PHYSICOCHEMICAL AND *IN VITRO* CATION RELEASE RELEVANCFE OF THERAPEUTIC MUDS "MADURATION"

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Abstract

Therapeutic muds are used in the treatment of illnesses of the locomotor apparatus, including osteoarthritis and rheumatologic diseases. The mechanisms of action of this therapy are a matter of discussion, mainly for the different traditions of pelotherapy centers. Heat plays a fundamental role in the beneficial effects of thermal mud therapy together with the possible transfer across the skin barrier of chemical elements presented in the mud. Preparation procedures of therapeutic muds have been orally transmitted since ancient times, being accepted that muds require a "maturation" process to achieve the desired therapeutic results. Pharmaceutical research of maturation is crucial to ascertain the possible changes induced by this operation in the properties of muds. In particular, it is necessary to verify the changes associated with physical and/or chemical therapeutic mechanisms that sustain the traditional use of maturation in the preparation of therapeutic muds. Two clay samples were used to prepare thermal muds with mineral medicinal water from the thermal spring of Graena (Cortes y Graena, Granada, Spain). Muds were matured for three months and characterized over time for those properties considered relevant in view of their topical administration (rheological properties and particle size distribution) and possible mechanisms of action (composition, pH, cation exchange capacity, thermal properties and amount of cations released). Maturation of the studied therapeutic muds did not induce alteration of clay minerals, even if a decrease in amplitude of particle size distribution, changes in pH and disappearance of thixotropic behavior were observed. Maturation increased the release of cations from therapeutic muds but did not improve their thermal properties. In the studied case, thermophysical activity did not require maturation. Conversely, maturation increased the amount of cations released from the muds, appearing as a beneficial process for possible chemical therapeutic effects associated with the ionic content of these systems. Maturation could therefore explain the differential chemical effects associated with the use of therapeutic muds compared to other thermotherapeutic agents.

Keywords: Therapeutic muds; Maturation; Clays; Cation release; Specific heat.

1. Introduction

Clays are used in several European thermal centres to prepare semisolid suspensions with mineral medicinal water (therapeutic muds) that are topically administered to treat osteoarthritis and other musculoskeletal disorders (Grassi et al., 2003; Veniale et al., 2007; Gomes et al., 2007, 2013; Bellometti et al., 2007; Evcik et al., 2007; Giacomino and De Michele, 2007; Fraioli et al., 2011; Beer et al., 2013; Espejo-Antúnez et al., 2013). These semisolid remedies, in which the clay behaves as vehicle of the mineral medicinal water, are inorganic structured gels formed by the interactions between clay particles suspended in mineral medicinal water (Viseras et al., 2007). The properties of these systems greatly depend on the solid-liquid interfacial phenomena occurring during "maturation" (Aguzzi et al., 2013). Maturation is an ethnopharmaceutical procedure that involves the contact between clay particles and thermal waters for a certain period of time (generally months), following local traditional protocols that greatly vary depending on the thermal centres (Veniale et al., 2004; Baschini et al., 2010; Pozo et al., 2013). It is assumed that virgin muds require maturation to optimise their therapeutic effects, achieving the category of "matured muds" or "therapeutic muds" ready to be used in pelotherapy (Gomes et al., 2013). Veniale et al. (2004) compared the mineralogical composition and properties of virgin and matured clays from different thermal centres of northern Italy concluding that the observed changes were mainly due to the composition of the water. Smectite/water muds are considered optimal due to the ability of smectitic clays to retain high amounts of water. This is consistent with the correlation observed between thermal properties of muds and their relative amount of water (Caridad et al., 2014). Cara et al. (2000a) found that therapeutic muds could be improved by addition of smectite. Maturation of illitic-smectitic clays in bicarbonate and sulphate rich waters induced reduction in the crystallinity of clay minerals, dissolution of carbonates, precipitation of gypsum and changes in granulometry and conductivity (Sánchez et al., 2002). Changes in crystallinity and granulometry were also found in saponite and montmorillonite matured in seawater (Carretero et al., 2007), kaolinite-saponite in sodium potassium chloride water (Gamiz et al., 2009) and kaolinite and bentonite in bicarbonate rich waters (Fernández-González et al., 2013). Tateo et al. (2010) described in detail the mineralogical changes occurring during short term (up to 2 months) and long term (up to 15 months) maturation of different clays in sulphate mineral water, concluding the need for the study of each particular water-clay pair on case-by-case basis. Pozo et al. (2013) compared matured muds from different Spanish

thermal stations and found pronounced differences in composition and properties, even if the muds were used for similar clinical purposes.

