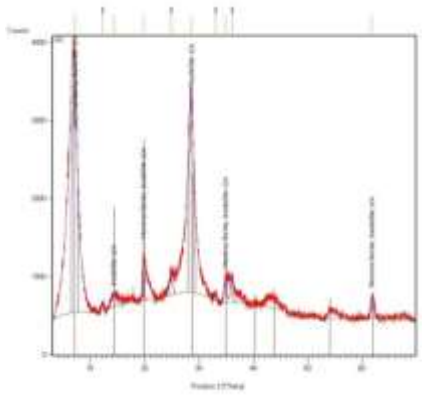

Figure2 . *XRD of activated bentonite M4*

III.2. Chemical composition
The elemental analysis of the majority oxides in the raw material and those of the activated sample M4 are presented in Table 1. Slight decrease in SiO_2

and increase in Al_2O_3 after activation confirms the suppression of a quantity of quartz and the proportional increase in clay minerals. The Na_2O content also increases and the CaO content has decreased significantly in accordance with the conversion of calcium bentonite to sodium bentonite. On the other hand, the MgO content remained unaffected. Small amounts of K_2O and CaO are still present in the purified sample. The K_2O is most likely due to illite and the CaO to trace amounts of calcite and/or feldspar. Finally, the percentages of Fe_2O_3, TiO_2 and MnO remain

Table 1. *Oxydes Composition of Maghnia bentonite (M0) and activated sample (M4)*

Sample	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	TiO_2	K_2O	MnO
M0	62.61	16.15	2.80	3.15	4.53	1.10	0.22	1.60	0.04
M4	61.12	17.83	1.22	1.79	3.90	3.61	0.20	1.50	0.04

unchanged or almost unchanged in the activated sample, which increases the purity and whiteness of the purified product. Trace elements Pb and As, detected by atomic absorption ICP-AES (0.25 ppm for As and 0.07 ppm for Pb), significantly lower than limits allowed by the pharmacopoeia, 15 and 3 ppm for purified bentonite respectively (US Pharmacopeia, 2015b). the amount of Cd is also insignificant <0.02 ppm.

III.3. Infrared Analysis

The FTIR spectra of the M0 and M4 bentonite samples are shown in Fig. 3 and in fig. 4. The infrared spectra show the presence of stretching vibrations at 3618 cm-1 for the structural OH groups present in the clay mineral samples (illite, smectite, interbedded clay minerals, kaolinite). A wide band close to 3425 cm-1 is attributed to OH water bodies. The band observed around 1633 c m-1 is attributed to the OH deformation mode of the water. A broad band centered at about 1035 cm-1 is attributed to Si–O stretching vibrations, while Si–O–Al bending vibrations are found at 515 cm-1. The band at 450 cm-1 is due to Si-O-Si bending vibrations (Pentrak.M et al)

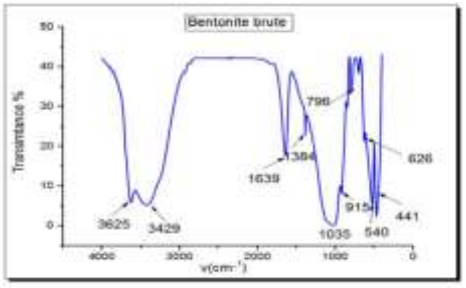

Figure3 . *Infrared Spectrum of raw sample M0*

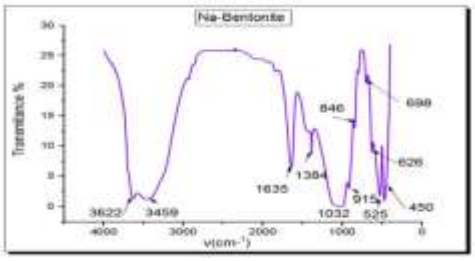

Figure4 . *Infrared Spectrum of sample M4*

III.4. SEM Observations

SEM analysis was performed to probe the effects of the activation process on the surface morphology of bentonite clay. Differences are observed in the micrographs for the raw M0 bentonite and the activated bentonite M4.

