

1 **Organo-modified bentonite for gentamicin topical application: Interlayer structure and in vivo**  
2 **skin permeation**

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6 **Highlights**

7 An organo-modified raw bentonite was developed as novel antibacterial material.

8 Thermal reactions could support drug intercalation occurrence.

9 MD simulations showed gentamicin monolayer arrangement within Mnt interlayer.

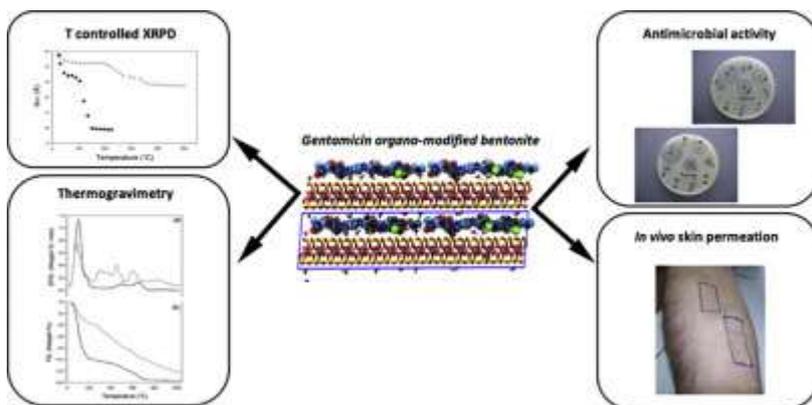
10 *Trans*-epidermal route was favored by drug intercalation as arisen from *in vivo* data.

11

12 **Abstract**

13 Recent [biomedical applications](#) of clay materials have included organically modified clays or clay minerals  
14 with the purpose of modifying and improving drug biological activity. The present research aims to explore  
15 the potential benefits provided by a raw [bentonite](#) (Bt) modified by gentamicin (GM) adsorbed within  
16 [montmorillonite interlayers](#) in the management of cutaneous infectious diseases. Information arisen from  
17 controlled X-ray powder diffraction, [thermogravimetry](#) coupled with evolved gas [mass spectrometry](#), and  
18 molecular dynamics simulations pointed out GM monolayer arrangement within montmorillonite  
19 framework without producing substantial effects on the layer periodicity. Concerning skin biomedical  
20 application, unlike the pure antibiotic permeating along the trans-follicular pathway across *stratum*  
21 *corneum*, the organo-modified Bt/GM would favor the trans-epidermal route along inter-cluster [corneocyte](#)  
22 region, as *in vivo* skin penetration studies by means of tape stripping test indicated. Based on the results  
23 obtained, GM [intercalation](#) could represent a potential advantageous approach allowing a long-term  
24 Bt/GM reservoir for sustained antibacterial activity.

25 **Graphical abstract**



26

27 **Keywords**

28 Bentonite, Gentamicin, Intercalation, Thermal analyses, Molecular dynamics, *In vivo* skin penetration

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## 31 1. Introduction

32 There is a strong demand to identify new strategies in order to set optimal drug delivery systems for  
33 antibiotic treatments. [Intercalation](#) of organic molecules into layered inorganic solids provides a useful and  
34 convenient approach to prepare hybrids that show properties of both the inorganic host and organic guest  
35 in a single material ([Aguzzi et al., 2007](#); [Rodrigues et al., 2013](#)). In the last five decades the ability of both  
36 raw and synthetic [smectites](#) to exchange cations with several organic compounds has been exploited in  
37 many application fields. An archetypical example of such [versatility](#) is represented by the polymeric  
38 [nanocomposites](#) employing organo-modified [bentonites](#) ([Benelli et al., 2017](#); [Franchini et al., 2008](#); [2011](#);  
39 [Morgan and Wilkie, 2007](#)).

40 More recently, smectites have been proposed as materials for modulating drug delivery or improving  
41 dissolution of poorly water-soluble drugs ([Aguzzi et al., 2005](#); [Iannuccelli et al., 2015](#); [Joshi et al., 2009](#)).  
42 Among smectites, the 2:1 layered montmorillonite is probably the most investigated clay mineral. The  
43 reasons that drive this interest mainly arise from its high specific surface area, swelling and [adsorptive](#)  
44 [capacity](#), high cation exchange capacity (CEC), specific rheological properties, drug-carrying capability and  
45 ability to modulate drug release ([World Health Organization, 2005](#)). Montmorillonite is mainly used as  
46 auxiliary material in the [pharmaceutical industry](#) for oral or topical dosage forms, recorded in the United  
47 States, European, and British Pharmacopeias. Montmorillonite, following to its high [swelling behavior](#), can  
48 intercalate therapeutic compounds between the layers generating a host for oral or topical drug delivery  
49 ([Aguzzi et al., 2005](#); [Bello et al., 2015](#); [Bonina et al., 2007](#), p. 200; [de Paiva et al., 2008](#); [Forni et al., 1987](#);  
50 [Iannuccelli et al., 2015](#); [Iliescu et al., 2011](#); [Joshi et al., 2009](#); [Kant and Datta, 2016](#); [Katti et al., 2010](#); [Kim et](#)  
51 [al., 2016](#); [Mohamed et al., 2014](#); [Rapacz-Kmita et al., 2015](#)). Concerning topical use, montmorillonite has  
52 beneficial effects in dermatological and cosmetic applications (geotherapy, paleotherapy) ([Carretero, 2002](#);  
53 [López-Galindo et al., 2007](#)).

54 The present work focuses on the assessment of a raw bentonite (Bt), a montmorillonite rich clay recently  
55 characterized in a previous work ([Iannuccelli et al., 2016](#)), for the development of a novel gentamicin/clay  
56 hybrid material for the topical use. Gentamicin (GM) is an aminoglycoside antibiotic widely used in the  
57 treatment of severe infections, caused by many Gram-negative and Gram-positive bacteria, such as  
58 meningitis, nephritis, and post-operative infections. Although it presents a very broad spectrum of action,  
59 its use is limited to serious infections caused by Gram-negative bacteria because of its high toxicity.  
60 Gentamicin is commonly administered as injections, topical and ophthalmic dosage forms because of poor  
61 absorption following the oral administration. The well-known poor gastrointestinal membrane permeability  
62 and the consequent low [bioavailability](#) (class III of the biopharmaceutical classification system) are likely  
63 connected to the high polarity of this cationic compound. Various approaches have been investigated in  
64 order to increase GM oral bioavailability, including the co-administration of absorption-enhancing agents  
65 such as surfactants ([Hu et al., 2001](#); [Ito et al., 2005](#)), bile salts and glucosteroids ([Axelrod et al., 1998](#)), and  
66 liposaccharides ([Ross et al., 2004](#)). Although good gastrointestinal absorption enhancing effects were  
67 demonstrated, cytotoxicity and damage to the mucosa have been reported ([Aungst, 2000](#); [Ross et al., 2004](#);  
68 [Swenson et al., 1994](#)). Another strategy aiming to promote GM oral bioavailability could involve the use of  
69 microparticulate carriers to be taken up by the intestinal lymphoid tissue ([des Rieux et al., 2007](#); [Hussain et](#)  
70 [al., 2001](#); [Iannuccelli et al., 2011](#); [McClellan et al., 1998](#); [Moyes et al., 2007](#)) or to be implanted for bone  
71 infection treatment also exploiting drug interaction with anionic polymers ([Iannuccelli et al., 1996, 2011](#)).  
72 Gentamicin is extensively used topically against severe microbial infections especially in burns and wounds  
73 ([Chang et al., 2006](#)), but also in the treatment of impetigo, infected bed sores, nasal staphylococcal carrier  
74 state, pyoderma, infections of the external eye, and adnexa ([Nishijima and Kurokawa, 2002](#)). Gentamicin  
75 applied to the skin has only a low systemic absorption due to the difficult penetration through the deep  
76 layers of the skin, related, probably, to its cationic nature; for this reason, its use is limited to the local  
77 effect that involves mainly the most superficial skin layers. Despite its benefits, GM short-life, bacterial

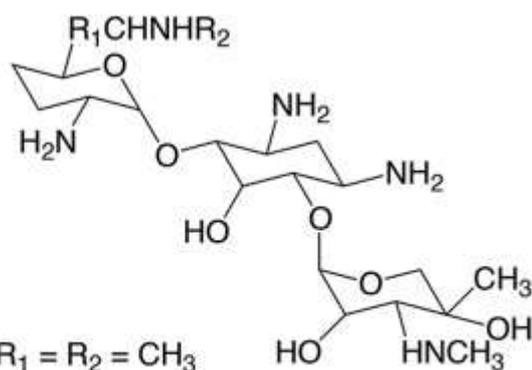
78 barriers and adverse effects such as nephrotoxicity, ototoxicity, and neurotoxicity upon prolonged use limit  
 79 GM daily dosage (Roberts, 2007). In fact, many clinicians are reluctant to use it, even for a short term  
 80 (Drusano, 2007). Efforts have been made to reduce toxicity associated with prolonged use by means of  
 81 liposomes, [micellar systems](#), hydrogels, [microgels](#), or [nanospheres](#) (Ahangari et al., 2013; Ayhan and Ozkan,  
 82 2007; Changez et al., 2003; Eljarrat-Binstock et al., 2004; Jia et al., 2008; Nnamani et al., 2013; Sökmen et  
 83 al., 2008; Umeyor et al., 2012). Local delivery of GM can solve the major disadvantages of the [systemic](#)  
 84 [administration](#) by maintaining a high local antibiotic concentration for an extended time (Zalavras et al.,  
 85 2004). Particularly, drug delivery systems exhibiting high initial release rate followed by a sustained release  
 86 at an effective antibiotic concentration may allow local control of infection while minimizing side effects  
 87 and preventing bacterial resistance (Aviv et al., 2007; Persson et al., 2006).

88 The preparation of a GM-based organo-modified bentonite (Bt/GM) may therefore represent a valuable  
 89 alternative to assure safer and more effective utilization of GM for topical treatment. Based on these  
 90 premises, the present research includes a thorough characterization of Bt/GM by means of several  
 91 instrumental analyses as well as the comparison of the experimental results with Molecular Dynamics  
 92 simulations (MD modeling) to provide a more detailed understanding about the [interlayer](#) arrangement  
 93 and interactions promoted by the organic guest molecules confined in the montmorillonite framework.  
 94 Moreover, GM [antimicrobial activity](#), *in vitro* desorption, and *in vivo* skin [permeation](#) on human beings  
 95 were assessed in the perspective of contribution to a novel antibiotic material.

## 96 2. Experimental part

### 97 2.1. Materials

98 A [bentonite](#) (Bt) of volcanic origin from Iglesias (Sardinia, Italy) deposit (average [mineralogical composition](#)  
 99 from the producer's [datasheet](#): [montmorillonite](#) 80%, quartz 13%, illite-kaolinite 5%, [plagioclase](#) 2%) was  
 100 donated by Eurit srl (Colorobbia Group, Sovigliana Vinci, Italy). Gentamicin sulfate (GM, [Fig. 1](#)), composed  
 101 of gentamicin C1 ( $C_{21}H_{43}N_5O_7 \cdot H_2SO_4$ , <45%), gentamicin C1a ( $C_{19}H_{39}N_5O_7 \cdot H_2SO_4$ , <35%), and gentamicin C2  
 102 ( $C_{20}H_{41}N_5O_7 \cdot H_2SO_4$ , <25%), pKa = 12.55 in acidic condition; 10.18 in basic condition, was purchased by  
 103 Polichimica (Bologna, Italy). All the chemicals and reagents were of analytical grade (Sigma-Aldrich, Milan,  
 104 Italy).



**C<sub>1</sub>** (< 45%) R<sub>1</sub> = R<sub>2</sub> = CH<sub>3</sub>

**C<sub>2</sub>** (< 25%) R<sub>1</sub> = CH<sub>3</sub>; R<sub>2</sub> = H

**C<sub>1a</sub>** (< 35%) R<sub>1</sub> = R<sub>2</sub> = H

105

106 Fig. 1. Molecular structure of gentamicin [sulfate](#).

107

### 108 2.2. Bt activation

109 Bt activation and thus the implementation of its organophilic behavior are provided by the saturation of the  
 110 montmorillonite [interlayers](#) with a homogeneous cationic population through the cation exchange reaction

111 hereafter detailed. A defined amount of Bt was grinded by a vibratory [ball mill](#) (Fritsch GmbH, Idar-  
112 Oberstein, Germany) for 10 h to remove particle aggregates. Batches of dispersions were prepared mixing  
113 1 g of milled Bt and 25 mL of NaCl 0.1 M and were shaken with a [magnetic stirrer](#) at room temperature for  
114 24 h. The supernatant was centrifuged (mod. 4235, 188 ALC International, Milan, Italy) at 2115  $\times g$  for  
115 20 min and the solid was twice subjected to the same treatment. The separated solids were washed several  
116 times with 35 mL of distilled water under magnetic stirring at room temperature for 4 h followed by  
117 centrifugation at 2115  $\times g$  for 2 h. The solid was dried under vacuum at room temperature and the  
118 supernatant analyzed for NaCl absence by [titration](#) with 0.1 M [silver nitrate](#) solution according to [U.S.](#)  
119 [Pharmacopeia](#). The [activation process](#) was carried out in triplicate.

