

1 **CHARACTERIZATION OF NATURAL CLAYS FROM ITALIAN DEPOSITS WITH**  
2 **THE FOCUS ON ELEMENTAL COMPOSITION AND EXCHANGE ESTIMATED**  
3 **BY EDX ANALYSIS FOR POTENTIAL PHARMACEUTICAL AND COSMETIC**  
4 **USES**

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28

29 **Abstract**

30

31 Purification processes performed on natural clays to select specific clay minerals are complex  
32 and expensive as well as leading to deposit overexploitation. In this context, the present study  
33 aims to examine physico-chemical (mineralogy, morphology, size, surface charge, chemical  
34 composition, Cation Exchange Capacity, and pH value) and hydration properties (swelling,  
35 wettability, water sorption, and rheological behavior) of three native clays from Italian  
36 deposits for potential pharmaceutical and cosmetic uses due to the presence of phyllosilicate  
37 minerals. A special focus was given to Energy Dispersive X-ray (EDX) microanalysis,  
38 coupled with the cesium method, as a novel methodology to assay clay elemental composition  
39 and Cation Exchange Capacity (CEC). One bentonite of volcanic origin (BNT) and two  
40 kaolins, a kaolin of hydrothermal origin (K-H) and a kaolin of lacustrine and fluvial origin  
41 (K-L), were evaluated in comparison with a commercial purified bentonite. CEC assay  
42 revealed the complete substitution of exchangeable cations ( $\text{Na}^+$  and  $\text{Ca}^{2+}$ ) by  $\text{Cs}^+$  in BNT  
43 sample and CEC values consistent with those of typical smectites ( $100.64 \pm 7.33$  meq/100).  
44 As regards kaolins, a partial substitution of  $\text{Na}^+$  cations occurred only in K-L sample owing to  
45 the interstratified mineral component giving in any case low CEC values ( $11.13 \pm 5.46$   
46 meq/100 for K-H sample and  $14.75 \pm 6.58$  meq/100 for K-L sample). The degree of  
47 isomorphous substitution of  $\text{Al}^{3+}$  by  $\text{Mg}^{2+}$  affected the hydration properties of BNT in terms  
48 of swelling, water sorption, and rheology, whereas both the low-expandable kaolins exhibited  
49 high water adsorptive properties. EDX microanalysis has proved of considerable interest to  
50 provide more information about clay properties in comparison with other commonly used  
51 methods and to identify the role played by both chemical and mineralogical composition of  
52 natural clays for their appropriate use in pharmaceutical and cosmetic fields.

53

54 **Key Words** - Bentonite, Cation Exchange Capacity, Energy Dispersive X-ray Analysis,  
55 Kaolin, Rheology, Water Sorption.

56

57

## INTRODUCTION

58

59         Phyllosilicate minerals constitute the most important and abundant class of soil  
60 minerals mined worldwide having unique physico-chemical features due to their layered  
61 structure that justifies their wide and diversified applications (ceramics, oil, and animal waste  
62 absorbing, as a bonding agent in animal feeds, purifying wastewater, clarifying wine,  
63 decoloration agents, metal, paints, paper industries, pharmaceuticals, and cosmetics) (Lopez-  
64 Galindo *et al.*, 2007; Murray, 1991). ~~Clays such as~~ Bentonite, ~~rock term used to describe a~~  
65 ~~smectite-rich material for industrial mineral commodities where the most usual smectite is~~  
66 ~~mainly composed of smectite minerals, mainly~~ montmorillonite (2:1 Si:Al ratio) ~~and~~ kaolin,  
67 ~~rock term used to describe a soil rich in kaolinite (1:1 Si:Al ratio) for industrial mineral~~  
68 ~~commodities, mainly composed of kaolinite~~ are extensively used for several applications  
69 according to their structural and chemical characteristics (Velde, 1992). Both ~~these clays~~  
70 ~~bentonite and kaolin~~ are included ~~as monographs~~ in the current editions of both U.S. and  
71 European Pharmacopeias also receiving special attention from the World Health Organization  
72 in the Environmental Health Criteria 231 (WHO, 2005). Bentonite and kaolin are common  
73 ingredients in pharmaceutical formulations used as excipients (adsorbent, binder, anticaking  
74 or disintegrant for solid dosage forms, dispersion stabilizers in semisolid dosage forms) or as  
75 active compounds for the oral administration route (antacid, antitoxic, antidiarrheal, and  
76 gastric protectant) and for topical applications also in cosmetic field (rheology control agents,  
77 adsorbents of skin exudates or bacterial toxins, peloid thermal muds) (Lopez-Galindo *et al.*,  
78 2007; Veniale *et al.*, 2007; Viseras *et al.*, 2010). Recently, bentonite has been proposed for

79 organo-clay development due to its interaction with several drugs affecting their release or  
80 bioavailability (Aguzzi *et al.*, 2007; de Paiva *et al.*, 2008; Katti *et al.*, 2010; Iannuccelli *et al.*,  
81 2015; White and Hem, 1983) and for nanocomposites, constituted by nano-scale dispersions  
82 of clays into polymers, exhibiting increased strength and heat resistance (Katti *et al.*, 2010;  
83 Jin *et al.*, 2010; Rajkumar *et al.*, 2011; Rodrigues *et al.*, 2013; Sharma and Thakur, 2010;  
84 Tran *et al.*, 2006).

85 The special properties of bentonite are derived from **montmorillonite** crystal  
86 imperfections, i.e. isomorphic Si and Al atom substitution in tetrahedral and octahedral sheets,  
87 respectively, with lower positive valence atoms, producing an excess of negative charges in  
88 the lattice which is balanced by cations (mainly Na<sup>+</sup> and Ca<sup>2+</sup>) in the interlayer space of  
89 **smectite**. These cations are exchangeable with inorganic or organic cationic compounds due  
90 to their loose electrostatic interactions giving a typically high Cation Exchange Capacity  
91 (CEC) (about 100 meq/100 g) (WHO, 2005; Pusch, 2015). Moreover, interruption of  
92 montmorillonite periodic structure confers to the edge surface an amphoteric character and  
93 also the capacity to react at the level of these sites with inorganic or organic cations or anions.  
94 **Bentonite Montmorillonite** structural characteristics result in an ability to form thixotropic  
95 gels with water, to adsorb rapidly large quantities of water and expand, as well as to exhibit  
96 high CEC with both inorganic and organic cationic compounds (WHO, 2005).