The nature and importance of the mineralogical, textural and chemical changes induced by the maturation process and mentioned above does not seem likely to have a decisive influence on the therapeutic properties of therapeutic muds. In other words, the mineralogical, textural and chemical differences observed by these authors hardly could explain alone the clinical need to perform maturation of the mud. Recently, the importance of elemental characterization of peloids has been evidenced and the necessity of determining the bioavailability of soluble mineral species when the peloids are applied has been suggested (Suárez Muñoz et al., 2015).

Maturation must be studied from a pharmaceutical point of view to understand its importance in achieving therapeutic muds. The pharmaceutical development of a matured therapeutic mud should be focused on achieving products suitable to the purpose for which they are intended (Cerezo et al., 2014). In particular, as health care products, therapeutic muds must be developed fulfilling technological, biopharmaceutical and clinical features in order to ensure their stability, effectiveness and safety.

With these premises, aim of this work was to evaluate the influence of maturation on therapeutic muds prepared with two clay samples, commonly used in spa centers of southern European/Mediterranean countries.

2. Materials and methods

2.1. Materials

Two clay samples (I, II) were used to prepare the thermal muds. Sample I was Therapeutic mud Minerale® from SO.MI.ES. (Italy) and sample II came from Jebel Aidoudi deposits (Tunisia). Both samples were fully characterized in a previous work in terms of identity, richness and purity (Sánchez-Espejo et al., 2014). The samples were identified as “high purity clays”, as the sum of smectites, kaolinite and illite was N 70% w/w, with 13% w/w of calcite in sample I and 11% w/w of gypsum in sample II as main mineral impurities. Both samples are used in several spas in Italy and Tunisia.

Mineral medicinal water used for the preparation of therapeutic muds came from thermal spring of Graena (Cortes y Graena, Granada, Spain). Its physicochemical characteristics were determined in a previous work (Aguzzi et al., 2013).

2.2. Preparation of therapeutic muds

1:2 (w/w) clay/water muds were prepared by using a turbine stirrer (Silverson LT, U.K.) (4000 rpm, 5 min) and allowed to swell for 48 hours (samples I_0 and II_0). The resultant systems were then stored at room temperature in airtight polyethylene containers for three months. At the end of each month, the muds were manually stirred for 15 min by means of a glass rod performing planetary movements and aliquots were taken to be characterized (samples I_1, I_2, I_3, II_1, II_2 and II_3).

2.3. Characterization of therapeutic muds

Chemical and physical integrity of muds during maturation must be controlled by measuring different parameters, including pH, cation exchange capacity, viscosity and when necessary mineralogical and chemical compositions (Cerezo et al., 2014).

2.3.1. Mineralogical and chemical composition

X-Ray diffraction (XRD) data and chemical analysis (major elements) were done following López-Galindo et al. (1996). XRD analysis were done by using a Philips® X-Pert (Philips, Holanda) diffractometer equipped with automatic slit (CuKα, 4-70º 2θ, 6º/min, 40kV). Random powder diffraction was used on silt–clay fraction (the muds were dispersed in water and the silt–clay fraction separated and then dried), and air-dried/ethylene glycol solvated oriented- aggregates of the clay fractions were prepared on glass slides. All oriented clay fractions were submitted to thermal treatments (550°C, 2 h). Data were analyzed with the Xpowder® software package (Martín-Ramos, 2004). Major elements were determined by X-ray fluorescence (XRF), using a Bruker® S4 Pioneer equipment, with a Rh X-ray tube (60 kV, 150 mA).

2.3.2. Water content, pH and particle size distribution

The water content of therapeutic muds was determined by weight loss on drying of 1 g of mud. pH of the muds was measured by using a pHmeter (Crison, pH 25+) equipped with a semisolid sensor (5052T).

Particle size changes of the solid phases, as a result of maturation, were measured with a

Malvern® Mastersizer 2000 LF granulometer. The muds were dispersed in water under sonication. Data were online collected and statistical particle diameters (d_{10}, d_{50}, d_{90}) were calculated. SPAN factor was also calculated following Gavini et al. (2008) as an index of the amplitude of particle size distribution. Three replicates were performed for each sample.