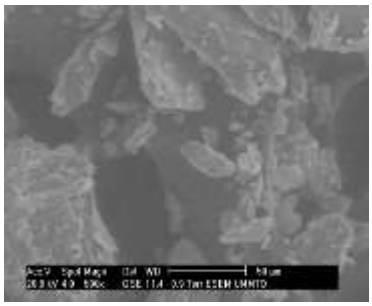

Figure 5a. *SEM of Sample M0*

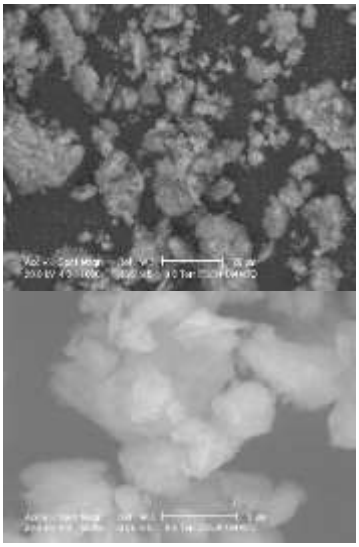

Figure 5b. *SEM of Sample M4 (Magnification of 50 and 5 µm)*

The original bentonite is composed of massive aggregates of irregular shapes of smectite, usually 5 to 100 µm (Fig. 5a). Most of the particles not being connected to each other. the raw bentonite has a compact and smooth morphology, which would consequently decrease the surface porosity. After purification and activation with Na_2CO_3, the surface morphology is significantly modified (fig. 5b), it can also be observed that the surfaces of the activated sample (M4) are more porous than those of the sample (M0), which corroborates with the measurements of the specific surfaces (Table 3).

III.5. Specific surface and porosity

The isotherms observed are of type IV showing the hysteresis loop H4, suggesting a slot-like porosity between particles (fig.6a, 6b). The hysteresis loop is due to capillary condensation of nitrogen in the form of a mesopore slit. The small hysteresis below 400mmHg and the steep slope between 640 and 720mmHg suggest the presence of micropores and macropores respectively. The isotherms of samples M0 and M4 are comparable; the only difference to note is the increase in the area of the hysteresis loop in the activated sample, indicating an increase in mesoporosity and higher adsorption volume than the original sample (Table2). According to the adsorption isotherm, the total pore volumes calculated by the BJH method increase after activation (Table 2). The results reveal the increase in the specific surface area of the activated sample (M4) (Table 2).

The cation exchange capacity of the raw sample (M0) is 75 meq/100 g of bentonite. it is quite high, an increase is observed for the activated samples (Table 3), but this is rather due to the purification, therefore decrease in the rate of quartz and not to the sodium activation. the sodium activation.

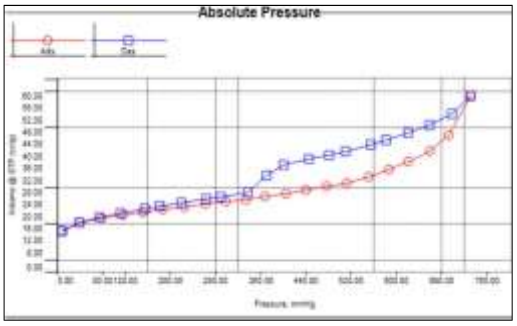

Figure 6a . *BET of raw sample M0*

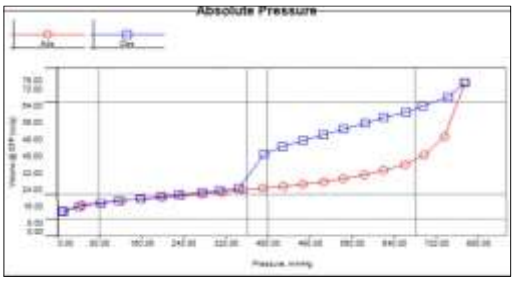

Figure 6b. *BET of activated sample M4*

Table 2. *BET values of M0 et M4*

Proprieties	M0	M4
Specific surface area (m^2/g)	58.63	86.84
Volum of micro pores (cm^3/g)	0.025	0.029
Total Volum (cm^3/g)	0.078	0.090
Pore size (nm)	1.833	1.878

III.6. Cation Exchange Capacity

The CEC values for the two bentonite samples are considered to be very high since they are greater than 40 meq/100g of sample. We notice that the CEC value of the purified sample (sodium) is higher than that of the natural sample. This can be explained by the fact that natural bentonite has a relatively high degree of soluble salts (impurities) which prevent the complete saturation of its exchangeable sites. On the other hand, the exchangeable sites of sodium bentonite (mont-Na) are occupied by sodium which is easy to move The Relatively high CEC value of the sample at 4% of activating agent may allow pharmaceutical use

III.7. Microbiological analysis

Na-activated samples are free of pathogens and total bacterial counts are within the range allowed by different pharmacopoeias, i.e. $<10^3$ cfu/g established for safe use of bentonite in the preparation of pharmaceutical products . Absence of contamination by *E. coli* and *salmonella*, requirement fulfilled by the bentonite studied (US Pharmacopoeia, 2015a).