97 Unlike **montmorillonite bentonite**, kaolinite is a non- or low-expandable clay **mineral**  
98 in aqueous solution because of the **kaolinite exhibits** very poor isomorphic substitution in the  
99 lattice and, consequently, a CEC value (about 2-10 meq/100 g) considerably smaller than that  
100 of **bentonite montmorillonite**. Nevertheless, **kaolinite kaolin** was found to be able to adsorb  
101 lipids, proteins, bacteria, and viruses on the external planes or edge of the particle surface,  
102 owing to broken bonds and exposed hydroxyl groups (Grim, 1968; Huertas *et al.*, 1998; Van  
103 Olphen, 1997), exploited for its use in medicine.

104 Clay specific function in each particular formulation depends on both mineralogical  
105 and chemical composition influencing several physico-chemical properties (particle size and  
106 shape, surface area, surface chemistry and charge, CEC, water sorption, swelling, and  
107 rheological properties) (Grim, 1968; Hosterman and Patterson, 1992; Lopez-Galindo *et al.*,  
108 2007; Patterson and Murray, 1983; Tateo and Summa, 2007). Moreover, clay properties can  
109 be significantly modified by the industrial processes performed on the raw materials to  
110 remove impurities, obtain regulatory requirements or modify the type of exchangeable ions in  
111 clay crystal lattice. However, for large-scale industrial applications, purification processes  
112 performed on natural clays to select specific clay minerals are complex and expensive as well  
113 as leading to deposit overexploitation (Bergaya *et al.*, 2012; Bergaya and Lagaly, 2013; Alves  
114 *et al.*, 2016).

115 Based on these premises, in this work, three native clays (one bentonite and two  
116 kaolins from Italian deposits) were investigated to assess physico-chemical properties, namely  
117 mineralogy, particle size, zeta potential, elemental composition, CEC value, wettability,  
118 swelling, water sorption, and rheology, in order to establish their potential applications in  
119 pharmaceutical and cosmetic fields without being subjected to modification in mineralogical  
120 composition. In addition, a novel application of Energy Dispersive X-ray (EDX)  
121 microanalysis was proposed to determine cesium CEC values and acquire more information  
122 about the elements involved in the exchange process than that achievable by means of the  
123 commonly used methods.

124

## 125 METHODS

### 126 *Materials*

127 Bentonite (BNT) of volcanic origin from Iglesias (Sardinia, Italy) deposit (average  
128 mineralogical composition from the producer's datasheet: montmorillonite 80%, quartz 13%,

129 illite-kaolinite 5%, plagioclase 2%), kaolin of hydrothermal origin (K-H, average  
130 mineralogical composition from the producer's datasheet: kaolinite 56%, K-feldspar 18%,  
131 quartz 16%, illite 8%, plagioclase 2%), and kaolin of lacustrine and fluvial origin (K-L,  
132 average mineralogical composition from the producer's datasheet: kaolinite 15%,  
133 interstratified clay mineral 30%, K-feldspar 11%, quartz 42%, plagioclase 2%) from deposits  
134 of Piloni di Torriella (Roccastrada, Tuscany, Italy) were kindly donated by Eurit (Colorobbia  
135 Group, Porto Azzurro, Italy). The terms "bentonite" and "kaolin", used as rock terms and  
136 pharmacopeial names throughout the text, indicate soils rich in montmorillonite and  
137 kaolinite, respectively. For comparison, a purified bentonite (MM) (Veegum R, USP/NF  
138 Magnesium Aluminum Silicate Type IA, mineralogical composition from the producer's  
139 datasheet: montmorillonite >90%) supplied by Vanderbilt Minerals, LLC (Norwalk,  
140 Connecticut, USA) was used. All clay materials comply with the requirements of European  
141 and United States Pharmacopoeias for chemical limitations, alkalinity, loss on drying and  
142 microbial contaminations (total viable aerobic count and absence of *Escherichia coli*) as  
143 reported on the material datasheets. For CEC determination, cesium chloride (CsCl) was  
144 purchased from Sigma-Aldrich (Milan, Italy). All the other chemicals were of analytical grade  
145 (Carlo Erba, Milan, Italy).

146

#### 147 *X-ray Powder Diffraction*

148 The identification of clay minerals in BNT, K-H, and K-L samples was studied using  
149 X-ray Powder Diffraction (XPRD) at the Department of Engineering Enzo Ferrari (University  
150 of Modena and Reggio Emilia). The samples were analyzed using a conventional Bragg-  
151 Brentano diffractometer (Philips-Panalytical, PW3710) with Ni-filtered CuK $\alpha$  radiation. All  
152 XRD data were collected under the same experimental conditions, in the angular range  $5^\circ <$

153  $2\theta < 60^\circ$  (step size  $0,02^\circ$  and 1 second counting time for each step). The phases were identified  
154 from peak positions and intensities using reference JC-PDF database.

155

#### 156 *Morphology, size, and surface charge*

157 Clay morphology was evaluated by means of Environmental Scanning Electron  
158 Microscopy (ESEM, Quanta 200, Fei, The Netherlands). Particle size, expressed as the  
159 diameter of the most represented dimensional class (main class), was determined both before  
160 and after milling process by a vibratory ball mill (FRITSCH GmbH, Idar-Oberstein,  
161 Germany) for 10 h. The analysis was carried out on 10 mg/ml clay water suspensions by laser  
162 granulometer (Mastersizer 2000, Malvern Instruments Ltd, Worcs, UK). Clay surface charge  
163 values were determined by using Photon Correlation Spectroscopy (PCS) (Zetasizer version  
164 6.12, Malvern Instruments Ltd) equipped with a 4mW He-Ne laser (633 nm) and DTS  
165 software (Version 5.0). The determinations were carried out in triplicate from three different  
166 batches. All the analyses described below were performed on the milled samples.