2.3.3. Cation exchange capacity (CEC)

Dried mud powders (1 g) were dispersed in 25 mL tetramethylammonium bromide aqueous solution (1 M), in order to displace their constituent cations. Dispersions were shaken overnight at 50 rpm and then filtered. Cations in solution were assayed by ICP-OES (Optima 8300 ICP-OES Spectrometeer, Perkin Elmer, USA) and CEC was calculated as the sum of exchangeable cations, expressed in meq/100 g of dried mud.

2.3.4. Rheological properties

Rheological analysis was carried out with a Controlled Rate Viscometer (Thermo Scientific HAAKE, RotoVisco 1) connected to a "personal computer" to set analysis parameters, process and record data by means of HAAKE RheoWin software. A plate/plate combination (Plate Ø 20 mm serrated PP20/S sensor system) was used as measuring system. Measurements were carried out at 25ºC after a rest time of 90 s. A Peltier temperature controlled measuring plate for parallel plate (TCP/P, HAAKE unit) was used to control measurement temperature. Rheological properties of the samples were measured in the shear rate range 10-800 s^{-1}. Shear rates were selected as representative of the stress produced by common operations like skin spreading (10–200 s^{-1}), manual mixing (100–200 s^{-1}) or container removal (400–2000 s^{-1}) (Schott, 1995). Rheological characterization included thixotropic behavior, yield points and apparent viscosities of the samples. Six replicates were performed on each sample.

2.3.5. Thermal studies

Cooling kinetics were studied following Cara et al. (2000b). Briefly, known amounts of muds were conditioned at 50°C in a cylindrical polyethylene terephthalate cell and then immersed in a thermostatic bath at 25ºC, measuring the cooling of the samples up to 32ºC, by means of a thermometric probe located in the center of the cell. Experimental cooling data were fitted by using the Newton law, describing thermal exchange between two bodies in contact at different temperatures.

$$(T - T_{min}) = (T_{max} - T_{min})e^{-kt} \qquad (1)$$

where T_{min} was the room temperature (25ºC), T_{max} was the initial temperature (50ºC), t was the time in minutes and k was a constant that depend on the material and apparatus, given by:

$$k = \frac{P}{C} = \frac{P}{mC_p} \qquad (2)$$

where P is the instrumental constant of the apparatus, C the heat capacity of the heated material, m the heated mass and C_p the specific heat. The apparatus constant was obtained following Cara et al. (2000b) by fitting of cooling data obtained with a known amount of a reference water suspension of TiO_2. Experimental thermal parameters of the studied samples were then obtained by using equation 1 and 2.

2.3.6. In vitro release of cations

In vitro release experiments were performed on 5 mg of therapeutic muds by means of Franz diffusion cells (FDC40020FF, Crown Bio Scientific Inc., Clinton, Permeagear, USA) (contact area 0.64 cm^2). The donor and receptor chambers were separated by a dialysis membrane (cut-off 12–14 kDa). Before its use, the membrane was boiled in distilled water for 10 min. Purified water, thermostated at 32 °C, degassed and filtered, was used as receptor phase. At the end of the experiments (20 minutes; minimum typical time of application of mud-packs), the receptor phase was withdrawn, and amount of cations released was dosed by ICP-OES (Optima 8300 ICP-OES Spectrometer, Perkin Elmer, USA).

2.4. Statistical analysis

One-way analysis of variance (ANOVA) with post hoc Sheffé test for multiple comparisons was performed using the software Siphar 4.0 (France). Differences between groups were considered to be significant at a level of P less than 0.05.

3. Results and Discussion

3.1. Mineralogy

Mineralogical compositions of the therapeutic muds at different maturation times were obtained on the basis of XRD patterns (Figs. 1 and 2) and chemical compositions (Table 1) and compared to those of the initial raw clay materials. XRD patterns of the bulk samples seem to evidence some alteration in the d001 reflection of smectite (Figs. 1A and 2A). In sample I, reflection corresponding to the basal spacing of the Na^{+} smectite was altered to broader diffraction reflec- tions in the matured muds. Nevertheless, XRD patterns of the oriented aggregates clearly show that there were no significant changes as a result of maturation (Figs. 1B and 2B). The observed modifications in the bulk XRD patterns were ascribed to a progressive diminution in the crystallite sizes or in the diffraction domain of the smectites. Similar be- havior was also observed by Sánchez et al. (2002). In sample II, during the first 48 h of maturation, the Ca^{+2} ions in the smectite interlayer were exchanged by Na^{+} ions coming from the halite dissolution, resulting in a decrease of the interlayer space from ≅13.5 Å (≅6.0 2-theta) (II) to ≅12 Å (≅7.5 2-theta) (II0) (Fig. 2). During the rest of the maturation process, a similar progressive diminution to that previously described for sample I was observed. As regard the mineral impurities, the low amount of dolomite detected in sample I seems to disappear after 48 h of maturation. Similarly, the amount of gypsum in sample II greatly decreases during the first hours of maturation and halite completely dissolved in the first maturation month.