### 120 **2.3. Bt/GM preparation**

121 Gentamicin was adsorbed onto both activated and non-activated Bt at constant drug concentration  
122 corresponding to about two times Bt CEC measured for activated Bt/GM (aBt/GM) and non-activated  
123 Bt/GM (Bt/GM), respectively. Glass tubes filled with 20 mL GM water solution (1 mg/mL) and 100 mg of  
124 milled Bt were horizontally shaken in the darkness for 24 h, a time suitable to fully saturate the  
125 montmorillonite interlayer with GM. The dispersions were centrifuged (2115  $\times g$ , 20 min) and solids washed  
126 twice with 35 mL [deionized water](#) under magnetic stirring for 15 min. The obtained organo-modified clays  
127 were dried under vacuum at room temperature and stored in the darkness. Three batches were prepared  
128 for each sample.

### 129 **2.4. Gentamicin adsorption measurements**

130 In this paper, the term “adsorption” was used to generally refer to the [immobilization](#) of GM onto Bt thus  
131 without distinguish between [intercalation](#) in the interlayer of montmorillonite and adsorption on the outer  
132 surface of montmorillonite and illite-kaolinite. However, when dealing with each single mineral phase the  
133 term adsorption and intercalation will be suitably used.

134 The amount of GM adsorbed onto Bt in both aBt/GM and Bt/GM was calculated as the difference between  
135 the initial GM concentration and that in the supernatants obtained during organo-modified clay  
136 preparation. GM was derivatized by reaction with *o*-phtaldialdehyde of an aliquot of 1 mL from the  
137 supernatants according to Sampath and Robinson method ([Sampath and Robinson, 1990](#)) and determined  
138 spectrophotometrically (Lambda 3B, Perkin-Elmer, Norwalk, CT, USA) at 274 nm wavelength. The GM  
139 concentrations were expressed as drug/clay weight percentage as well as yield (actual/theoretical drug)  
140 percentage on three determinations from three different batches.

### 141 **2.5. Size, surface charge, and pH value**

142 Bt/GM particle size, [Polydispersity Index](#) (PDI), and Z-potential were determined on 10 mg/mL organo-  
143 modified [clay water](#) dispersion by using Photon Correlation [Spectroscopy](#) (PCS) (Zetasizer version 6.12,  
144 Malvern Instruments Ltd) equipped with a 4 mW He-Ne laser (633 nm) and a DTS software (Version 5.0)  
145 and compared with those of Bt. pH value of 2% Bt/GM water dispersion was measured by [potentiometry](#)  
146 immediately after the preparation of the dispersion and after 1 h; obtained data were compared with the  
147 value of Bt dispersion, according to U.S. Pharmacopeia monograph for bentonite, and with 0.1% (w/v) GM  
148 water solution. The reported values were averaged on three determinations from three different batches.

149

### 150 **2.6. CHN elemental microanalysis**

151 CHN elemental [microanalysis](#) (Elemental analyzer, mod. 1106, Carlo Erba, Milan, Italy) was performed on  
152 Bt/GM in comparison with bulk Bt and GM. The analysis was carried out in triplicate.

## 153 2.7. Elemental composition by EDX analysis

154 Clay elemental composition was determined by Energy Dispersive X-ray (EDX, Oxford INCA-350, FEI  
155 Company-Oxford Instruments, Oregon, USA) analysis coupled with an Environmental Scanning Electron  
156 Microscopy (ESEM, Quanta 200 Fei Company-Oxford Instruments). Elements can be identified qualitatively  
157 and semi-quantitatively in function of the X-ray energy emitted by their electrons transferring from a higher  
158 energy shell to a lower energy one. X-ray emission from  $K\alpha$  or  $K\beta$  levels of the atoms calcium, potassium,  
159 oxygen, sodium, magnesium, aluminum, silicon, and other elements with atomic numbers from 4 were  
160 recorded by the selected area method related to whole clay particles from samples mounted without a  
161 [conductive coating](#) on carbon stubs with the following experimental settings: low vacuum (0.70 Torr),  
162 [accelerating voltage](#) 12 kV, spot size 3, element detection limit  $\sim 0.05$  wt%, spatial resolution  $0.1 \mu\text{m}$ , total  
163 spectrum counts  $>250,000$ , accuracy within  $\pm 5\%$  relative errors by reference to standards. [EDX spectra](#)  
164 representing the plots of X-ray counts (intensity) vs. energy peak (keV) of each element were acquired and  
165 semi-quantitative compositions, obtained by a standardless method of acquisition and expressed as  
166 relative weight percentage of each element, were calculated. The carbon peak at low energy level, related  
167 to the hydrocarbon contamination growing the carbon stub signal, was not considered ([Rolland et al.,](#)  
168 [2004](#)). The reported data were averaged on three determinations for each sample.

## 169 2.8. FT-IR measurements

170 FT-IR measurements were performed using a Perkin-Elmer FT-IR 1600 (abscissa accuracy of  $0.01 \text{ cm}^{-1}$  using  
171 HeNe laser reference; resolution from 2 to  $16 \text{ cm}^{-1}$ ; lithium [tantalite](#) temperature-stabilized detector) on  
172 bulk GM, bulk Bt, Bt/GM, and Bt/GM physical mixture at GM content corresponding to that of the organo-  
173 modified clay. The samples were maintained in a drier, dispersed in a Nujol mull (typically 2% w/w) and  
174 measured. Spectra were collected in air in the MID Infra-Red (mid-IR) region. The analyses were performed  
175 in triplicate.

## 176 2.9. X-ray powder diffraction

177 X-ray powder diffraction (XRD) was employed mainly to detect basal periodicity variation in  
178 montmorillonite before and after treating Bt with GM. The XRD patterns were recorded from (00 $l$ ) oriented  
179 mounts, in the temperature range from 25 to  $500 \text{ }^\circ\text{C}$  with a [heating rate of](#)  $10 \text{ }^\circ\text{C}/\text{min}$  using a PANalytical  
180 X'Pert PRO [diffractometer](#) equipped with X'Celerator detector. Before measurements all the samples were  
181 simultaneously equilibrated at the same environmental conditions. Experimental conditions were: Incident  
182 beam: monochromatic  $\text{Cu } K\alpha_1$  radiation ( $1.54060 \text{ \AA}$ ), 40 kV and 40 mA; filter, nickel; Soller slits,  $0.04 \text{ rad}$ ;  
183 anti-scatter mask, 20 mm; anti-scatter slit,  $1/4^\circ$ ; divergence slit,  $1/4^\circ$ . [Diffracted beam](#): X-ray detector,  
184 X'Celerator (Position Sensitive Detector, PSD); anti-scatter mask, 5.0 mm; Soller slits,  $0.04 \text{ rad}$ ; integration  
185 time, 20 s in continuous scanning (PSD length of  $2.12^\circ 2\theta$  corresponding to a step size of  $0.0170^\circ 2\theta$ ).  
186 [Diffraction patterns](#) were recorded from  $3$  to  $75^\circ (2\theta)$  at room temperature, and from  $3$  to  $20^\circ (2\theta)$  when  
187 measuring in non-ambient temperature conditions. NIST corundum was used as calibrating standard.

## 188 2.10. Thermogravimetric measurements coupled with evolved gas mass spectrometry

189 Thermogravimetric analyses were performed with a Seiko SSC 5200 thermal analyzer equipped with a  
190 [quadrupole mass spectrometer](#) (ESS, GeneSys Quadstar 422) to analyze gases evolved during [thermal](#)  
191 [reactions](#) (MSEGA). This device samples gases *via* an inert, fused silicon capillary system, heated to prevent  
192 gas condensation. Analyses of evolved gas phases were carried out in multiple ion detection mode (MID),  
193 which allows the qualitative determination of evolved masses vs. temperature or time. MID analyses were  
194 carried out measuring the  $m/z$  ratios 17 and 18 for  $\text{H}_2\text{O}$ , 28 and 44 for  $\text{CO}_2$ , 30 for  $\text{NO}$  and  $\text{NO}_2$ , 34 for  $\text{H}_2\text{S}$ ,  
195 46 for  $\text{NO}_2$ , and 48, 64, 66 for  $\text{SO}_2$ , where  $m/z$  is the ratio between the mass number and the charge of an  
196 ion; SEM (Secondary Electron Multiplier) and FARADAY detectors operating at 900 V were employed with  
197 1 s of integration time on each measured mass.

198 Measurements were performed on Bt, Bt/GM, and bulk GM air-dried samples at the following experimental  
199 conditions: heating rate: 10 °C/min for Bt and Bt/GM, and 20 °C/min for pure GM; heating range: 25–  
200 1000 °C (25–900 °C for pure GM); data measurement: every 0.5 s; purging gas: ultrapure helium, at a flow  
201 rate of 100 µL/min.

202

### 203 **2.11. Differential scanning calorimetry**

204 Bt/GM was subjected to thermal analysis on a Differential Scanning [Calorimeter](#) (DSC-4, Perkin-Elmer) and  
205 compared with GM and Bt/GM physical mixture at GM content corresponding to that of the organo-  
206 modified clay. The samples (6–7 mg) were accurately weighed in crimped aluminum pans and heated from  
207 30 °C to 280 °C at a scanning rate of 10 °C/min under dry nitrogen flow (30 mL/min). All the thermograms  
208 were obtained in triplicate.

### 209 **2.12. Molecular dynamics simulations**

210 A  $X_{0.75}[\text{Si}_{7.75}\text{Al}_{0.25}][\text{Al}_{3.5}\text{Mg}_{0.5}]\text{O}_{20}(\text{OH})_4$  (X = monovalent cation) ideal montmorillonite model was used for the  
211 present study. This model shows  $-0.75e$  per unit cell, leading to a CEC of 101.9 meq/100 g, a value close to  
212 the experimental one. The simulation box consists of one layer made of 64 unit-cells corresponding to a  
213 basal surface of 4.14 nm × 7.17 nm. In order to mimic the partial cation exchange process obtained in the  
214 experiments, we consider the total negative charge of the layer to be counterbalanced by ~79.2% by C-  
215 1(GM) molecules and ~20.8% of sodium ions (see Supporting information for details). A certain amount of  
216 water molecules was also inserted in the interlayer (corresponding to the 2% wt.). In order to  
217 accommodate the organic cations, the [interlayer distance](#) was pre-expanded to 2.5 nm and the C-1 and  
218 water molecules and Na ions were randomly inserted. An initial [energy minimization](#) and MD simulation in  
219 the NPT-ensemble of 1 ns (298 K, 1 atm, time step 1 fs) have been used to optimize the interlayer region  
220 reaching an equilibrium density. Different initial starting configurations have been tested (in terms of  
221 organic, water, and sodium ions placement) and in all cases for a given system they converge to similar final  
222 arrangements and layer-to-layer distances. Then, the ones with the lowest energy have been further  
223 simulated for 1 ns in the NVT-ensemble. The last 0.5 ns have been used for the data analyses in terms of  
224 density profiles, pair correlation functions, ion coordinations. In all the simulations, both the atoms of the  
225 montmorillonite layer as well as the atoms confined in the interlayer were allowed to move and the  
226 periodic boundary conditions were applied. MD simulations were performed using the Discover module of  
227 the Materials Studio package (v. 5.0, Accelrys Inc.).

### 228 **2.13. Antimicrobial activity**

229 A microbiological agar well diffusion method was performed ([Giamarellou et al., 1975](#)) on Bt/GM in  
230 comparison with the respective physical mixture as well as pure GM and Bt. Tryptic soy agar (15 mL) and  
231 [Staphylococcus aureus](#) (*S. aureus*) strain ATCC 6538 or [Pseudomonas aeruginosa](#) (*P. aeruginosa*) strain ATCC  
232 27853 ( $10^5$  CFU/mL) were used as growth medium and indicator [microorganisms](#), respectively. The wells in  
233 [agar plates](#) were filled with the samples in water dispersions (5 µg/mL, 100 µL). The glass plates were  
234 incubated at 37 °C overnight and zone inhibition diameters determined and related to GM concentration of  
235 standard water solutions. The analyses were made in triplicate.