167

#### 168 *Elemental composition by EDX analysis*

169 Clay elemental composition was determined by Energy Dispersive X-ray (EDX)  
170 analysis (INCA 350, Oxford Instruments, Abingdon, UK) coupled with ESEM at the  
171 Interdepartmental Large Instruments Center (University of Modena and Reggio Emilia,  
172 Modena, Italy). Elements can be identified qualitatively and semi-quantitatively in function of  
173 the X-ray energy emitted by their electrons transferring from a higher energy shell to a lower  
174 energy one. X-ray emission from  $K\alpha$  or  $K\beta$  levels of the atoms calcium, potassium, oxygen,  
175 sodium, magnesium, aluminum, silicon, and other elements with atomic numbers from 4 were  
176 recorded by the selected area method related to whole clay particles from samples mounted  
177 without a conductive coating on carbon stubs with the following experimental settings: low

178 vacuum (0.70 Torr), accelerating voltage 12 kV, spot size 3, element detection limit  
179 ~0.05wt%, spatial resolution 0.1  $\mu\text{m}$ , total spectrum counts >250,000, accuracy within  $\pm 5\%$   
180 relative errors by reference to standards. EDX spectra representing the plots of X-ray counts  
181 vs. element and semi-quantitative results, expressed as relative weight percentage of the  
182 elements present in the specimen, were recorded. The reported data were averaged on three  
183 determinations for each sample.

184

#### 185 *Cation Exchange Capacity by EDX analysis*

186 CEC value of each clay sample was measured by placing 100 mg clay in a 0.1 M  
187 solution of CsCl (125 ml) under magnetic stirring for 24 h. After centrifugation (mod. 4235,  
188 ALC International, Milan, Italy) at 3,000 rpm for 15 min, the clay was rinsed with water,  
189 vacuum dried, compressed in a hydraulic press (Perkin-Elmer, Norwalk, CT, USA) at 200  
190  $\text{kg}/\text{cm}^2$  for 1 min using 12.5 mm diameter punches, mounted on carbon stubs without  
191 conductive coating, and assayed by Energy Dispersive X-ray (EDX) analysis coupled with  
192 ESEM. In this case, EDX analysis allowed for determining clay CEC and exploiting the  
193 peculiar affinity of phyllosilicate minerals for some elements, among which was cesium. X-  
194 ray emission of Cs atoms was evaluated at the intensity characteristic of this element ( $L\alpha =$   
195 4.2865 keV) and at the same working conditions described in the section above. CEC value  
196 was determined through the weight percentage of  $\text{Cs}_2\text{O}$ , applying the following equation  
197 (Hillier, 1992):

198

$$199 \text{ CEC (meq/100 g of clay) = (1000} \times \text{weight\% Cs}_2\text{O)/(282/2)}$$

200

201 The reported value was averaged on three determinations from three different batches.



202

203 *Cation Exchange Capacity by ammonium acetate method*

204 CEC value of BNT sample was determined by the slightly modified ammonium  
205 acetate method (Thomas, 1982). In practice, 500 mg BNT was shaken with 50 ml of 1 M  
206 ammonium acetate water solution for 12 h. The suspension was centrifuged at 4,000 rpm for  
207 20 min. The sediment, dispersed in 50 ml of water under mechanical stirring for 12 h and  
208 recovered by centrifugation, was subjected to two further rounds of exchange-washing in the  
209 same conditions mentioned above using 40 ml of ammonium acetate water solution. The  
210 excess of ammonium ions was removed in 50 ml water under stirring for 12 h. The recovered  
211 sediment was subjected to four washes with 95% ethanol and dried in an oven at 65°C for 2 h.  
212 Then, BNT water suspension (100 mg/25 ml) was loaded into the bulb of Kjeldahl apparatus  
213 with 50 ml of 30% w/w sodium hydroxide solution and distillation was run. The distillate was  
214 collected in 25 ml of 0.1 M nitric acid solution and titrated with 0.1 M sodium hydroxide.  
215 CEC value was calculated by applying the following equation (Hillier, 1992):

216

$$217 \quad \text{CEC (meq/100 g of clay)} = (1000 \times \text{weight \% NH}_4^+)/18$$

218

219 The reported values were averaged on three determinations from three different batches.

220

221 *pH value*

222 The pH value of 2% clay water suspension, according to U.S. Pharmacopeia  
223 monograph for bentonite, was determined by potentiometry both just after the preparation of  
224 the suspension and again after 1 h. The reported values were averaged on three determinations  
225 from three different batches.

226

227 *Wettability*

228 Clay wettability was determined on 500 mg clay sample compressed in a hydraulic  
229 press (Perkin-Elmer) at 200 kg/cm<sup>2</sup> for 1 min using 12.5 mm diameter punches by direct  
230 measurement of the contact angle, the tangent angle at the contact point between a deionized  
231 water drop and the tablets, at room temperature. The determinations were carried out in  
232 triplicate from three different batches.

233

234 *Swelling extent and water sorption*

235 Clay expansion extent was determined by monitoring the size of clay particles upon  
236 contact with deionized water at 25 ± 1°C through an optical microscope (N-400FL, Optika  
237 Microscopes, MAD Apparecchiature Scientifiche, Bergamo, Italy) by measuring the diameter  
238 change up to the equilibrium of at least 200 particles on recorded computerized images. Water  
239 uptake was evaluated at room temperature on 100 mg of clay sample by means of the Enslin  
240 apparatus (Enslin, 1933) up to 24 h. These analyses were carried out in triplicate from three  
241 different batches.

242

243 *Rheological behavior*

244 The rheological behavior of each clay water suspension was analyzed by coaxial  
245 cylinder rheometer (Rotovisco HAAKE RV12, Karlsruhe, Germany) on 10% and 25% w/v  
246 solid content. After 24 h, flow curves were obtained by using a defined program of gradient  
247 flow (from 48 to 1536 s<sup>-1</sup>) generating ascending and descending rheograms at a temperature  
248 of 25 ± 1°C. The reported values were averaged on three determinations from three different  
249 batches.

250

251 *Statistical analysis*

252 Data obtained were evaluated statistically using one-way analysis of variance  
253 (ANOVA). Differences at p values (calculated probability) <0.05 were considered significant.

