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Research paper

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

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ABSTRACT

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

## 1. Introduction

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

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

The present work focuses on the assessment of a raw bentonite (Bt), a montmorillonite rich clay recently characterized in a previous work (Iannuccelli et al., 2016), for the development of a novel gentamicin/clay hybrid material for the topical use. Gentamicin (GM) is an aminoglycoside antibiotic widely used in the treatment of severe infections, caused by many Gram-negative and Gram-positive bacteria, such as

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meningitis, nephritis, and post-operative infections. Although it presents a very broad spectrum of action, its use is limited to serious infections caused by Gram-negative bacteria because of its high toxicity. Gentamicin is commonly administered as injections, topical and ophthalmic dosage forms because of poor absorption following the oral administration. The well-known poor gastrointestinal membrane permeability and the consequent low bioavailability (class III of the biopharmaceutical classification system) are likely connected to the high polarity of this cationic compound. Various approaches have been investigated in order to increase GM oral bioavailability, including the co-administration of absorption-enhancing agents such as surfactants (Hu et al., 2001; Ito et al., 2005), bile salts and glucosteroids (Axelrod et al., 1998), and liposaccharides (Ross et al., 2004). Although good gastrointestinal absorption enhancing effects were demonstrated, cytotoxicity and damage to the mucosa have been reported (Aungst, 2000; Ross et al., 2004; Swenson et al., 1994). Another strategy aiming to promote GM oral bioavailability could involve the use of microparticulate carriers to be taken up by the intestinal lymphoid tissue (des Rieux et al., 2007; Hussain et al., 2001; Iannuccelli et al., 2011; McClean et al., 1998; Moyes et al., 2007) or to be implanted for bone infection treatment also exploiting drug interaction with anionic polymers (Iannuccelli et al., 1996, 2011). Gentamicin is extensively used topically against severe microbial infections especially in burns and wounds (Chang et al., 2006), but also in the treatment of impetigo, infected bed sores, nasal staphylococcal carrier state, pyoderma, infections of the external eye, and adnexa (Nishijima and Kurokawa, 2002). Gentamicin applied to the skin has only a low systemic absorption due to the difficult penetration through the deep layers of the skin, related, probably, to its cationic nature; for this reason, its use is limited to the local effect that involves mainly the most superficial skin layers. Despite its benefits, GM short-life, bacterial barriers and adverse effects such as nephrotoxicity, ototoxicity, and neurotoxicity upon prolonged use limit GM daily dosage (Roberts, 2007). In fact, many clinicians are reluctant to use it, even for a short term (Drusano, 2007). Efforts have been made to reduce toxicity associated with prolonged use by means of liposomes, micellar systems, hydrogels, microgels, or nanospheres (Ahangari et al., 2013; Ayhan and Ozkan, 2007; Changez et al., 2003; Eljarrat-Binstock et al., 2004; Jia et al., 2008; Nnamani et al., 2013; Sòkmen et al., 2008; Umeyor et al., 2012). Local delivery of GM can solve the major disadvantages of the systemic administration by maintaining a high local antibiotic concentration for an extended time (Zalavras et al., 2004). Particularly, drug delivery systems exhibiting high initial release rate followed by a sustained release at an effective antibiotic concentration may allow local control of infection while minimizing side effects and preventing bacterial resistance (Aviv et al., 2007; Persson et al., 2006).

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

## 2. Experimental part

### 2.1. Materials

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

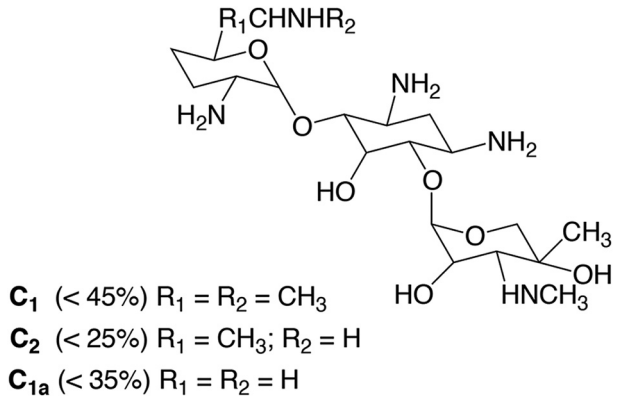


**Fig. 1.** Molecular structure of gentamicin sulfate.

### 2.2. Bt activation

Bt activation and thus the implementation of its organophilic behavior are provided by the saturation of the montmorillonite interlayers with a homogeneous cationic population through the cation exchange reaction hereafter detailed. A defined amount of Bt was grinded by a vibratory ball mill (Fritsch GmbH, Idar-Oberstein, Germany) for 10 h to remove particle aggregates. Batches of dispersions were prepared mixing 1 g of milled Bt and 25 mL of NaCl 0.1 M and were shaken with a magnetic stirrer at room temperature for 24 h. The supernatant was centrifuged (mod. 4235, 188 ALC International, Milan, Italy) at 2115 $\times g$ for 20 min and the solid was twice subjected to the same treatment. The separated solids were washed several times with 35 mL of distilled water under magnetic stirring at room temperature for 4 h followed by centrifugation at 2115 $\times g$ for 2 h. The solid was dried under vacuum at room temperature and the supernatant analyzed for NaCl absence by titration with 0.1 M silver nitrate solution according to U.S. Pharmacopeia. The activation process was carried out in triplicate.

### 2.3. Bt/GM preparation

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

### 2.4. Gentamicin adsorption measurements

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

The amount of GM adsorbed onto Bt in both aBt/GM and Bt/GM