3.2. Water content, pH and particle size distribution

Water content of the samples did not significantly change during maturation (Table 2). Potential changes in solid/liquid percentages associated to monthly agitation were therefore neglected.

pH of the samples was in the region of the isoelectric point of smectite edges (Avena and de Pauli, 1998), ranging from 7.27 to 8.09 (Table 2). These values were similar to those recently measured in analogous systems and related to the formation of three-dimensional band-type networks (Aguzzi et al., 2013). Is noteworthy that the pH of the samples changed slightly but significantly ($P < 0.001$) as a result of maturation. In both therapeutic muds the pH down slightly and then ascended up to the initial values, suggesting changes in the balance between acid and base cations adsorbed by the solid phase of the muds. Initial decrease in pH during the first maturation month indicated partial dissolution of montmorillonite structure and consequent release of Al^{3+} into the

solution as described by several authors (Wieland and Stumm, 1992; Fürrer et al., 1993; Bickmore et al., 2001; Sondi et al., 2008). Slow pH increase during the rest of maturation is coherent with the re-adsorption of Al^{3+} cations on the new mixed layer illite-smectite structures forming in the second and third months of maturation (Decarreau, 1985; Goluvev et al., 2006).

As regard of the aggregation states of the particles during maturation, neither d_{90}, nor d_{50} showed significant changes compared to the initial values, whereas d_{10} increased only after two (sample I, $P < 0.01$) or three months (sample II, $P < 0.05$) of maturation (Table 3). These results are in agreement with those observed in very concentrated clay suspensions in which long maturation time reduced the percentage of individual clay particles (Aguzzi et al., 2013). As a result of the mineralogical changes in crystallite sizes, the amplitude in particle size distribution decreased during maturation, as confirmed by the reduction in SPAN factor values (Table 3).

3.3. Rheological properties

As concentrated suspensions of flocculated clay particles, all the therapeutic muds showed typical non-Newtonian viscoplastic flow curves, whatever the maturation time (Fig. 3). The profile of the curves increased with maturation, especially after two months. At the beginning of maturation, the curves showed hysteresis area (especially in the case of sample II) in agreement with the smectite content. The thixotropy of the systems greatly decreased with maturation, corresponding with the alteration of smectites discussed previously.

From the flow curves, it was possible to obtain the apparent viscosities (at 200 s^{-1}) and yield point values (calculated according to the Bingham model as the intercept of the linear portion of the flow curve with the stress axis) (Table 4). Both apparent viscosities and yield point values increased significantly ($P < 0.001$) with maturation times. These results are consistent with those observed in concentrated laminar clay gels by Aguzzi et al. (2013). Calcium ions, presented in the liquid phase, promoted face(−)/face(−) contacts and stabilized band-like structures (Lagaly, 2006).

3.4. Thermal studies

Experimental specific heats of the therapeutic muds are shown in Table 5. In the table the time required to achieve 32ºC (t_{32}) and the mud temperature after 20 minutes (T_{20min}) (minimum typical time of application of mud-packs), calculated by linear

regression of equation 1 ($R^2 > 0.9999$ in all cases), are also included. The experimental values of specific heats were similar to those measured in analogous systems by other authors (Cara et al., 2000b; Legido et al., 2007; Casas et al., 2011, 2013; Caridad et al., 2014; Khiari et al., 2014). In all samples, t_{32} was around 30 min and T_{20min} was 35ºC approximately. These values were able to assure heat transfer between the samples and the skin in normal application procedures. No influence of the composition, neither of the maturation time was observed in the studied thermal parameters ($P > 0.05$). This is congruent with the hypothesis that thermal behaviour of clay muds is mainly dependent of their water content (Cara et al., 2000b).