254

## 255 RESULTS AND DISCUSSION

256

257 Pharmaceutical and cosmetic sectors make extensive use of clays and clay minerals, in  
258 particular phyllosilicate minerals. For these uses phyllosilicate minerals should possess, in  
259 addition to the pharmacopeial requirements, other properties based on interlayer reactions,  
260 swelling, water sorption, and rheological behavior that justify their use either as excipients in  
261 both conventional dosage forms and controlled release formulations, or as biological active  
262 agents also in combination with water (geotherapy) or minero-medicinal waters (Carretero,  
263 2002; Lopez-Galindo *et al.*, 2007). Clay properties, related to their mineralogical and  
264 chemical compositions, depending in their turn on the geological context of the different  
265 deposits, usually dictate their appropriate use. Hence, a bentonite of volcanic origin (BNT) as  
266 well as two kaolins of different geological origin, a kaolin of hydrothermal origin (K-H) and a  
267 kaolin of lacustrine and fluvial origin (K-L), were investigated for physico-chemical  
268 properties (mineralogy, particle size, surface charge, elemental composition, CEC, pH value)  
269 and hydration properties (wettability, swelling, water sorption, rheology) in order to envisage  
270 their possible application in pharmaceutical or cosmetic field. The name kaolin was also  
271 conferred to the sample K-L despite the low content of kaolinite (Bloodworth *et al.*, 1993).  
272 The characterization results were compared with those obtained from a pharmaceutical grade  
273 bentonite (MM).

274

275 *Physico-chemical properties*

276 Original unmilled clay particles exhibited an irregular shape (Figure 1) and a broad  
277 size distribution in the fine-grained range of about 1-100  $\mu\text{m}$ , regardless of the sample.  
278 Although these dimensions are considered proper for several pharmaceutical and cosmetic  
279 applications, particle size is known to affect CEC values, water sorption, swelling, and  
280 rheological behavior (Adeyinka *et al.*, 2009; Chemani, 2015; Lloyd and Conley, 1970;  
281 Stepkowska *et al.*, 2001; Stul and van Leemput, 1982). For that reason, all clays including  
282 MM, used for comparison, were subjected to a milling process that provided uniform particle  
283 dimensions among the samples in the range of about 1-3  $\mu\text{m}$  (Table 1).

284 The identification of the clay minerals composing the clayey materials was performed  
285 by XRD (Figure 2, ~~Table 2~~). The diffraction peaks of the BNT sample show the following  
286 composition: montmorillonite, a mineral of the smectite group with chemical formula  
287  $(\text{Na,Ca})_{0.33}(\text{Al,Mg})_2(\text{Si}_4\text{O}_{10})(\text{OH})_2 \cdot n\text{H}_2\text{O}$  (Mnt, ~~JCPDS 00-003-0010~~) (Anthony *et al.*, 1995);  
288 quartz, a tectosilicate with formula  $\text{SiO}_2$ , present, ~~JCPDS 01-085-1054~~) in different  
289 polymorphic phases of cristobalite (Crs, ~~JCPDS 01-076-0941~~) (Anthony *et al.*, 1995) and  $\alpha$ -  
290 quartz (Qz) (Anthony *et al.*, 1995); albite, a plagioclase feldspar mineral with formula  
291  $\text{NaAlSi}_3\text{O}_8$  (Ab, ~~JCPDS 01-078-1995~~) (Anthony *et al.*, 1995); illite, a non-expanding clay  
292 crystalline mineral with chemical formula  $(\text{K,H}_3\text{O})(\text{Al,Mg,Fe})_2(\text{Si,Al})_4\text{O}_{10}[(\text{OH})_2,(\text{H}_2\text{O})]$  (Illt,  
293 ~~JCPDS 00-026-0911~~) (Rieder *et al.*, 1998) and ~~in mixed layers with~~ kaolinite, another clay  
294 crystalline mineral with composition  $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$  (Kln, ~~JCPDS 01-074-1784~~). The  
295 mineralogical composition of BNT corresponds with a sodium bentonite, an aluminium  
296 phyllosilicate clay consisting mostly of montmorillonite with quartz, feldspar, illite, and other  
297 minerals as minor components (Nones *et al.*, 2015). The K-H sample is kaolin clay. It  
298 contains mainly: kaolinite (Kln (~~JCPDS 01-075-0938~~); sanidine, a potassium feldspar with  
299 formula  $\text{K}(\text{AlSi}_3\text{O}_8)$  (Sa, ~~JCPDS 01-089-2650~~) (Anthony *et al.*, 1995); quartz (Qz (~~JCPDS 01-~~  
300 ~~086-1629~~); intermediate Albite (Ab (~~JCPDS 01-076-0757~~), and illite (Illt), and ~~in mixed layers~~

301 ~~with montmorillonite (Mnt (JCPDS 00-007-0330)) are present as secondary minerals.~~ K-L  
302 sample was found composed mainly of quartz (Qz (JCPDS 01-086-1629), kaolinite (Kln  
303 (JCPDS 00-001-0527), sanidine (Sa (JCPDS 00-019-1227), illite (Ilt (JCPDS 00-002-0056),  
304 and montmorillonite (Mnt (~~JCPDS 00-002-0009~~)). These results were consistent with the  
305 mineralogical analysis from the producer's datasheet.

306 Clay elemental composition was identified by Energy Dispersive X-ray (EDX)  
307 analysis obtaining the relative quantitative abundance of each element present in the samples  
308 (Figure 3, Table 2). For all the clays the results showed the characteristic  $\text{Si}^{4+}$  and  $\text{Al}^{3+}$  ions of  
309 the tetrahedral and octahedral sheets, respectively. As expected, BNT exhibited a partial  
310 substitution of  $\text{Al}^{3+}$  by  $\text{Mg}^{2+}$  ( $2.07 \pm 0.03\%$ ) in the octahedral sheet, even if less than that of  
311 MM ( $9.59 \pm 0.15\%$ ), used as term of comparison. Such a substitution was absent or less than  
312 1% in K-H or K-L sample, respectively. Given that  $\text{Al}^{3+}$  isomorphous substitution is seldom  
313 in Kln (Grim, 1968), the origin of  $\text{Mg}^{2+}$  in K-L sample could lie in its component of  
314 interstratified clay minerals containing Mnt.