### 236 **2.14. In vitro gentamicin desorption**

237 Gentamicin dissolution and desorption from the organo-modified clay were examined under sink  
238 conditions using the flow-through cell, USP Apparatus 4 (Disotest CE-1, Sotax, Basel, Switzerland) on  
239 exactly weighed samples in 100 mL of phosphate buffer solutions (pH 5.4 or 7.4 according to the European  
240 Pharmacopeia) at a temperature of  $37.0 \pm 0.5$  °C under a flow rate of 25 mL/min. The dissolved or desorbed  
241 concentration of drug was determined spectrophotometrically (Lambda 3B, Perkin-Elmer) following

242 derivatization according to Sampath and Robinson ([Sampath and Robinson, 1990](#)) at fixed time intervals for  
243 3 h. The reported data were averaged on three determinations.

## 244 **2.15. *In vivo* skin permeation**

245 *In vivo* skin [permeation](#) study was carried out following the application of Bt/GM, Bt, and GM, mechanically  
246 mixed into petroleum jelly immediately before skin application, on both the two shaved volar forearms and  
247 forehead of 3 healthy Caucasian volunteers (2 females and 1 male, aged 20–62), free of any dermatological  
248 disorder after obtaining informed consent for the experimentation following the recommended guidelines  
249 as set out in the Declaration of Helsinki. A delineated area of 2 × 5 cm received 200 mg of each formulation  
250 containing pure GM (4 mg), Bt/GM (65 mg) both corresponding to GM dose of 0.4 mg/cm<sup>2</sup>, or pure Bt  
251 (65 mg). The samples were homogeneously distributed by means of rubber gloves. After an application  
252 time of 60 min, which had been found to have a predictive value for penetration resulting from longer  
253 times of application ([Howes et al., 1996](#)), the *stratum corneum* (SC) was stripped twelve times by using an  
254 [adhesive tape](#) (Scotch Film Tape 600-3M). This number of stripped tapes is considered proper by Food and  
255 Drug Administration bioequivalence guidelines ([Shah et al., 1998](#)). The tapes were applied to the skin with a  
256 constant pressure by a 500 g roller. The first stripped tape was not considered in the penetration study  
257 because it represents unabsorbed materials. Twelve tapes stripped from SC that has received pure  
258 petrolatum jelly as well as from untreated SC were also assayed as controls. The tapes were combined into  
259 2 groups (group 1: tapes 2–6; group 2: tapes 7–12) in order to increase determination sensitivity and  
260 subjected to an extraction procedure by [isopropyl alcohol](#) to determine GM according to the method  
261 described above. Data were expressed in penetrated GM percentage of the applied dose. A further tape-  
262 stripping test was conducted on the volunteers under the same conditions after a resting period of 14 days.  
263 Tapes n. 2, 6, and 12 were assayed by Energy Dispersive X-ray (EDX) analysis coupled with an Environmental  
264 Scanning Electron Microscopy (ESEM) using the selected area method. An area of 1.25 cm<sup>2</sup> of each tape  
265 was cut from the center of the tape, mounted without conductive coating on a carbon stub. X-ray emission  
266 from K $\alpha$  and K $\beta$  levels of the atoms carbon, oxygen, aluminum, silicon, and sulfur were registered under the  
267 experimental setting described above. EDX spectra representing the plots of X-ray counts vs. elements and  
268 semi-quantitative results expressed as relative weight percentage of the elements present in the specimen  
269 were obtained. The reported data were averaged on the results obtained from the volunteers.

## 270 **2.16. Statistical analysis**

271 Data obtained were evaluated from a statistical point of view using ANOVA one-way. Differences at *p*-  
272 values < .05 were considered significant.

## 273 **3. Results and discussion**

274 Adsorption of GM onto [Bt](#) is essentially driven by the CEC of the clay that is related to isomorphous  
275 substitution in octahedral and tetrahedral sheets of [montmorillonite](#). To modulate drug [bioavailability](#)  
276 through interactions with bentonite (Bt), the cationic gentamicin (GM) was selected. Each of the three  
277 major components of GM complex (C1, C1a, C2) contains five basic amino functions exhibiting change in  
278 [protonation](#) state of the amino groups as function of pH. At the acidic pH value of Bt/GM preparation, GM  
279 molecules carry almost fully protonated charges (+5 and +4) ([Lesniak et al., 2003](#)) that are appropriate for  
280 efficacious cation exchange on montmorillonite. The Bt selected to develop Bt/GM organo-modified clay  
281 was previously characterized providing information on mineralogical and physico-chemical features  
282 ([Iannuccelli et al., 2016](#)). Gentamicin capacity to be intercalated in a commercial montmorillonite to be  
283 used as general drug carrier has been demonstrated by [Rapacz-Kmita et al. \(2015, 2017\)](#). In the present  
284 work, more extensive knowledge about the specific interactions between GM and Bt was supplied  
285 providing information on the arrangement of the guest molecule in the montmorillonite [interlayer](#) as well  
286 as the potentiality of a topical application.

287 **3.1. Interlayer structure**

288 The first step of the research was to verify the usefulness of Bt activation procedure. Clay activation did not  
 289 offer advantages in terms of GM adsorption extent (aBt/GM = 4.49 ± 0.56%, yield% = 25.13 ± 0.62;  
 290 Bt/GM = 7.16 ± 0.91%, yield% = 35.29 ± 0.51). Therefore, the study was performed only on the non-  
 291 activated Bt/GM.

292 Bentonite treatment with GM generated an increase of clay average particle size ( $p > .05$ ) (Table 1) though  
 293 remaining in the size range considered proper for several dermatological and cosmetic purposes (Lein and  
 294 Oussoren, 2015). Both Bt and Bt/GM exhibited negative surface charge with a greater magnitude in Bt  
 295 compared to Bt/GM ( $p < .05$ ) (Table 1). Bentonite net surface charge is mainly due to montmorillonite pH-  
 296 independent permanent structural charges accounting for 90–95% of the total charges (Au and Leong,  
 297 2016; Pecini and Avena, 2013). Furthermore, montmorillonite is characterized by a surface charge due to  
 298 the hydrolysis of Si—O and Al—OH bonds on the external surfaces of tetrahedral sheets as well as along  
 299 the edges. Consequently, the lower Bt/GM Z-potential in absolute value is probably ascribable to GM  
 300 interactions with the edges of clay particles and/or acidic pH medium of GM water solution (Delgado et al.,  
 301 1986; Furukawa et al., 2009). Upon contact with water, Bt provided alkaline dispersions (pH of about 9,  
 302 Table 1), in agreement with the values required by both U.S. and European Pharmacopeias. Alkalinity, that  
 303 remained unchanged after 1 h, is generated by quick diffusion from the interlayer surfaces of exchangeable  
 304 Na<sup>+</sup> ions retained by electrostatic attraction. Conversely, Bt/GM water dispersions exhibited pH values  
 305 consistent with those of pure GM suggesting the occurred exchange process between montmorillonite  
 306 interlayer cations and GM.

**Table 1**  
 Size, polydispersity index (PDI), surface charge, and pH values of Bt, GM, and Bt/GM. Mean values ± SD.

Sample	Z-average (µm) ± SD	PDI	Z-potential (mV) ± SD	pH value
GM	—	—	—	4.52 ± 0.05
Bt	3.64 ± 0.98	0.42 ± 0.15	-28.40 ± 0.98	9.43 ± 0.48 (time = 0) 9.30 ± 0.07 (time = 1 h)
Bt/GM	6.82 ± 0.75	0.62 ± 0.12	-17.60 ± 1.30	4.66 ± 0.13 (time = 0) 4.91 ± 0.10 (time = 1 h)

307

308 In order to define the arrangement of GM molecules within montmorillonite lattice and elucidate the  
 309 interaction mechanism occurring in Bt/GM, a suite of analyses was performed.

310 Elemental CHN analysis carried out on Bt/GM showed a C/N ratio in nice agreement ( $p < .05$ ) with that of  
 311 GM alone (Table 2) and therefore evidenced that the drug was present in the organo-modified clay.

**Table 2**  
 CHN microanalysis and C/N ratios of Bt, GM, and Bt/GM. Mean values ± SD.

Sample	C%	H%	N%	C/N
Bt	—	0.90 ± 0.21	—	—
GM	51.80 ± 1.05	8.91 ± 0.52	15.12 ± 0.83	3.43 ± 0.11
Bt/GM	2.28 ± 0.20	1.26 ± 0.23	0.63 ± 0.005	3.62 ± 0.30

312

313 The occurrence of a possible exchange process between GM and the exchangeable cations of  
 314 montmorillonite as a preliminary evidence of GM intercalation was provided by Energy Dispersive X-ray  
 315 (EDX) analysis carried out on Bt/GM compared with Bt. The identification and relative quantification  
 316 analysis (Table 3, see Fig. S1) showed the Si and Al elements of tetrahedral and octahedral sheets,  
 317 respectively, as well as Mg due to Al partial isomorphous substitution. Isomorphous substitutions in the  
 318 octahedral sheets create an excess of negative structural charge within the lattice that is balanced by  
 319 inorganic cations (mainly Na<sup>+</sup> and Ca<sup>2+</sup>). A significant ( $p < .05$ ) less abundance of Ca<sup>2+</sup> ions in Bt/GM  
 320 compared with those of Bt suggests the almost complete substitution of Ca<sup>2+</sup> ions by the cationic form of  
 321 GM and, consequently, a possible drug arrangement within montmorillonite interlayers. The detection of a

322 minimal content of S in the mixture suggests, however, a non-negligible intercalation of GM without losing  
323 the [sulfate](#) group.

**Table 3**

EDX semi-quantitative analysis of the elements present in Bt and Bt/GM samples. All data are expressed as relative percentage of the elements > 0.5%. Mean values  $\pm$  SD.

	Bt	Bt/GM
O	32.25 $\pm$ 3.24	59.48 $\pm$ 0.63
Na	1.13 $\pm$ 0.73	0.90 $\pm$ 0.25
Mg	0.79 $\pm$ 0.59	0.71 $\pm$ 0.20
Al	11.01 $\pm$ 0.76	7.32 $\pm$ 0.23
Si	46.92 $\pm$ 2.42	27.99 $\pm$ 0.45
S	–	0.26 $\pm$ 0.20
K	2.38 $\pm$ 0.60	1.47 $\pm$ 0.17
Ca	2.30 $\pm$ 0.59	0.44 $\pm$ 0.16
Fe	3.22 $\pm$ 0.86	1.43 $\pm$ 0.27

324

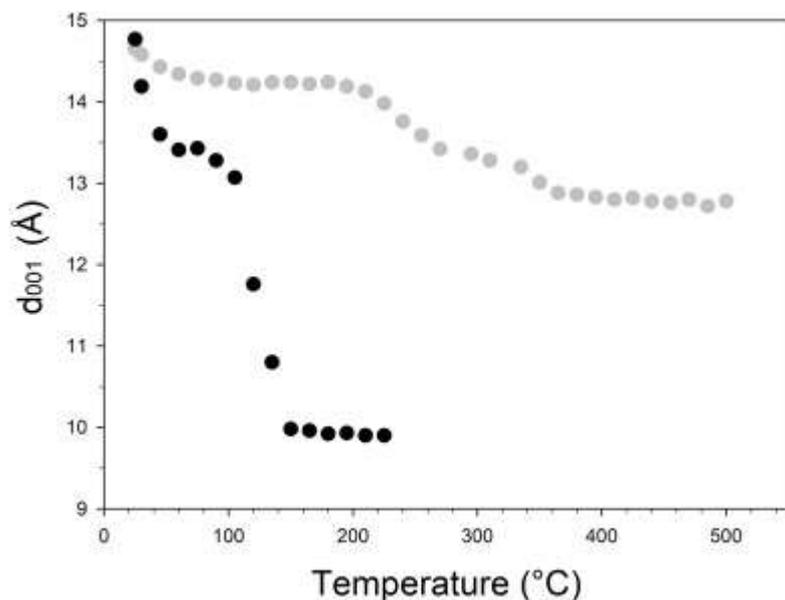
325 Nonetheless, it has to be considered that GM could interact also with additional negative polar sites at the  
326 broken edges as well as by exposed hydroxyl end-groups on the terminated planes.