Based on these results, the raw and activated form are microbiologically sound and can be used in the pharmaceutical field. (US Pharmacopoeia, 2015a).

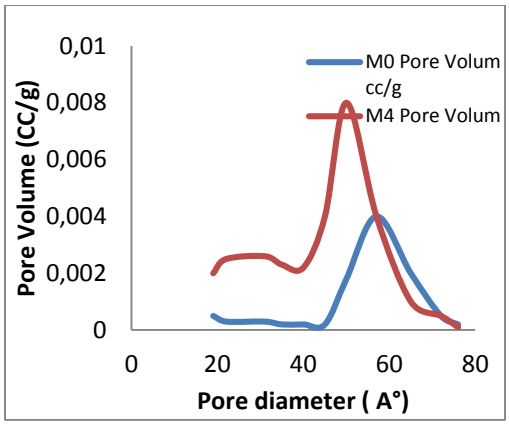

Figure 8 . *Mesopore size distribution (dV/dD) curves from BJH method for M0 and M4 samples.*

The two figures show a significant presence of mesopores in the two samples analyzed. It can be seen that the diameters of the pores, and particularly the mean diameter of mesopores, did not change, while the number of mesopores increased with purification**.**

III.8. Pharmacopoeial requirements

The pH, sedimentation volume or gel formation and swelling capacity of samples M0, M2, M4, M6 and M8 are listed in Table 4.

III.8.1. Swelling volum

The swelling volume (mL) of Maghnia bentonite samples before and after activation by Na_2CO_3 with different concentrations are shown in Table 4, the swelling volume of the original bentonite is 6ml. The swelling volume increased to 25 ml following the increase in activation with Na_2CO_3 up to 4 g/100 g (M4) (Fig. 3). The increase in swelling volume with increasing Na_2CO_3 concentration is due to the gradual replacement of exchangeable Ca^{+2} by Na^+ thus facilitating swelling due to the weak electrostatic interaction between Na^+ ions and the negatively charged smectite layer, allowing the insertion of excess water molecules. This volume is nearly constant at high concentrations.

Table 3 . *Cation Exchange Capacity (CEC) of raw bentonite and activated samples*

Echantillon	**M0**	**M2**	**M4**	**M6**
CEC (meq/100g)	75	75	85	85

III.8.2. Sedimentation and pH

The sediment volume measurement also meets pharmacopoeial specifications, with a value less than 2 ml (Table 4). Finally, the pH of the M0 sample is 7.5 and that of M4 is 9.2 after activation and thus meets the requirement of the pharmacopoeias

Table4 . *Physical properties with pharmacopeial requirements*

Propriétés	M0	M2	M4	M6	M8	Limits of Pharmacopoeia
pH (4g/200mL)	7.5	8.2	9.2	9.5	10.2	9-10.5
Swelling Volum (2g/100mL)	6mL	13mL	25mL	26mL	27mL	Minimum 24mL
Gel Formation(6g/100mL)	2mL	<1mL	<1mL	1mL	1mL	Minimum 2 mL

IV. Conclusion

Sodium bentonite (M4) is chemically and microbiologically pure and meets pharmacopoeial requirements, M4 is suitable for use as an excipient for absence of *E. coli*, total content of aerobic microbes and physico-chemical properties such as swelling volume, pH and sedimentation volume. . The low content of oligominerals As and Pb suggets the tolerability at oral pharmaceutical formulations .Purified Maghnia bentonite could be used in topical applications as a suspending agent

due to its high swelling volume and sedimentation volume according to pharmacopoeias. The high swelling volume of activated bentonite also suggests its use as a disintegrating agent in tablet formulation. The CEC and high surface area of the activated sample (M4), the absence of pathogens also allows its use as a drug adsorbent, and as a gelling agent in cosmetics.

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