315 Isomorphous substitutions in the octahedral sheets create an excess of negative structural  
316 charge within the lattice that is balanced by inorganic cations (mainly  $\text{Na}^+$  and  $\text{Ca}^{2+}$ ).  
317 Additional negative polar sites available for inorganic cation adsorption are constituted by  
318 octahedral Al-OH and tetrahedral Si-OH groups located at the broken edges as well as by  
319 exposed hydroxyl end-groups on the terminated planes. Clay abundance of  $\text{Na}^+$  and  $\text{Ca}^{2+}$ ,  
320 summed together, was found in the following decreasing order: MM>BNT>K-L>K-H. For  
321 smectites (MM and BNT) the broken edge surface contributes for only the 20% of the total  
322 surface area involved in cation adsorption and hence cation concentration may be mainly  
323 related to the degree of isomorphic substitution indicated by the abundance of  $\text{Mg}^{2+}$  ions. In  
324 between the two exchangeable cations, both kaolins contained only  $\text{Na}^+$  ions which are  
325 reasonably ascribable to three components: compensating cations adsorbed on the crystal

326 edges or the external surfaces where terminal hydroxyl groups are present, interstratified clay  
327 minerals containing Mnt and Na-feldspar mineral (Ab) as a mineralogical component of K-H  
328 sample.

329 In addition, all the samples contained iron (ranging from about 1.2 to 2.9%) that is often  
330 present in soils as free iron oxide at low concentrations (Singh and Gilkes, 1991) and non-  
331 exchangeable  $K^+$  ions.  $K^+$  ion percentages were higher in kaolins (about 3.5%, regardless of  
332 the sample,  $p > 0.05$ ) than those in both bentonites ( $p < 0.05$ ) reasonably owing to greater 1:1  
333 phases, a 2:1 clay mineral with  $K^+$  as the main cation in the interlayer, and the presence of K-  
334 feldspar (Sa) phases in both kaolins. Clay elemental composition was found consistent with  
335 the average chemical analysis from the producer's datasheet.

336         The tendency of clays to exchange the inorganic cations located in the interlayer space  
337 with chemical moieties on which they come in contact is quantified by the value of CEC.  
338 CEC arises mainly from lattice isomorphous substitution, i.e. substitution of  $Si^{4+}$  by  $Al^{3+}$  and  
339 of  $Al^{3+}$  by  $Mg^{2+}$ , producing mainly  $Na^+$  or  $Ca^{2+}$  adsorption in the interlayer space as charge  
340 compensation, and it is affected by clay chemical composition varying from one original clay  
341 deposit to another (He *et al.*, 2010). CEC may also be due to cations dissociating from  
342 hydroxyl end-groups located at the external surfaces and at the edge surfaces or to cations  
343 originating from broken bonds around the crystal edges. Clay CEC was assayed by using  $Cs^+$   
344 as the exchange cation and by evaluating the modification of the elements with respect to  
345 those exhibited by the untreated clays through EDX analysis. Cesium interacts strongly and  
346 semi-permanently with phyllosilicate minerals removing the exchangeable cations and  
347 binding to the interlayer spaces, probably owing to its low hydration tendency (Motokawa *et*  
348 *al.*, 2014). The element identification and quantification (Figure 4, Table 3) revealed the  
349 complete substitution of  $Na^+$  and  $Ca^{2+}$  cations by  $Cs^+$  in BNT clay as well as the complete and  
350 almost complete substitution of  $Na^+$  and  $Ca^{2+}$  cations, respectively, in MM clay. Contrary to

351 this, substitution of Na<sup>+</sup> cations did not occur in K-H kaolin and it was only partial in K-L  
352 kaolin. Considering the impossibility to separate Kln aluminosilicate units, the partial  
353 exchange in K-L kaolin is attributable to the greater Mnt phase present in K-L sample.  
354 Accordingly, CEC values were high for bentonites (100.64 ± 7.33 meq/100 g for BNT, 84.81  
355 ± 3.45 meq/100 g for MM), in good agreement with typical **smeelite** **montmorillonite** CEC  
356 values (Donahue *et al.*, 1977), and low for kaolins (11.13 ± 5.46 meq/100 g for K-H, 14.75 ±  
357 6.58 meq/100 g for K-L) without significant difference (p >0.05) between them. CEC  
358 determinations obtained by using cesium exchange method combined with EDX analysis  
359 were validated on BNT samples by means of the standard ammonium acetate method giving a  
360 comparable CEC value of 95.90 ± 4.70 (p <0.05).

361 Upon contact with water, both BNT and MM provided alkaline suspensions (pH of about 10),  
362 in accordance with the values required by both U.S. and European Pharmacopeias, whereas  
363 both kaolins provided almost neutral suspensions (pH of about 7.5) (Table 4). All clay pH  
364 values remained unchanged after 1 h of dispersion in water. Unlike kaolins, **smeelite**  
365 **bentonite** alkalinity is generated by **Mnt** exchangeable Na<sup>+</sup> ions that are retained by  
366 electrostatic attraction and can quickly diffuse from the interlayer surfaces.