327 Another preliminary evidence was provided by mid-IR spectroscopy (see Fig. S2). The most relevant feature  
328 of the mid-IR spectra is the band at about 3400  $\text{cm}^{-1}$ , related to the overlapping of asymmetric  $\nu_3$  and  
329 symmetric  $\nu_1(\text{H}\text{---}\text{O}\text{---}\text{H})$  stretching vibrations of water bound by [hydrogen bonds](#) in the interlayer  
([Farmer, 1974](#)). Unlike in Bt and Bt/GM physical mixture, the band position in the Bt/GM shift to a lower  
330 value, suggesting a decrease of the coordinated water amount induced by the substitution of the  
331 exchangeable cations of montmorillonite by GM. This conclusion is supported by the shift of the shoulder at  
332 about 3250  $\text{cm}^{-1}$  related to the [overtone](#) ( $2\nu_2$ ) of the bending mode of the solvated water ([Farmer, 1974](#))  
333 and by the positions of the [absorption bands](#) at about 1635  $\text{cm}^{-1}$  attributable to  $\nu_2(\text{H}\text{---}\text{O}\text{---}\text{H})$  [bending](#)  
334 [vibration](#). The position of this band is the same in Bt and Bt/GM physical mixture ( $\sim 1637 \text{ cm}^{-1}$ ), it is instead  
335 moved to a lower value ( $\sim 1628 \text{ cm}^{-1}$ ) in the Bt/GM suggesting a decrease of the coordinated water amount  
336 as demonstrated also by [Madejová et al. \(2002\)](#) in samples where the amount of water has been  
337 decreased. Other features of the mid-IR spectra can be related both to montmorillonite and to other  
338 mineralogical phases as well ([Iannuccelli et al., 2016](#)). More in detail we refer to the bands at about  
339 ([Madejová and Komadel, 2001](#)): i) 3692 and 3620  $\text{cm}^{-1}$  related to OH stretching of structural hydroxyl  
340 groups of kaolinite and montmorillonite, respectively; ii) 916  $\text{cm}^{-1}$ , a band related to (AlAlOH) bending in  
341 montmorillonite; iii) 1100, 795 and 690  $\text{cm}^{-1}$  related to the Si–O vibration of quartz; iv) 1030  $\text{cm}^{-1}$  related  
342 to Si–O stretching vibration in montmorillonite. These features, however do not significantly change in Bt,  
343 Bt/GM and Bt/GM physical mixture suggesting that they are unaffected by the presence of the antibiotic.  
344

345 The main technique for detecting structural variations such as changes in the basal periodicity of  
346 montmorillonite, is obtained by [XRD](#). The comparison between XRD pattern of Bt and Bt/GM did not  
347 highlight considerable differences except for a minor reduction of  $d_{001}$  value in Bt/GM montmorillonite  
348 ( $d_{001} = 14.19 \text{ \AA}$ ) respect to Bt montmorillonite ( $d_{001} = 14.95 \text{ \AA}$ ). Hence, temperature controlled XRD analysis  
349 was performed to acquire more information about GM arrangement within montmorillonite framework. In  
350 fact, in montmorillonite the thickness of the interlayer depends greatly on the type of occupying molecules  
351 (e.g., cations, organic molecules, etc.) and on the amount of solvating water. Conversely, by measuring the  
352 variation of the distances between the stacked layers along the  $c$  axis (*i.e.*, the  $d$  values of the (001)  
353 reflections) as a function of temperature, information can be acquired on the thermal stability of the  
354 molecules occupying the interlayer.

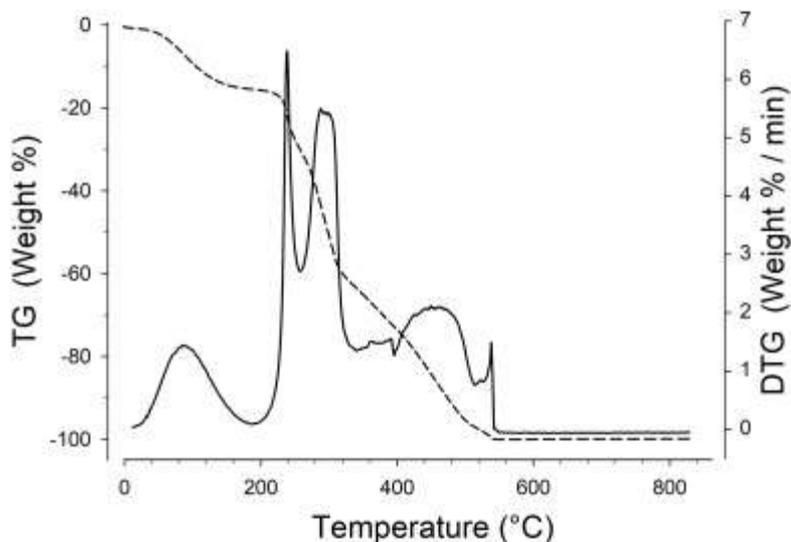
355 [Fig. 2](#) compares the trends of  $d_{001}$  reflections of montmorillonite in Bt and Bt/GM samples. The most  
356 significant data of the comparison is the persistence of the periodicity of Bt/GM montmorillonite despite

357 the rapid decrease observed for Bt montmorillonite. In fact, at 150 °C Bt montmorillonite interlayer  
358 exhibited the typical value of the completely dehydrated interlayer ( $d_{001} = \sim 10 \text{ \AA}$ ), and this value did not  
359 change until the occurring of framework collapse at about 600 °C. Conversely, the reduction of the  
360 interlayer spacing in Bt/GM montmorillonite only starts after 210 °C. Thermal analyses here after reported,  
361 in nice agreement with these finding, will better highlight that at about this temperature begins the  
362 [thermal decomposition](#) of the antibiotic.



363  
364 Fig. 2. Plot of  $d_{001}$  values for [Bt](#) (black circle) and Bt/GM (gray circle) [montmorillonite](#) as a function of  
365 temperature. The position of each (001) peak has been determined at the mid-height of the reflection,  
366 through the use of the software X-Pert High Score Plus. The error of the measurement falls within the  
367 dimensions of the used symbol.

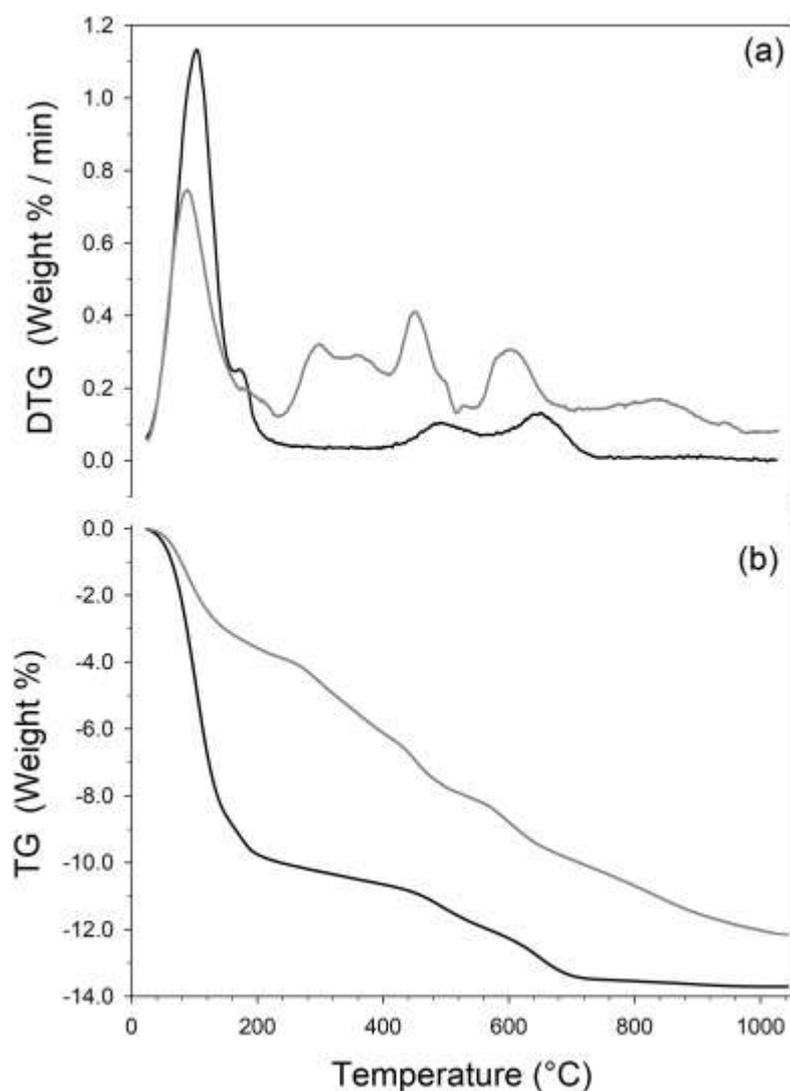
368 Thermogravimetric (TG) and its first derivative (DTG) curves for GM are shown in [Fig. 3](#). Five main reactions,  
369 with maxima at 80, 260, 317, 500, and 582 °C (DTG curve), lead to a nearly complete thermal  
370 decomposition of the antibiotic. The weight loss with maximum at 80 °C (mass loss of 14.7%, TG curve) is  
371 related to the removal of free water molecules, whereas reactions at higher temperature are related to the  
372 thermal decomposition of both the organic fraction and the [sulfate group](#). The asymmetry and/or band-like  
373 shape of the DTG peaks, in particular for the thermal event that occurs at  $T > 200 \text{ °C}$ , maybe related to the  
374 different forms (C1, C1a, and C2) of GM that thermally decomposed at significantly different temperature  
375 values.



376

377 Fig. 3. TG (dashed line) and DTG curves (solid line) of GM.

378 The [thermal behaviors](#) of Bt and Bt/GM are compared in [Fig. 4](#). The DTG curve ([Fig. 4a](#)) of Bt shows four  
 379 [thermal reactions](#) with maxima at 103, 165, 485 and 645 °C. The first two reactions occurred between 25  
 380 and 210 °C and are attributed to the dehydration of montmorillonite with the removal of two water layers  
 381 differently bound to the interlayer cation (maxima at 103 and 165 °C with mass losses of 9.0 and 0.84%,  
 382 respectively - [Fig. 4b](#)). This finding is in agreement with the presence of [divalent cations](#) ( $\text{Ca}^{2+}$ ) as major  
 383 interlayer species ([Iannuccelli et al., 2016](#); [Mackenzie, 1970](#)) and with XRD data ([Fig. 2](#)). The two reactions  
 384 at higher temperature are ascribable to the dehydroxilation of the octahedral sheets of kaolinite (maximum  
 385 at 485 °C, mass loss 1.2%) and montmorillonite (maximum at 645 °C, mass loss 1.9%). Differential thermal  
 386 analyses (not reported) additionally evidenced an [endothermic reaction](#) with maximum at 573 °C related to  
 387 the transition from the trigonal  $\alpha$  to the hexagonal  $\beta$  form of quartz ([Iannuccelli et al., 2016](#)).



388

389 Fig. 4. DTG (a) and TG (b) curves of [Bt](#) (black line) and Bt/GM (gray line).

390 The TG and DTG curves of Bt/GM nearly parallel those of Bt, but with some major differences, arising from  
 391 the presence of GM, that are: i) a drastic reduction of the mass loss related to the removal of hydration  
 392 water (reactions between 25 and 235 °C, mass loss 3.85%); ii) two additional thermal events with maxima  
 393 at 298 and 360 °C (reactions between 220 and 405 °C, mass loss 2.30%); iii) a shift toward lower  
 394 temperature values of the two dehydroxilation reactions; iv) an additional thermal events with maximum at  
 395 about 830 °C.

396 The lower amount of water in Bt/GM compared to Bt may explain the small difference in layer periodicity  
 397 of montmorillonite measured by XRD at room temperature. The two reactions between 220 and 405 °C  
 398 occur nearly in the same temperature of those observed for pure GM, however with a lower decomposition  
 399 rate (DTG curve). As already pointed out, it is not negligible that the reaction at temperature at which starts  
 400 the first of the two reactions (220 °C) is about the same at which begins the reduction of layer periodicity  
 401 ([Fig. 2](#)). This finding may be thus definitively attributed to the beginning of the thermal decomposition of  
 402 the intercalated antibiotic, as moreover suggested by MS-EGA curves.