367 Exchangeable cations adsorbed onto **smeelite** **Mnt** interlayer surfaces compensate the  
368 permanent structural negative charge usually originated from the isomorphous substitutions in  
369 **smeelite** **Mnt** octahedral sheets. In addition to the permanent charge, clay minerals are also  
370 characterized by a surface charge due to the hydrolysis of Si-O and Al-OH bonds on the  
371 external surfaces of tetrahedral sheets in 2:1 clays or in both tetrahedral and octahedral sheets  
372 in 1:1 clays as well as along the edges of both 2:1 and 1:1 clays. The net surface charge of the  
373 clay samples was investigated by determining particle zeta potential values. All clay samples  
374 exhibited a negative net surface charge (Table 3) with a greater magnitude in kaolins (about -  
375 42 mV, without significant difference between the two samples, p >0.05) compared with

376 bentonites (about -28 mV in BNT and -35 mV in MM,  $p < 0.05$ ). The net surface charge of  
377 kaolins is related to their mineralogical composition, with the contribution from Kln,  
378 interstratified minerals containing Mnt, and Qz. The edge surface charges of Mnt and Kln  
379 have been estimated to be very close to zero at neutral pH medium (Furukawa *et al.*, 2009)  
380 and the degree of dissociation in aqueous solution of exchangeable cations at Mnt interlayers  
381 makes a relatively small contribution (Low, 1981). Based on this consideration, the main  
382 effect on the net surface charge of kaolins arises from the dissociation of the exposed  
383 hydroxyls in Kln and Qz at their basal surfaces (20% and 100% of the total surface,  
384 respectively). For bentonite, the basal surface of Mnt is close to 5% (WHO, 2005) whereas  
385 the permanent negative charge at the interlayer spaces represents more than 90% of the  
386 surface charge. At the level of Mnt interlayer, the compensating exchangeable cations having  
387 low dissociation degree provide a poor contribution to the net surface and account for the high  
388 CEC value of bentonite. On the other hand, the net surface charge of kaolins deriving from  
389 exposed basal hydroxyl groups in Kln and Qz contributes to the CEC values of kaolins, even  
390 though low (Ma and Eggleton, 1999; White and Zelazny, 1988), together with the CEC  
391 arising from Mnt in the interstratified minerals.

392

### 393 *Hydration properties*

394 Clay hydration properties, in terms of water sorption capacity and swelling extent, are  
395 strictly related to clay chemical composition, isomorphous substitution degree, as well as  
396 amounts and nature of exchangeable cations. Clay samples were evaluated for wettability,  
397 swelling extent, and water sorption. Upon contact with water, all the clay samples expanded  
398 reaching a plateau value in a few minutes (Figure 5). Because of the hydrophilicity conferred  
399 by hydroxyl groups present on the broken edges or terminated surfaces, water spread quickly  
400 on all sample surfaces giving contact angles of zero degrees. BNT as well as MM swelled



401 greatly in water achieving particle dimensions from 13 to 18 times larger than the original  
402 one, respectively (Table 1) owing to water adsorption in the interlayer space by osmotic  
403 effects and exchangeable cation diffusion leading to sheet repulsion. Conversely, kaolins  
404 increased their diameter of only 2.5 (K-H) or 4 (K-L) times. The greater swelling extent of K-  
405 L compared with K-H sample may be reasonably related to its greater Mnt phase. The  
406 swelling extent was found to be in agreement with clay structural characteristics and  
407 consistent with the definition of “expandable” for smectites and “non-expandable” or “low-  
408 expandable” for ~~kaolinitic~~-kaolinite-rich clays (Holtz and Kovac, 1981; Miranda-Trevino  
409 and Coles, 2003; Taylor and Smith, 1986). Despite the poor crystal expansion, both kaolins  
410 were able to adsorb a higher volume of water (about 0.65 ml, regardless of the sample,  $p$   
411  $>0.05$ ) than BNT ( $0.21 \pm 0.02$  ml) ( $p <0.05$ ) (Table 1, Figure 6), but slightly lower than that  
412 adsorbed by MM ( $0.78 \pm 0.05$  ml) ( $p <0.05$ ). ~~Kaolins are~~ Kln is known to possess high  
413 adsorptive properties provided by the highly ionizable cations placed at the level of the  
414 terminated planes and edges (Foster, 1954). The lower water adsorption along with the lesser  
415 swelling extent of BNT compared with MM may be attributed to the less  $Mg^{2+}$  abundance, i.e.  
416 isomorphous substitution of  $Al^{3+}$  by  $Mg^{2+}$  in BNT octahedral sheet, producing less amount of  
417 exchangeable cations considered as the main factor influencing water sorption. Moreover,  
418 influences may arise from the greater content in impurities of BNT, such as Qz (Dontsova *et*  
419 *al.*, 2004).

420 Relevant differences in rheological properties among the analyzed clays were also  
421 recorded. As is common knowledge, the flow behavior of a clay suspension has a significant  
422 impact upon its processability, applications, and storage in several fields (Abu-Jdayil, 2011).  
423 The shear stress versus shear rate curves for clay suspensions with 10 or 25% solid content  
424 were recorded (Figure 7). All the samples exhibited non-Newtonian pseudoplastic patterns  
425 with the decrease in apparent viscosity with increasing shear rate. Flow curves of BNT, K-H,

426 and K-L suspensions with 10% solid content were not significantly different from each other  
427 ( $p > 0.05$ ). The rheology of these clays was weak with viscosity values ranging approximately  
428 from 6 mPa·s to 3 mPa·s at low shear rate ( $43.28 \text{ s}^{-1}$ ), and decreasing to about 1 mPa·s at high  
429 shear rate ( $1384.96 \text{ s}^{-1}$ ), without displaying yield stress as well as thixotropic properties  
430 (overlapped ascending and descending rheograms). Conversely, MM suspensions exhibited  
431 higher viscosities, ranging approximately from 140 mPa·s at low shear rate ( $43.28 \text{ s}^{-1}$ ) to 36  
432 mPa·s at high shear rate ( $1384.96 \text{ s}^{-1}$ ) with yield stress of about 9 Pa and a hysteresis loop  
433 formed by the descending curve higher than that of the ascending one indicating a negative  
434 thixotropic behavior, i.e. a rapid increase in recovery of the original viscosity. The rheological  
435 properties of clay suspensions are known to be related to the association between clay  
436 particles (Galindo-Rosales and Rubio-Hernandez, 2006). When 2:1 clay minerals are  
437 dispersed in polar media, a highly structured system is formed as a consequence of face-to-  
438 face and edge-to-face repulsive forces (Luckham and Rossi, 1999; Kennedy and Kennedy  
439 2007) causing platelets to exfoliate and delaminate. This leads to high viscosity, yield stress,  
440 and thixotropy that characterize suspensions of bentonites composed of 70-90% Mnt, from a  
441 3% solid content (Luckham and Rossi, 1999). The reduction in viscosity with the increase of  
442 shear rate can be attributed to the progressive orientation of the individual platelets in the flow  
443 direction as well as to the breaking of the bonds between clay mineral platelets (Heller and  
444 Keren, 2001). BNT, however, showed rheological behavior significantly weaker with lower  
445 viscosity values, compared to MM. Since the rheological properties of bentonites are  
446 determined by the swelling components (Au and Leong, 2013), this finding can be attributed  
447 to the lower water adsorption along with the lesser swelling extent of BNT in comparison  
448 with MM, as described above. Moreover, BNT contains other clay minerals in addition to  
449 Mnt, in particular Qz, as well as a  $\text{Na}^+/\text{Ca}^{2+}$  ratio less than MM that could mitigate the  
450 rheological properties (Benea and Gorea, 2004; Packter, 1956). With regard to 1:1 clay