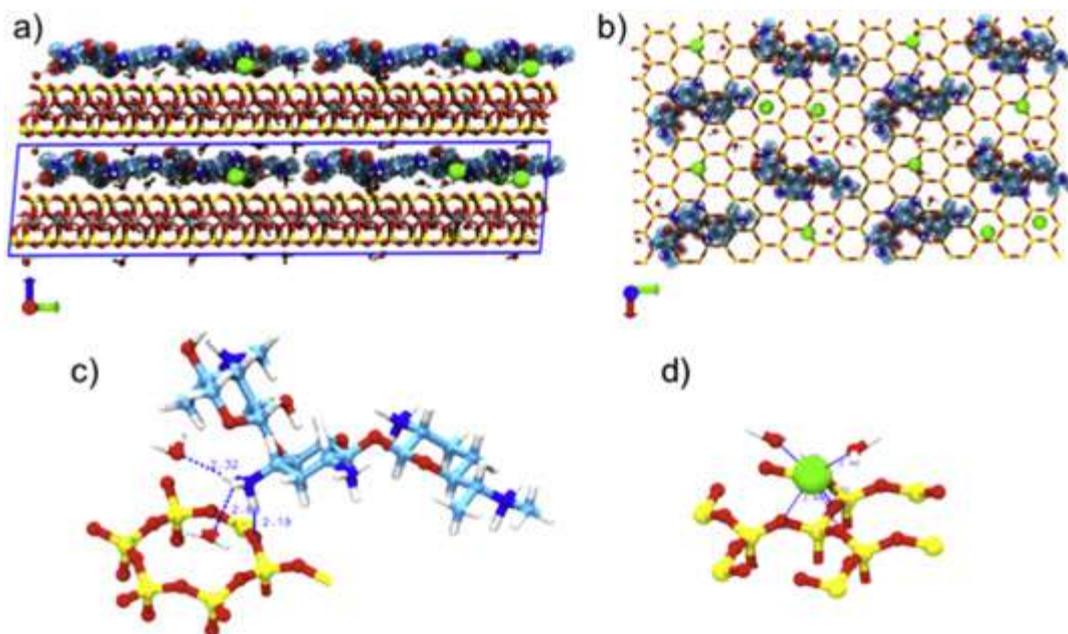
403 The thermal decomposition of pure GM is complete at about 600 °C ([Fig. 3](#)); however, TG curves indicated  
 404 that the intercalated antibiotic follows a different thermal path. More in detail, MS-EGA curves (see [Fig. S3](#))  
 405 indicated that the two just mentioned reactions lead to the release of H<sub>2</sub>O (m/z = 18), NO (m/z = 30), and  
 406 CO<sub>2</sub> ([Fig. S3](#)), further supporting the intercalation of GM. The detection of a MS-EGA signal for SO<sub>2</sub>

407 highlights, however, the presence of marginal content of GM in its sulfate form, in accordance with the  
408 elemental analysis. XRD measurements showed that the layer periodicity of Bt/GM montmorillonite after  
409 the reaction with maximum at 360 °C was about 13 Å, higher than that in Bt montmorillonite (*i.e.*, 9.9 Å). It  
410 may be concluded that after the two thermal reactions occurring between 220 and 405 °C, a pillar-like  
411 residue, that thermally decomposes at higher temperature, forms in the interlayer of montmorillonite.  
412 However, as above mentioned, all these high temperature reactions (included the modification of the  
413 temperature at which occurred dehydroxylation) have not been taken into account in the present work, as  
414 they are related to the [thermal evolution](#) of the already decomposed antibiotic and mineral. Nevertheless,  
415 they well support the hypothesis of intercalation.

416 Further evidence supporting the intercalation of gentamicin is provided by [DSC](#) measurements that showed  
417 the absence of GM endothermic reaction in Bt/GM. On the opposite, this reaction is visible when DSC  
418 curves are collected for pure GM and physical mixture of Bt and GM.

419 Comparison of experimental measurements with MD simulations provided a more detailed understanding  
420 of the arrangement and bonding promoted by the GM molecules intercalated in montmorillonite. A  
421 computational approach attempted to simulate the interactions in the inter-structure of an ideal model of  
422 montmorillonite ( $X_{0.75}[Si_{7.75}Al_{0.25}][Al_{3.5}Mg_{0.5}]O_{20}(OH)_4$  which corresponds to a CEC of 101.9 meq/100 g),  
423 gentamicin molecules (using C1 as a molecule model), sodium ions and [solvent molecules](#) (H<sub>2</sub>O).  
424 Gentamicin components present both hydroxyl and amino groups and this affects the total positive charge  
425 (from 1 to 5 possible positive charge) of each molecule depending on the pH of the solution ([Lesniak et al.,  
2003](#)). In this study the system was simulated considering a pH ~ 5 [aqueous environment](#) corresponding to  
427 the preparation pH value at which GM molecules carry almost fully protonated charges (~85% of +5 and  
428 ~15% of +4), taking into account the distribution diagram of the different protonated species of component  
429 C1. This was achieved by inserting into the interlayer ~78.9% of molecules with +5 and ~21.1% molecules  
430 with 4+ charges and a certain content of water molecules (~2 wt%). Plus, in order to mimic the partial  
431 exchange process obtained experimentally a certain amount of sodium ions were also inserted. The  
432 simulated interlayer is made of ~79.2% of GM and ~20.8% of sodium ions with respect to the theoretical  
433 CEC. In this situation, the content of GM molecules useful to balance the negative charge of  
434 montmorillonite corresponds to an organic content of ~7.7% by weight.

435 Modeling results clearly show that Bt/GM is characterized by a monolayer arrangement of the GM (C1)  
436 molecules with a resulting layering periodicity of  $14.2 \pm 0.3$  Å, a value in fair agreement with XRD finding  
437 (14.19 Å). The side and top views of a typical configuration of Bt/GM hybrid system optimized by simulation  
438 are shown in [Fig. 5a](#) and b. The analyses of density profiles show clear peaks for all the intercalated species.  
439 On the basis of [radial distribution functions](#), the interatomic distance between the individual components  
440 of the sub-networks was further assessed. In particular, the ammonium H atoms of the GM molecules  
441 resulted located within the 2.1–2.9 Å range from the oxygen atoms of the montmorillonite surfaces, a  
442 distance slightly larger than those found in alkyl ammonium ions-based [organoclays](#) (1.8–2.5 Å) ([Liu et al.,  
2007](#)). This difference can be ascribed to the larger molecular flexibility of the alkyl [ammonium cation](#) due  
444 to its alkyl chain with respect to the more rigid and sterically hindered nature of the cyclic GM (C1)  
445 molecules. Both Na ions and the ammonium H atoms promote strong interactions with the oxygen atoms  
446 of the tetrahedral sheets ([Fig. 5c](#) and d), being mostly located above the surface [siloxane](#) rings (*i.e.* six-  
447 member rings). Water molecules partially affect such interactions by competing with the oxygen atoms of  
448 the tetrahedral sheets in promoting H-bonding with GM (C1) ammonium H atoms and hydroxyl groups and  
449 solvating the inner- and outer-sphere Na complexes ([Brigatti et al., 2011](#)).



450

451 Fig. 5. a) Side view of [montmorillonite](#) in the Bt/GM hybrid system optimized by MD modeling. The  
 452 simulated [supercell](#) is shown within the blue line and it is replicated twice along the z direction for  
 453 clearness. b) Top view of the local arrangement of GM and water molecules and Na ions within the  
 454 montmorillonite [interlayer](#). c) Local environment around the GM ammonium group where the interaction  
 455 between its [hydrogen atoms](#) with the oxygen of the tetrahedral sheets and the oxygen of water molecules  
 456 are highlighted with a blue dashed line. d) Local coordination environment of a Na ion located on top of  
 457 tetrahedral Al (Si substitution, charged site). For clearness, only the bottom [siloxane](#) sheet surface is shown.  
 458 Color legend: Si atoms, yellow; O atoms, red; H atoms, white; C atoms, cyan; N atoms, blue; Na ions, green.  
 459 (For interpretation of the references to color in this figure legend, the reader is referred to the web version  
 460 of this article.)

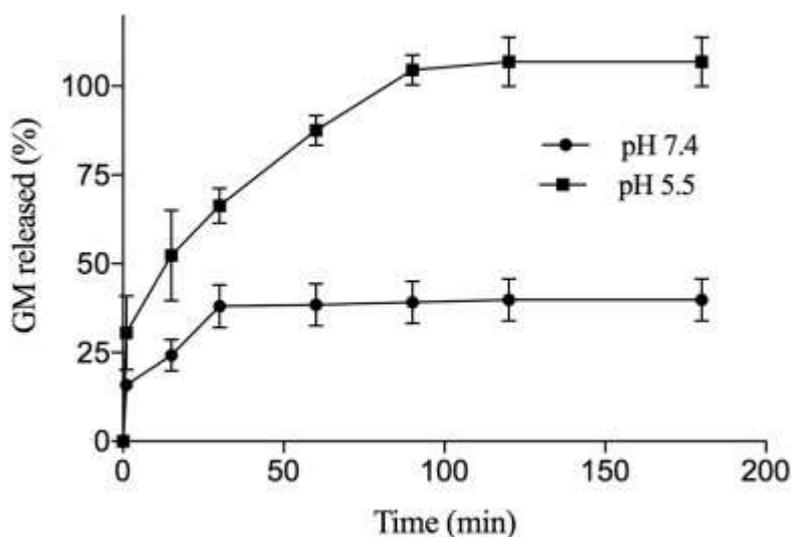
461

### 462 3.2. Antibacterial activity and *in vivo* skin permeation

463 In a perspective of Bt/GM use as cutaneous drug delivery system for local or systemic control of infections,  
 464 the study involved the evaluation of GM biological activity within the organo-modified Bt, *in vitro*  
 465 desorption, and *in vivo* skin [permeation](#) on human beings. The biological activity of GM within Bt was  
 466 assessed to point out a possible synergistic clay action. In fact, clay minerals, in particular smectites, have  
 467 [antimicrobial activity](#) due to their high absorbing/adsorbing power with respect to water forming  
 468 unfavorable hydrophobic conditions for the growth of [microorganisms](#) (Kim et al., 2016; Williams and  
 469 Haydel, 2010). Antimicrobial activity of Bt/GM was determined on *S. aureus* or *P. aeruginosa* and compared  
 470 with that of pure GM, pure Bt and the physical mixture of the antibiotic with the clay in the same ratio as  
 471 Bt/GM.

472 The results obtained highlighted the absence of antimicrobial activity of the pure Bt and an almost  
 473 complete activity of the physical mixture compared with pure GM ( $96.59 \pm 5.68\%$  against *S. aureus*,  
 474  $88.01 \pm 2.90\%$  against *P. aeruginosa*). On the other hand, Bt/GM exhibited an activity against both the  
 475 strains less than those obtained for GM solutions having the same antibiotic concentration ( $50.37 \pm 1.59\%$   
 476 against *S. aureus*,  $50.07 \pm 1.88\%$  against *P. aeruginosa*). It is reasonable therefore to suppose that GM,  
 477 though maintaining antibacterial activity against both Gram-positive and Gram-negative bacteria, desorbs  
 478 from Bt/GM within the culture media of the [agar plate](#) incompletely indicating that drug intercalation  
 479 between montmorillonite interlayer reduces drug availability (Ambrogi et al., 2017).

480 Nevertheless, complete GM desorption from Bt/GM was obtained at pH 5.5 buffer solution mimicking the  
481 acidic environment of skin surface (Fig. 6). Unlike the pure GM dissolving within 1 min regardless of the pH  
482 value, drug desorption profile involved a burst phase corresponding to about 40% of GM adsorbed onto Bt  
483 followed by a sustained phase reaching 100% drug delivered in about 2 h. Conversely, following a burst  
484 phase in which about 15% GM diffused into the medium, drug desorption at pH 7.4 was incomplete  
485 reaching a plateau corresponding to about 50% of GM payload after about 30 min. Burst phases could be  
486 related to GM fraction deposited or weakly linked to Bt particle surface whereas the subsequent phases to  
487 an exchange process between the intercalated GM and the cations present in the media reaching  
488 equilibrium according to the available cations (Joshi et al., 2009).



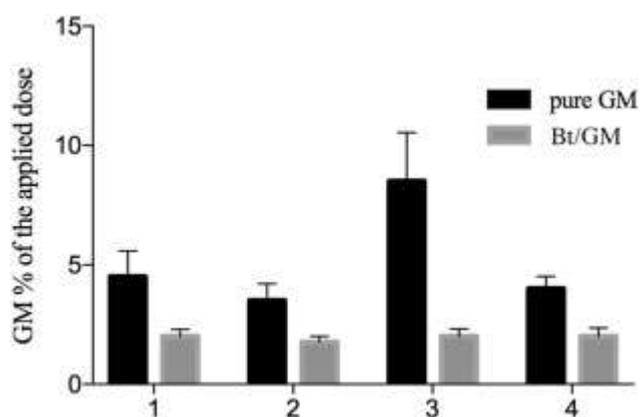
489

490 Fig. 6. Profiles of gentamicin desorption from Bt/GM in pH 5.5 and pH 7.4 media.

491 The higher GM percentage amounts desorbed at pH 5.5 with respect to pH 7.4 may reasonably arise from a  
492 competition by H<sup>+</sup> ions present in the [assay medium](#) on the same interaction sites of GM (Iannuccelli et al.,  
493 2015; Joshi et al., 2009). In biological fluids, physiological counter-ions can displace differently the drug  
494 from the substrate and deliver it into the body (Aguzzi et al., 2007).