451 minerals, no water can penetrate between the layers in Kln due to the strong hydrogen bonds,  
452 no charge deficiency is present in their structures and the surface area is extremely lower than  
453 that of bentonites. Consequently, kaolins display low viscosity except at high solid content (>  
454 40%) (Au and Leong, 2013). The increase in solid content to 25% determined increased  
455 viscosities only for BNT suspensions with values ranging from approximately 12 mPa·s at  
456 low shear rate ( $43.28 \text{ s}^{-1}$ ) to 5 mPa·s at higher shear rate (from 346.24 to  $1384.96 \text{ s}^{-1}$ ), without  
457 yield stress as well as thixotropy (overlapped ascending and descending rheograms), whereas  
458 rheograms of both kaolins showed no changes in function of the solid content. At 25% solid  
459 percentage, the handling of MM suspensions was impracticable due to the extreme viscosity.

460

461

## CONCLUSIONS

462

463 The characterization of three Italian native clays, one bentonite and two kaolins, with  
464 the aim of establishing their possible use in pharmaceutical and cosmetic formulations, has  
465 revealed different properties according to the mineralogical and elemental composition of  
466 each clay. In this regard, EDX analysis has proved of considerable interest to identify and  
467 quantify the elements within clay lattice structure. Bentonite from the Iglesias deposit  
468 exhibited hydration properties in terms of swelling, water sorption, and viscosity lower than  
469 those typical of clays having Mnt as the main component. The CEC value, however, was  
470 excellent and proper to be proposed for drug interactions in modified drug delivery systems or  
471 thermal muds following clay maturation into contact with thermal and/or mineral waters.  
472 Both kaolins from the Roccastrada deposit proved to be good adsorbents, then useful in  
473 pharmaceuticals as dermatological protectors, or in cosmetics as deodorants, protectants, bath  
474 powders, and face masks for their capacity to increase the adherence of the preparation as  
475 well as adsorb undesired substances like skin exudates. Despite the weak rheological

476 properties, all samples could be used for the stabilization of pharmaceutical and cosmetic  
477 dispersions because of their presence on the interface boundaries between the formulation  
478 phases. Nevertheless, for topical application, samples could require some treatments to reduce  
479 the amount of abrasive materials as quartz or feldspar.

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481

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482

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486

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Table 1. Particle size, swelling extent and water sorption capacity of bentonite (BNT), hydrothermal kaolin (K-H), and lacustrine kaolin (K-L), in comparison with a pharmaceutical grade bentonite (MM). Mean values  $\pm$  SD.

	Size after milling ( $\mu\text{m} \pm \text{SD}$ )	Equilibrium swelling size ( $\mu\text{m} \pm \text{SD}$ )	Equilibrium swelling ratio	Water sorption (ml)
BNT	$1.26 \pm 0.38$	$16.00 \pm 3.03$	1 : 13	$0.21 \pm 0.02$
K-H	$2.76 \pm 1.14$	$7.40 \pm 1.15$	1 : 2.5	$0.60 \pm 0.05$
K-L	$1.91 \pm 0.66$	$8.10 \pm 1.32$	1 : 4	$0.66 \pm 0.03$
MM	$1.06 \pm 0.31$	$18.65 \pm 3.22$	1 : 18	$0.78 \pm 0.05$

725

726 ~~Table 2. Values of d-spacing (Å) and 2θ (°) from XRD patterns of BNT, K-H, and K-L~~727 ~~samples.~~

728

BNT		K-H		K-L	
2θ	d-spacing (Å)	2θ (°)	d-spacing (Å)	2θ	d-spacing (Å)
5.60	15.77	12.37	7.16	5.44	16.25
8.82	10.02	13.71	6.46	8.97	9.86
9.82	9.01	15.61	5.68	12.28	7.21
17.39	5.10	17.96	4.94	13.66	6.48
19.88	4.47	20.07	4.42	17.87	4.96
20.84	4.26	20.76	4.28	19.83	4.48
21.80	4.08	20.98	4.23	20.81	4.27
22.04	4.03	21.44	4.15	20.95	4.24
24.39	3.65	21.96	4.05	22.84	3.89
26.41	3.38	22.78	3.90	23.53	3.78
26.69	3.34	23.53	3.78	23.74	3.75
28.03	3.18	24.86	3.58	25.56	3.48
30.09	2.97	25.50	3.49	26.72	3.34
34.92	2.57	26.65	3.34	27.74	3.22
36.06	2.49	26.75	3.34	29.93	2.99
37.15	2.42	27.07	3.29	30.78	2.90
39.51	2.28	27.40	3.25	32.08	2.79
42.51	2.13	27.65	3.22	34.93	2.57
50.19	1.82	30.03	2.97	35.52	2.53
50.91	1.79	30.89	2.89	36.58	2.46
		32.42	2.76	37.72	2.39
		34.99	2.56	39.56	2.28
		36.59	2.45	40.34	2.24
		38.52	2.34	42.53	2.13
		39.43	2.28	45.83	1.98
		39.57	2.28	50.17	1.82
		40.36	2.23	54.90	1.67
		41.66	2.17	55.34	1.66
		42.50	2.13		
		45.85	1.98		
		47.77	1.90		
		50.18	1.82		
		50.94	1.79		
		52.37	1.75		
		54.92	1.67		
		55.38	1.66		
		59.08	1.56		

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Table 2. EDX semi-quantitative analysis of the elements present in bentonite (BNT), hydrothermal kaolin (K-H), and lacustrine kaolin (K-L), in comparison with a pharmaceutical grade bentonite (MM). All data are expressed as oxide relative percentage of the elements >0.5%. Mean values  $\pm$  SD

	BNT	K-H	K-L	MM
SiO <sub>2</sub>	75.06 $\pm$ 0.03	59.42 $\pm$ 1.63	74.60 $\pm$ 1.23	70.89 $\pm$ 0.47
Al <sub>2</sub> O <sub>3</sub>	15.89 $\pm$ 0.20	26.67 $\pm$ 1.90	15.09 $\pm$ 0.59	10.71 $\pm$ 0.04
FeO	2.94 $\pm$ 0.14	2.44 $\pm$ 0.10	2.48 $\pm$ 1.12	1.26 $\pm$ 0.03
TiO <sub>2</sub>	0.00	0.00	0.65 $\pm$ 0.28	0.00
CaO	1.67 $\pm$ 0.04	0.00	0.00	2.15 $\pm$ 0.16
MgO	2.07 $\pm$ 0.03	0.00	0.83 $\pm$ 0.09	9.59 $\pm$ 0.15
K <sub>2</sub> O	1.62 $\pm$ 0.12	3.38 $\pm$ 0.40	3.53 $\pm$ 0.68	0.66 $\pm$ 0.04
Na <sub>2</sub> O	0.47 $\pm$ 0.14	0.72 $\pm$ 0.67	1.14 $\pm$ 0.22	1.86 $\pm$ 0.02
SO <sub>3</sub>	0.00	7.36 $\pm$ 0.74	0.81 $\pm$ 0.14	0.00

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Table 3. EDX semi-quantitative analysis of the elements present in bentonite (BNT), hydrothermal kaolin (K-H), and lacustrine kaolin (K-L), in comparison with a pharmaceutical grade bentonite (MM) following cation exchange by cesium. All data are expressed as oxide relative percentage of the elements >0.5%. Mean values  $\pm$  SD

	BNT	K-H	K-L	MM
Cs <sub>2</sub> O	14.19 $\pm$ 1.27	1.57 $\pm$ 0.08	2.08 $\pm$ 0.17	11.28 $\pm$ 0.40
SiO <sub>2</sub>	64.87 $\pm$ 2.19	56.72 $\pm$ 0.43	73.81 $\pm$ 0.48	65.68 $\pm$ 0.10
Al <sub>2</sub> O <sub>3</sub>	13.65 $\pm$ 0.29	27.66 $\pm$ 0.18	14.42 $\pm$ 0.49	10.01 $\pm$ 0.20
FeO	3.68 $\pm$ 0.83	2.13 $\pm$ 0.09	3.44 $\pm$ 0.12	1.48 $\pm$ 0.34
TiO <sub>2</sub>	0.00	0.00	0.00	0.00
CaO	0.00	0.00	0.00	0.78 $\pm$ 0.12
MgO	1.34 $\pm$ 0.04	0.00	0.77 $\pm$ 0.09	8.53 $\pm$ 0.25
K <sub>2</sub> O	1.34 $\pm$ 0.09	3.04 $\pm$ 0.20	3.72 $\pm$ 0.19	0.50 $\pm$ 0.03
Na <sub>2</sub> O	0.00	0.58 $\pm$ 0.07	0.55 $\pm$ 0.02	0.00
SO <sub>3</sub>	0.00	7.25 $\pm$ 0.14	0.70 $\pm$ 0.09	0.00

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Table 4. pH values and zeta potential of bentonite (BNT), hydrothermal kaolin (K-H), and lacustrine kaolin (K-L), in comparison with a pharmaceutical grade bentonite (MM). Mean values  $\pm$  SD.

	pH value (mean $\pm$ SD)	pH value after 1 h (mean $\pm$ SD)	Z-potential (mV $\pm$ SD)
BNT	9.43 $\pm$ 0.48	9.30 $\pm$ 0.23	-28.40 $\pm$ 0.98
K-H	7.63 $\pm$ 0.43	7.41 $\pm$ 0.18	-41.17 $\pm$ 1.29
K-L	7.82 $\pm$ 0.26	7.35 $\pm$ 0.25	-42.50 $\pm$ 0.17
MM	10.24 $\pm$ 0.30	10.00 $\pm$ 0.30	-34.47 $\pm$ 0.49

776

777 **Figure captions**

778

779 Figure 1. ESEM images of clay samples before milling: bentonite BNT (a), hydrothermal  
780 kaolin K-H (b), and lacustrine kaolin K-L (c).

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782 Figure 2. X-ray diffraction patterns of bentonite BNT (a), hydrothermal kaolin K-H (b), and  
783 lacustrine kaolin K-L (c) samples.

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785 Figure 3. X-ray emissions (EDX spectra) from the elements contained in bentonite (BNT),  
786 hydrothermal kaolin (K-H), and lacustrine kaolin (K-L), in comparison with a pharmaceutical  
787 grade bentonite (MM).

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789 Figure 4. X-ray emissions (EDX spectra) from the elements contained in bentonite (BNT),  
790 hydrothermal kaolin (K-H), and lacustrine kaolin (K-L), in comparison with a pharmaceutical  
791 grade bentonite (MM), following cation exchange by cesium.

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793 Figure 5. Particle swelling of bentonite (BNT), hydrothermal kaolin (K-H), and lacustrine  
794 kaolin (K-L), in comparison with a pharmaceutical grade bentonite (MM), upon contact with  
795 water determined by optical microscopy.

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797 Figure 6. Water sorption of bentonite (BNT), hydrothermal kaolin (K-H), and lacustrine  
798 kaolin (K-L), in comparison with a pharmaceutical grade bentonite (MM), upon contact with  
799 water determined by means of Enslin apparatus.

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801 Figure 7. Flow behavior (shear stress *versus* shear rate) of bentonite (BNT), hydrothermal  
802 kaolin (K-H), and lacustrine kaolin (K-L), in comparison with a pharmaceutical grade  
803 bentonite (MM), determined by coaxial cylinder rheometer on 10% or 25% clay water  
804 suspension  
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