495 The understanding of the mechanism by which insoluble particles can cross the *stratum corneum* (SC) is  
496 relevant to both prevent any possible local side effects or systemic exposure and properly exploit their  
497 potential benefits such as the reservoir role inside the [hair follicles](#) (Wosicka and Cal, 2010) or the drug  
498 transport modulation (Scalia et al., 2013). Since the passive transport through intact skin is considered  
499 highly unlikely (SCCP, 2007), particle penetration is most likely along the intercellular route following the  
500 lipid channels between the [corneocytes](#) and the appendage route along the hair follicles. Unlike the open  
501 question concerning [nanoparticles](#), there is agreement that [microparticles](#) up to 10 μm can enter into the  
502 follicle orifices that can act as an efficient long-term drug reservoir (Lademann et al., 2007) from which  
503 soluble compounds could also diffuse into the viable epidermis (Borm et al., 2006). In relation to this, the  
504 density and size of hair follicles as well as the lipophilicity of the material applied on the skin have been  
505 assumed to contribute to differences in penetration rates (Feldmann and Maibach, 1967; Knorr et al., 2009;  
506 Otberg et al., 2004). To assess the mechanism by which the organo-modified bentonite enters the SC, the  
507 present study has considered the *in vivo* skin [penetration profile](#) of Bt/GM in comparison with that of pure  
508 GM and pure Bt applying the samples, incorporated in petroleum jelly, on human skin regions having two  
509 different hair follicle densities, the volar forearm (18 follicles/cm<sup>2</sup> corresponding to 0.09% skin surface,  
510 78 μm diameter of hair follicle orifice) and the forehead (292 follicles/cm<sup>2</sup> corresponding to 1.28% skin  
511 surface, 66 μm diameter of hair follicle orifice) (Otberg et al., 2004).

512 The investigation was performed using EDX analysis on twelve repetitive stripped tapes containing the  
 513 outermost layers of SC, generally the *stratum disjunctum* from the 2nd to the 5th tape, the *stratum*  
 514 *compactum* from the 6th to the 12th tape (Jacobi et al., 2005) by both GM extraction from combined tapes and  
 515 Bt detection on each tape. Skin exposure to pure GM at the level of the forehead region provided drug  
 516 concentrations higher than those obtained on the volar forearm region ( $p < .05$  concerning the first tape  
 517 group) suggesting the involvement of the trans-follicular route pathway across SC. This assumption is  
 518 consistent with the results from other Authors arguing that skin appendage route gained renewed interest  
 519 for hydrophilic drugs representing a significant access also for gentamicin (Barry, 2002; Fadli et al., 2015;  
 520 Ogiso et al., 2002). Moreover, GM levels are inclined to decrease with the increase of SC depth. The  
 521 interaction of GM with Bt decreased antibiotic permeation extent compared with pure GM permeation  
 522 ( $p < .05$ ) leading to a constant GM concentration (about 2% of the applied dose), regardless of SC depth  
 523 (Fig. 7).



524

525

526 Fig. 7. GM permeated dose (% of the applied dose) across SC from pure GM and Bt/GM: (1) volar forearm,  
 527 tape group 1 (tapes 2–6); (2) volar forearm, tape group 2 (tapes 7–12); (3) forehead, tape group 1 (tapes 2–  
 528 6); (4) forehead, tape group 2 (tapes 7–12).

529 It follows a hampering effect of GM permeation provided by its reaction with Bt and the irrelevance of the  
 530 application region, *i.e.* follicle density indicating presumably a different pathway. To monitor the possible  
 531 translocation and distribution of Bt/GM across SC, each stripped tape (tapes 2, 6, and 12) was assayed by  
 532 EDX analysis. All EDX spectra obtained from both pure Bt and Bt/GM skin exposure exhibited peaks from  
 533 carbon, oxygen, aluminum, silicon, and sulfur atoms. The presence of sulfur in all tapes removed from  
 534 untreated skin, attributable to the emission from SC keratin, prevented assessing the permeation of GM in  
 535 its sulfate form. The elements Si and Al that are not naturally occurring elements in SC entail the presence  
 536 of Bt (Cullander et al., 2000; Moretto et al., 1999). Accordingly, the presence of S atoms and the absence of  
 537 Si and Al atoms were pointed out in all the control tapes (untreated skin and skin treated with only  
 538 petroleum jelly). By measuring the intensity of characteristic X-rays spectra, the relative weight fraction of  
 539 Si and Al can be calculated (Table 4).

Table 4  
 EDX analysis: relative element weight percentage detected on tapes 2, 6, and 12 following skin exposure to pure Bt and Bt/GM. Mean values  $\pm$  SD.

Sample	Volar forearm		Forehead	
	Relative silicon weight	Relative aluminum weight	Relative silicon weight	Relative aluminum weight
pure Bt tape 2	29.3 $\pm$ 0.7	7.3 $\pm$ 0.3	13.7 $\pm$ 0.6	4.1 $\pm$ 0.5
pure Bt tape 6	3.9 $\pm$ 0.6	1.8 $\pm$ 0.6	5.5 $\pm$ 0.3	1.6 $\pm$ 0.3
pure Bt tape 12	5.3 $\pm$ 0.4	1.6 $\pm$ 0.4	5.3 $\pm$ 0.2	2.3 $\pm$ 0.2
Bt/GM tape 2	28.9 $\pm$ 0.4	7.0 $\pm$ 0.2	51.4 $\pm$ 0.9	10.8 $\pm$ 0.3
Bt/GM tape 6	17.6 $\pm$ 0.4	4.3 $\pm$ 0.3	18.0 $\pm$ 0.5	4.9 $\pm$ 0.3
Bt/GM tape 12	7.8 $\pm$ 0.5	2.4 $\pm$ 0.4	1.0 $\pm$ 0.5	1.6 $\pm$ 0.5

540

541 The detection of Si and Al atoms in all the tapes reveals the ability of clay particles to translocate across SC  
542 until the *stratum compactum* though Si and Al extents decreased with the increase of SC depth. Differences  
543 in quantitative distribution of Si and Al atoms between volar forearm and forehead application of both Bt  
544 and Bt/GM among the tapes were considered pointless to be argued. The limited influence of the sample  
545 exposure region is consistent with the results obtained by GM extraction from the tapes as the evidence of  
546 drug/clay association at the time of selecting SC pathway, plausibly different from the trans-follicular one.  
547 Besides skin appendages, SC is interrupted by inter-cluster corneocyte regions up to 100 µm in width, made  
548 of unsteady lipid packing generating openings having a low resistance to hydrophilic compounds ([Cevc and](#)  
549 [Vierl, 2010](#); [Dayan, 2005](#); [Iannuccelli et al., 2013](#)). Taking into account the size together with the [hydrophilic](#)  
550 [nature](#) of clayey samples, their motion along this trans-epidermal pathway could be considered as the  
551 favorite pathway. Moreover, Bt could increase SC hydration by means of the occlusive effect decreasing  
552 corneocyte packing and improving the clay transport. Such a route could also bypass the hindering effect  
553 on hydrophilic drug permeation provided by sebum along the trans-follicular route ([Verma et al., 2016](#)).  
554 Even if restricted to the superficial SC layers, it follows that a long-term Bt/GM reservoir for gradual GM  
555 release may be expected to perform leading to sustained antibacterial activity and minimized drug side  
556 effects.

#### 557 **4. Conclusions**

558 The approach consisting of new carriers redeveloping already-approved drugs and excipients was  
559 addressed to modulate gentamicin release and skin [permeation](#) by exploiting drug arrangement in the  
560 [interlayer](#) of [montmorillonite](#). Comprehensive examination of the organo-modified clay combined with a  
561 computational approach elucidated the mechanism of drug interaction with montmorillonite  
562 demonstrating the occurred [intercalation](#). From that, gentamicin sustained desorption and the possible  
563 pathway across inter-cluster [corneocyte](#) regions of the *stratum corneum* may be ensued in the perspective  
564 of contribution to a novel antibiotic material offering a potential more effective anti-infective therapy.

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#### 569 **References**

570 C. Aguzzi, C. Viseras, P. Cerezo, S. Rossi, F. Ferrari, A. López-Galindo, C. Caramella

#### 571 **Influence of dispersion conditions of two pharmaceutical grade clays on their interaction with some** 572 **tetracyclines**

573 Appl. Clay Sci., 30 (2005), pp. 79-86, [10.1016/j.clay.2005.03.007](#)

574 C. Aguzzi, P. Cerezo, C. Viseras, C. Caramella

#### 575 **Use of clays as drug delivery systems: possibilities and limitations**

576 Appl. Clay Sci., 36 (2007), pp. 22-36, [10.1016/j.clay.2006.06.015](#)

577 A. Ahangari, M. Salouti, Z. Heidari, A.R. Kazemizadeh, A.A. Safari

#### 578 **Development of gentamicin-gold nanospheres for antimicrobial drug delivery to Staphylococcal infected** 579 **foci**

580 Drug Deliv., 20 (2013), pp. 34-39, [10.3109/10717544.2012.746402](#)

581 V. Ambrogi, C. Carfagna, P. Cerruti, V. Marturano

582 **Additives in polymers**

583 C.F. Jasso-Gastinel, J.M. Kenny (Eds.), Modification of Polymer Properties, William Andrew Publishing  
584 (2017), pp. 87-108, [10.1016/B978-0-323-44353-1.00004-X](https://doi.org/10.1016/B978-0-323-44353-1.00004-X)

585 P.-I. Au, Y.-K. Leong

586 **Surface chemistry and rheology of slurries of kaolinite and montmorillonite from different sources**

587 KONA Powder Part. J., 33 (2016), pp. 17-32, [10.14356/kona.2016007](https://doi.org/10.14356/kona.2016007)

588 B.J. Aungst

589 **Intestinal permeation enhancers**

590 J. Pharm. Sci., 89 (2000), pp. 429-442, [10.1002/\(SICI\)1520-6017\(200004\)89:4<429::AID-JPS1>3.0.CO;2-J](https://doi.org/10.1002/(SICI)1520-6017(200004)89:4<429::AID-JPS1>3.0.CO;2-J)

591 M. Aviv, I. Berdicevsky, M. Zilberman

592 **Gentamicin-loaded bioresorbable films for prevention of bacterial infections associated with orthopedic**  
593 **implants**

594 J. Biomed. Mater. Res. A, 83A (2007), pp. 10-19, [10.1002/jbm.a.31184](https://doi.org/10.1002/jbm.a.31184)

595 H.R. Axelrod, J.S. Kim, C.B. Longley, E. Lipka, G.L. Amidon, R. Kakarla, Y.W. Hui, S.J. Weber, S. Choe, M.J.  
596 Sofia

597 **Intestinal transport of gentamicin with a novel, glyco steroid drug transport agent**

598 Pharm. Res., 15 (1998), pp. 1876-1881

599 F. Ayhan, S. Ozkan

600 **Gentamicin release from photopolymerized PEG diacrylate and pHEMA hydrogel discs and their in vitro**  
601 **antimicrobial activities**

602 Drug Deliv., 14 (2007), pp. 433-439, [10.1080/10717540701202911](https://doi.org/10.1080/10717540701202911)

603 B.W. Barry

604 **Drug delivery routes in skin: a novel approach**

605 Adv. Drug Deliv. Rev., 54 (Suppl. 1) (2002), pp. S31-40

606 M.L. Bello, A.M. Junior, B.A. Vieira, L.R.S. Dias, V.P. de Sousa, H.C. Castro, C.R. Rodrigues, L.M. Cabral

607 **Sodium montmorillonite/amine-containing drugs complexes: new insights on intercalated drugs**  
608 **arrangement into layered carrier material**

609 PLoS One, 10 (2015), p. e0121110, [10.1371/journal.pone.0121110](https://doi.org/10.1371/journal.pone.0121110)

610 T. Benelli, L. Mazzocchetti, E. D'Angelo, M. Lanzi, F. Saraga, L. Sambri, M.C. Franchini, L. Giorgini

611 **New nitrogen-rich heterocycles for organo-modified bentonites as flame retardant fillers in epoxy resin**  
612 **nanocomposites**

613 Polym. Eng. Sci., 57 (2017), pp. 621-630, [10.1002/pen.24565](https://doi.org/10.1002/pen.24565)

614 F.P. Bonina, M.L. Giannossi, L. Medici, C. Puglia, V. Summa, F. Tateo

- 615 **Adsorption of salicylic acid on bentonite and kaolin and release experiments**
- 616 Appl. Clay Sci, 36 (2007), pp. 77-85, [10.1016/j.clay.2006.07.008](https://doi.org/10.1016/j.clay.2006.07.008)
- 617 P.J. Borm, D. Robbins, S. Haubold, T. Kuhlbusch, H. Fissan, K. Donaldson, R. Schins, V. Stone, W. Kreyling, J.  
618 Lademann, J. Krutmann, D. Warheit, E. Oberdorster
- 619 **The potential risks of nanomaterials: a review carried out for ECETOC**
- 620 Part. Fibre Toxicol., 3 (2006), p. 11, [10.1186/1743-8977-3-11](https://doi.org/10.1186/1743-8977-3-11)
- 621 M.F. Brigatti, E. Galan, B.K.G. Theng
- 622 **Structure and mineralogy of clay minerals**
- 623 F. Bergaya, B.K.G. Theng, G. Lagaly (Eds.), Handbook of Clay Science (2011), pp. 19-86
- 624 M.I. Carretero
- 625 **Clay minerals and their beneficial effects upon human health. A review**
- 626 Appl. Clay Sci., 21 (2002), pp. 155-163, [10.1016/S0169-1317\(01\)00085-0](https://doi.org/10.1016/S0169-1317(01)00085-0)
- 627 G. Cevc, U. Vierl
- 628 **Nanotechnology and the transdermal route**
- 629 J. Control. Release, 141 (2010), pp. 277-299, [10.1016/j.jconrel.2009.10.016](https://doi.org/10.1016/j.jconrel.2009.10.016)
- 630 H.-I. Chang, Y. Perrie, A.G.A. Coombes
- 631 **Delivery of the antibiotic gentamicin sulphate from precipitation cast matrices of polycaprolactone**
- 632 J. Control. Release, 110 (2006), pp. 414-421, [10.1016/j.jconrel.2005.10.028](https://doi.org/10.1016/j.jconrel.2005.10.028)
- 633 M. Changez, K. Burugapalli, V. Koul, V. Choudhary
- 634 **The effect of composition of poly(acrylic acid)-gelatin hydrogel on gentamicin sulphate release: in vitro**
- 635 Biomaterials, 24 (2003), pp. 527-536
- 636 C. Cullander, S. Jeske, D. Imbert, P.G. Grant, G. Bench
- 637 **A quantitative minimally invasive assay for the detection of metals in the stratum corneum**
- 638 J. Pharm. Biomed. Anal., 22 (2000), pp. 265-279, [10.1016/S0731-7085\(99\)00248-4](https://doi.org/10.1016/S0731-7085(99)00248-4)
- 639 N. Dayan
- 640 **Pathways for Skin Penetration. Cosmetics & Toiletries® magazine 120**
- 641 (2005), pp. 67-76
- 642 A. Delgado, F. González-Caballero, J.M. Bruque
- 643 **On the zeta potential and surface charge density of montmorillonite in aqueous electrolyte solutions**
- 644 J. Colloid Interface Sci., 113 (1986), pp. 203-211, [10.1016/0021-9797\(86\)90220-1](https://doi.org/10.1016/0021-9797(86)90220-1)
- 645 G.L. Drusano
- 646 **Pharmacokinetics and pharmacodynamics of antimicrobials**

- 647 Clin. Infect. Dis., 45 (Suppl. 1) (2007), pp. S89-95, [10.1086/518137](https://doi.org/10.1086/518137)
- 648 E. Eljarrat-Binstock, F. Raiskup, D. Stepensky, A.J. Domb, J. Frucht-Pery
- 649 **Delivery of gentamicin to the rabbit eye by drug-loaded hydrogel iontophoresis**
- 650 Invest. Ophthalmol. Vis. Sci., 45 (2004), pp. 2543-2548, [10.1167/iovs.03-1294](https://doi.org/10.1167/iovs.03-1294)
- 651 A. Fadli, K. Jiyauddin, S.J. Siti Nur, A.D. Samer, S. Budiasih, A. Jawad, M. Kaleemullah, S. Rasha, A.G.
- 652 Mohammad Nizam, A. Ibrahim, H. Todo, K. Sugibayashi, Y. Eddy
- 653 **Determination of permeation pathway of gentamicin into pig's ear skin**
- 654 Int. Res. J. Pharm., 6 (2015), pp. 183-190
- 655 V.C. Farmer
- 656 **The Infrared Spectra of Minerals**
- 657 Mineralogical Society, Farmer, V.C., London, UK (1974)
- 658 R.J. Feldmann, H.I. Maibach
- 659 **Regional variation in percutaneous penetration of <sup>14</sup>C cortisol in man**
- 660 J. Invest. Dermatol., 48 (1967), pp. 181-183
- 661 F. Forni, V. Iannuccelli, G. Coppi, M.A. Vandelli, R. Cameroni
- 662 **Montmorillonite as a drug carrier: surface deposition of papaverine on the papaverine-veegum complex**
- 663 Boll. Chim. Farm., 126 (1987), pp. 342-346
- 664 M.C. Franchini, P. Fabbri, A. Frache, G. Ori, M. Messori, C. Siligardi, A. Ricci
- 665 **Bentonite-based organoclays as innovative flame retardants agents for SBS copolymer**
- 666 J. Nanosci. Nanotechnol., 8 (2008), pp. 6316-6324
- 667 M.C. Franchini, M. Messori, G. Ori, C. Siligardi
- 668 **Flame retardant SBS–clay nanocomposites**
- 669 V. Mittal (Ed.), Thermally Stable and Flame Retardant Polymer Nanocomposites (2011), pp. 360-382
- 670 Y. Furukawa, J.L. Watkins, J. Kim, K.J. Curry, R.H. Bennett
- 671 **Aggregation of montmorillonite and organic matter in aqueous media containing artificial seawater**
- 672 Geochem. Trans., 10 (2009), p. 2, [10.1186/1467-4866-10-2](https://doi.org/10.1186/1467-4866-10-2)
- 673 H. Giamarellou, V.M. Zimelis, D.O. Matulionis, G.G. Jackson
- 674 **Assay of aminoglycoside antibiotics in clinical specimens**
- 675 J. Infect. Dis., 132 (1975), pp. 399-406
- 676 D. Howes, R. Guy, J. Hadgraft, J. Heylings, U. Hoeck, F. Kemper, H. Maibach, J.P. Marty, H. Merk, J. Parra
- 677 **Methods for Assessing Percutaneous Absorption: The Report and Recommendations of ECVAM**
- 678 **Workshop 13**

679 Altern. Lab. Anim. ATLA (1996)

680 Z. Hu, R. Tawa, T. Konishi, N. Shibata, K. Takada

681 **A novel emulsifier, labrasol, enhances gastrointestinal absorption of gentamicin**

682 Life Sci., 69 (2001), pp. 2899-2910

683 N. Hussain, V. Jaitley, A.T. Florence

684 **Recent advances in the understanding of uptake of microparticulates across the gastrointestinal**

685 **lymphatics**

686 Adv. Drug Deliv. Rev., 50 (2001), pp. 107-142

687 V. Iannuccelli, G. Coppi, R. Cameroni

688 **Biodegradable intraoperative system for bone infection treatment. I. The drug/polymer interaction**

689 Int. J. Pharm., 143 (1996), pp. 195-201, [10.1016/S0378-5173\(96\)04703-5](https://doi.org/10.1016/S0378-5173(96)04703-5)

690 V. Iannuccelli, M. Montanari, D. Bertelli, F. Pellati, G. Coppi

691 **Microparticulate polyelectrolyte complexes for gentamicin transport across intestinal epithelia**

692 Drug Deliv., 18 (2011), pp. 26-37, [10.3109/10717544.2010.509362](https://doi.org/10.3109/10717544.2010.509362)

693 V. Iannuccelli, G. Coppi, M. Romagnoli, S. Sergi, E. Leo

694 **In vivo detection of lipid-based nano- and microparticles in the outermost human stratum corneum by**

695 **EDX analysis**

696 Int. J. Pharm., 447 (2013), pp. 204-212, [10.1016/j.ijpharm.2013.03.002](https://doi.org/10.1016/j.ijpharm.2013.03.002)

697 V. Iannuccelli, E. Maretti, M. Montorsi, C. Rustichelli, F. Sacchetti, E. Leo

698 **Gastroretentive montmorillonite-tetracycline nanoclay for the treatment of *Helicobacter pylori* infection**

699 Int. J. Pharm., 493 (2015), pp. 295-304, [10.1016/j.ijpharm.2015.06.049](https://doi.org/10.1016/j.ijpharm.2015.06.049)

700 V. Iannuccelli, E. Maretti, F. Sacchetti, M. Romagnoli, A. Bellini, E. Truzzi, P. Miselli, E. Leo

701 **Characterization of natural clays from Italian deposits with focus on elemental composition and**

702 **exchange estimated by Edx analysis: potential pharmaceutical and cosmetic uses**

703 Clay Clay Miner., 64 (2016), pp. 719-731, [10.1346/CCMN.2016.064038](https://doi.org/10.1346/CCMN.2016.064038)

704 R.I. Iliescu, E. Andronescu, G. Voicu, A. Ficai, C.I. Covaliu

705 **Hybrid materials based on montmorillonite and citostatic drugs: preparation and characterization**

706 Appl. Clay Sci., 52 (2011), pp. 62-68, [10.1016/j.clay.2011.01.031](https://doi.org/10.1016/j.clay.2011.01.031)

707 Y. Ito, T. Kusawake, M. Ishida, R. Tawa, N. Shibata, K. Takada

708 **Oral solid gentamicin preparation using emulsifier and adsorbent**

709 J. Control. Release, 105 (2005), pp. 23-31, [10.1016/j.jconrel.2005.03.017](https://doi.org/10.1016/j.jconrel.2005.03.017)

710 U. Jacobi, H.-J. Weigmann, J. Ulrich, W. Sterry, J. Lademann

- 711 **Estimation of the relative stratum corneum amount removed by tape stripping**  
712 Skin Res. Technol., 11 (2005), pp. 91-96, [10.1111/j.1600-0846.2005.00094.x](https://doi.org/10.1111/j.1600-0846.2005.00094.x)  
713 Y. Jia, H. Joly, A. Omri
- 714 **Liposomes as a carrier for gentamicin delivery: development and evaluation of the physicochemical**  
715 **properties**  
716 Int. J. Pharm., 359 (2008), pp. 254-263, [10.1016/j.ijpharm.2008.03.035](https://doi.org/10.1016/j.ijpharm.2008.03.035)  
717 G.V. Joshi, B.D. Kevadiya, H.A. Patel, H.C. Bajaj, R.V. Jasra
- 718 **Montmorillonite as a drug delivery system: intercalation and in vitro release of timolol maleate**  
719 Int. J. Pharm., 374 (2009), pp. 53-57, [10.1016/j.ijpharm.2009.03.004](https://doi.org/10.1016/j.ijpharm.2009.03.004)  
720 A. Kant, M. Datta
- 721 **Organo montmorillonite as drug delivery vehicle for the extended release of an antibiotic drug**  
722 World J. Pharm. Res., 6 (2016), pp. 574-586  
723 K.S. Katti, A.H. Ambre, N. Peterka, D.R. Katti
- 724 **Use of unnatural amino acids for design of novel organomodified clays as components of nanocomposite**  
725 **biomaterials**  
726 Philos. Trans. A Math. Phys. Eng. Sci., 368 (2010), pp. 1963-1980, [10.1098/rsta.2010.0008](https://doi.org/10.1098/rsta.2010.0008)  
727 M.H. Kim, G. Choi, A. Elzatahry, A. Vinu, Y.B. Choy, J.-H. Choy
- 728 **Review of clay-drug hybrid materials for biomedical applications: administration routes**  
729 Clay Clay Miner., 64 (2016), pp. 115-130, [10.1346/CCMN.2016.0640204](https://doi.org/10.1346/CCMN.2016.0640204)  
730 F. Knorr, J. Lademann, A. Patzelt, W. Sterry, U. Blume-Peytavi, A. Vogt
- 731 **Follicular transport route – research progress and future perspectives**  
732 Eur. J. Pharm. Biopharm., 71 (2009), pp. 173-180, [10.1016/j.ejpb.2008.11.001](https://doi.org/10.1016/j.ejpb.2008.11.001)  
733 J. Lademann, H. Richter, A. Teichmann, N. Otberg, U. Blume-Peytavi, J. Luengo, B. Weiß, U.F. Schaefer, C.-  
734 M. Lehr, R. Wepf, W. Sterry
- 735 **Nanoparticles – an efficient carrier for drug delivery into the hair follicles**  
736 Eur. J. Pharm. Biopharm., 66 (2007), pp. 159-164, [10.1016/j.ejpb.2006.10.019](https://doi.org/10.1016/j.ejpb.2006.10.019)  
737 A. Lein, C. Oussoren
- 738 **Dermal**  
739 Practical Pharmaceutics, Springer, Cham (2015), pp. 229-263, [10.1007/978-3-319-15814-3\\_12](https://doi.org/10.1007/978-3-319-15814-3_12)  
740 W. Lesniak, J. Mc Laren, W.R. Harris, V.L. Pecoraro, J. Schacht
- 741 **An isocratic separation of underivatized gentamicin components, <sup>1</sup>H NMR assignment and protonation**  
742 **pattern**  
743 Carbohydr. Res., 338 (2003), pp. 2853-2862

744 X. Liu, X. Lu, R. Wang, H. Zhou, S. Xu  
745 **Interlayer structure and dynamics of alkylammonium-intercalated Smectites with and without water: a**  
746 **molecular dynamics study**  
747 Clay Clay Miner., 55 (2007), pp. 554-564, [10.1346/CCMN.2007.0550602](https://doi.org/10.1346/CCMN.2007.0550602)  
748 A. López-Galindo, C. Viseras, P. Cerezo  
749 **Compositional, technical and safety specifications of clays to be used as pharmaceutical and cosmetic**  
750 **products**  
751 Appl. Clay Sci., 36 (2007), pp. 51-63, [10.1016/j.clay.2006.06.016](https://doi.org/10.1016/j.clay.2006.06.016)  
752 R.C. Mackenzie  
753 **Differential Thermal Analysis, Volume 1: Fundamental Aspects**  
754 Academic Press, London (1970)  
755 J. Madejová, P. Komadel  
756 **Baseline studies of the clay minerals society source clays: infrared methods**  
757 Clay Clay Miner., 49 (2001), pp. 410-432  
758 J. Madejová, M. Janek, P. Komadel, H.-J. Herbert, H.C. Moog  
759 **FTIR analyses of water in MX-80 bentonite compacted from high salinary salt solution systems**  
760 Appl. Clay Sci., 20 (2002), pp. 255-271  
761 S. McClean, E. Prosser, E. Meehan, D. O'Malley, N. Clarke, Z. Ramtoola, D. Brayden  
762 **Binding and uptake of biodegradable poly-DL-lactide micro- and nanoparticles in intestinal epithelia**  
763 Eur. J. Pharm. Sci., 6 (1998), pp. 153-163  
764 W.S. Mohamed, A.B. Mostafa, H.E. Nasr  
765 **Characterization and application of intercalated montmorillonite with verapamil and its polymethyl**  
766 **methacrylate nanocomposite in drug delivery**  
767 Polym.-Plast. Technol. Eng., 53 (2014), pp. 1425-1433, [10.1080/03602559.2014.909462](https://doi.org/10.1080/03602559.2014.909462)  
768 P. Moretto, J.E. Surleve-Bazeille, D. Licu, C. Michelet, P. Stedzel  
769 **Microanalysis of the human skin structure: preliminary results**  
770 Nucl. Instrum. Methods Phys. Res. B, 158 (1999), pp. 386-392, [10.1016/S0168-583X\(99\)00346-8](https://doi.org/10.1016/S0168-583X(99)00346-8)  
771 A.B. Morgan, C.A. Wilkie  
772 **Flame Retardant Polymer Nanocomposites**  
773 John Wiley & Son, Hoboken, New Jersey, USA (2007)  
774 S.M. Moyes, S.H. Smyth, A. Shipman, S. Long, J.F. Morris, K.E. Carr  
775 **Parameters influencing intestinal epithelial permeability and microparticle uptake in vitro**

776 Int. J. Pharm., 337 (2007), pp. 133-141, [10.1016/j.ijpharm.2006.12.036](https://doi.org/10.1016/j.ijpharm.2006.12.036)

777 S. Nishijima, I. Kurokawa

778 **Antimicrobial resistance of Staphylococcus aureus isolated from skin infections**

779 Int. J. Antimicrob. Agents, 19 (2002), pp. 241-243

780 P.O. Nnamani, F.C. Kenechukwu, C.L. Anugwolu, A.C. Obumneme, A.A. Attama

781 **Characterization and controlled release of gentamicin from novel hydrogels based on Poloxamer 407 and**  
782 **polyacrylic acids**

783 Afr. J. Pharm. Pharmacol., 7 (2013), pp. 2540-2552

784 T. Ogiso, T. Shiraki, K. Okajima, T. Tanino, M. Iwaki, T. Wada

785 **Transfollicular drug delivery: penetration of drugs through human scalp skin and comparison of**  
786 **penetration between scalp and abdominal skins in vitro**

787 J. Drug Target., 10 (2002), pp. 369-378, [10.1080/1061186021000001814](https://doi.org/10.1080/1061186021000001814)

788 N. Otberg, H. Richter, H. Schaefer, U. Blume-Peytavi, W. Sterry, J. Lademann

789 **Variations of hair follicle size and distribution in different body sites**

790 J. Invest. Dermatol., 122 (2004), pp. 14-19, [10.1046/j.0022-202X.2003.22110.x](https://doi.org/10.1046/j.0022-202X.2003.22110.x)

791 L.B. de Paiva, A.R. Morales, F.R. Valenzuela Díaz

792 **Organoclays: properties, preparation and applications**

793 Appl. Clay Sci., 42 (2008), pp. 8-24, [10.1016/j.clay.2008.02.006](https://doi.org/10.1016/j.clay.2008.02.006)

794 E.M. Pecini, M.J. Avena

795 **Measuring the isoelectric point of the edges of clay mineral particles: the case of montmorillonite**

796 Langmuir, 29 (2013), pp. 14926-14934, [10.1021/la403384g](https://doi.org/10.1021/la403384g)

797 G.R. Persson, G.E. Salvi, L.J.A. Heitz-Mayfield, N.P. Lang

798 **Antimicrobial therapy using a local drug delivery system (Arestin) in the treatment of peri-implantitis. I:**  
799 **microbiological outcomes**

800 Clin. Oral Implants Res., 17 (2006), pp. 386-393, [10.1111/j.1600-0501.2006.01269.x](https://doi.org/10.1111/j.1600-0501.2006.01269.x)

801 A. Rapacz-Kmita, E. Stodolak-Zych, M. Ziabka, A. Rozycka, M. Dudek

802 **Instrumental characterization of the smectite clay-gentamicin hybrids**

803 Bull. Mater. Sci., 38 (2015), pp. 1069-1078, [10.1007/s12034-015-0943-7](https://doi.org/10.1007/s12034-015-0943-7)

804 A. Rapacz-Kmita, M.M. Bućko, E. Stodolak-Zych, M. Mikołajczyk, P. Dudek, M. Trybus

805 **Characterisation, in vitro release study, and antibacterial activity of montmorillonite-gentamicin complex**  
806 **material**

807 Mater Sci Eng C Mater Biol Appl, 70 (2017), pp. 471-478, [10.1016/j.msec.2016.09.031](https://doi.org/10.1016/j.msec.2016.09.031)

808 A. des Rieux, V. Fievez, I. Théate, J. Mast, V. Préat, Y.-J. Schneider

- 809 **An improved in vitro model of human intestinal follicle-associated epithelium to study nanoparticle**  
810 **transport by M cells**
- 811 Eur. J. Pharm. Sci., 30 (2007), pp. 380-391, [10.1016/j.ejps.2006.12.006](https://doi.org/10.1016/j.ejps.2006.12.006)
- 812 M.S. Roberts
- 813 **Dermal Absorption and Toxicity Assessment**
- 814 (Second Edition), CRC Press (2007)
- 815 L.A. de S. Rodrigues, A. Figueiras, F. Veiga, R.M. de Freitas, L.C.C. Nunes, E.C. da Silva Filho, C.M. da Silva  
816 Leite
- 817 **The systems containing clays and clay minerals from modified drug release: a review**
- 818 Colloids Surf. B Biointerfaces, 103 (2013), pp. 642-651, [10.1016/j.colsurfb.2012.10.068](https://doi.org/10.1016/j.colsurfb.2012.10.068)
- 819 P. Rolland, V. Carlino, R. Vane
- 820 **Improved carbon analysis with Evactron plasma cleaning**
- 821 Microsc. Microanal., 10 (2004), pp. 964-965, [10.1017/S1431927604880504](https://doi.org/10.1017/S1431927604880504)
- 822 B.P. Ross, S.E. DeCruz, T.B. Lynch, K. Davis-Goff, I. Toth
- 823 **Design, synthesis, and evaluation of a liposaccharide drug delivery agent: application to the**  
824 **gastrointestinal absorption of gentamicin**
- 825 J. Med. Chem., 47 (2004), pp. 1251-1258, [10.1021/jm030474j](https://doi.org/10.1021/jm030474j)
- 826 S.S. Sampath, D.H. Robinson
- 827 **Comparison of new and existing spectrophotometric methods for the analysis of tobramycin and other**  
828 **aminoglycosides**
- 829 J. Pharm. Sci., 79 (1990), pp. 428-431
- 830 S. Scalia, E. Franceschinis, D. Bertelli, V. Iannuccelli
- 831 **Comparative evaluation of the effect of permeation enhancers, lipid nanoparticles and colloidal silica on**  
832 **in vivo human skin penetration of quercetin**
- 833 Skin Pharmacol. Physiol., 26 (2013), pp. 57-67, [10.1159/000345210](https://doi.org/10.1159/000345210)
- 834 SCCP (Scientific Committee on Consumer Products)
- 835 **Opinion on Safety of Nanomaterials in Cosmetic Products, SCCP/1147/07**
- 836 European Commission (2007)
- 837 V.P. Shah, G.L. Flynn, A. Yacobi, H.I. Maibach, C. Bon, N.M. Fleischer, T.J. Franz, S.A. Kaplan, J. Kawamoto,  
838 L.J. Lesko, J.P. Marty, L.K. Pershing, H. Schaefer, J.A. Sequeira, S.P. Shrivastava, J. Wilkin, R.L. Williams
- 839 **Bioequivalence of topical dermatological dosage forms—methods of evaluation of bioequivalence**
- 840 Pharm. Res., 15 (1998), pp. 167-171
- 841 N. Sökmen, F. Bican, F. Ayhan, H. Ayhan
- 842 **Chelating agent effect on the release of gentamicin from PEG-DA hydrogels**

843 Hacettepe J. Biol. Chem., 36 (2008), pp. 347-352

844 E.S. Swenson, W.B. Milisen, W. Curatolo

845 **Intestinal permeability enhancement: efficacy, acute local toxicity, and reversibility**

846 Pharm. Res., 11 (1994), pp. 1132-1142

847 E.C. Umeyor, F.C. Kenechukwu, J.D. Ogbonna, S.A. Chime, A. Attama

848 **Preparation of novel solid lipid microparticles loaded with gentamicin and its evaluation in vitro and in**  
849 **vivo**

850 J. Microencapsul., 29 (2012), pp. 296-307, [10.3109/02652048.2011.651495](https://doi.org/10.3109/02652048.2011.651495)

851 A. Verma, A. Jain, P. Hurkat, S.K. Jain

852 **Transfollicular drug delivery: current perspectives**

853 Res. Rep. Transdermal Drug Deliv., 5 (2016), pp. 1-17, [10.2147/RRTD.S75809](https://doi.org/10.2147/RRTD.S75809)

854 L.B. Williams, S.E. Haydel

855 **Evaluation of the medicinal use of clay minerals as antibacterial agents**

856 Int. Geol. Rev., 52 (2010), pp. 745-770, [10.1080/00206811003679737](https://doi.org/10.1080/00206811003679737)

857 World Health Organization

858 **Bentonite, kaolin, and selected clay minerals**

859 Environmental Health Criteria 231, World Health Organization, Geneva (2005)

860 H. Wosicka, K. Cal

861 **Targeting to the hair follicles: current status and potential**

862 J. Dermatol. Sci., 57 (2010), pp. 83-89, [10.1016/j.jdermsci.2009.12.005](https://doi.org/10.1016/j.jdermsci.2009.12.005)

863 C.G. Zalavras, M.J. Patzakis, P. Holtom

864 **Local antibiotic therapy in the treatment of open fractures and osteomyelitis**

865 Clin. Orthop. (2004), pp. 86-93

866