3.5. Cation exchange capacity and in vitro cation release

The total CEC of the therapeutic muds was independent of matura- tion time (Table 6). However, some significant changes occurred in the exchanged amounts of certain cations (Table 6). Exchangeable Ca^{2+} and Mg^{2+} decreased in sample I ($P < 0.05$) as a result of dissolution of dolomite and Na^+ values change only after the third month. In sample II, Na^+ values are the only ones changing with time. The amount of exchangeable Na^+ increased during the first maturation month ($P < 0.01$), in agreement with the mineralogical changes previously described (halite dissolution and simultaneous exchange of the Ca^{2+} with Na^+ in the smectite interlayer).

The amount of cations released in the Franz cell experiments is reported in Table 7. In all cases, the amount of cations released was lower than the CEC. However, in both samples, the release increased significantly achieved three months of maturation. Release of K^+, Ca^{2+} and Mg^{2+} increased progressively, whereas the release of Na^+ showed a sharp increase starting from the second month of maturation. Apparently, the alteration of the smectites by diminution in crystallite sizes improved the release of Na^+ cations presented in the smectite interlayer. To explain the reasons of the observed differences in the behavior of the studied cations it would be necessary to perform complementary studies focusing on the possible influence of other metallic cations presented in the systems.

4. Conclusions

Maturation of the studied clay samples in the mineral medicinal water of Graena did not induce important mineral alterations except the expected disappearance of soluble minerals. Maturation resulted in a decrease in amplitude of particle size distribution as

well as changes in pH of the muds and disappearance of thixotropic behavior of initial smectite gels. Nevertheless, the viscosity and yield point values of the muds increased and the thermal properties remained unaltered. Matu- ration hardly changed the CEC of the muds, but the amount of cations released, in particular of Na^+, greatly increased during maturation. According to our results, in the studied systems, maturation makes sense as an operation that increases the release of cations from the therapeutic muds but does not improve their thermal properties. Consequently, in the studied cases, the therapeutic effects associated with thermophysical mechanisms do not require mud maturation. Maturation was only relevant in order to explain the possible chemical effects associated with the use of the therapeutic muds. According to our results, the mud will be mature at two months (maximum of cation release), being necessary to examine the clinical differences associated with that maturation period.

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335 **References**

336 Aguzzi, C., Sánchez-Espejo, R., Cerezo, P., Machado, J., Bonferoni, C., Rossi, S.,
337 Salcedo, I., Viseras, C., 2013. Networking and rheology of concentrated clay
338 suspensions "matured" in mineral medicinal water. International Journal of
339 Pharmaceutics 453, 473– 479.

340 Avena, M.J and De Pauli, C.P., 1998. Proton adsorption and electrokinetics of an
341 Argentinian montmorillonite. Journal of Colloid and Interface Science 202, 195-204.

342 Baschini, M.T., Pettinari, G.R., Vallés, J.M., Aguzzi, C., Cerezo, P., López-Galindo, A.,
343 Setti, M., Viseras, C., 2010. Suitability of natural sulphur-rich muds from Copahue
344 (Argentina) for use as semisolid health care products. Applied Clay Science 49, 205-
345 212.

346 Beer, A.M., Fetaj, S., Lange, U., 2013. Peloid therapy: An overview of the empirical
347 status and evidence of mud therapy. Zeitschrift für Rheumatologie 72, 581-589.

348 Bellometti, S., Gallotti, C., Pacileo, G., Rota, A., Tenconi, M.T., 2007. Evaluation of
349 outcomes in SPA-treated osteoarthrosic patients. Journal of Preventive Medicine and
350 Hygiene 48, 1-4.

351 Bickmore, B.R., Bosbach, D., Hochella, M.F., Charlet, L., Rufe, E., 2001. In situ atomic
352 force microscopy study of hectorite and nontronite dissolution: Implications for
353 phyllosilicate edge surface structures and dissolution mechanisms. American
354 Mineralogist 86, 411-423.

355 Cara, S., Carcangiu, G.F., Padalino, G., Palomba, M., Tamanini, M., 2000a. The
356 bentonites in pelotherapy: chemical, mineralogical and technological properties of
357 materials from Sardinia deposits (Italy). Applied Clay Science 16, 117–124.

358 Cara, S., Carcangiu, G., Padalino, G., Palomba, M., Tamanini, M., 2000b. The
359 bentonites in pelotherapy: thermal properties of clay pastes from Sardinia (Italy).
360 Applied Clay Science 16, 125-132.

361 Caridad, V., Ortiz de Zárate, J.M., Khayet, M., Legido, J.L., 2014. Thermal
362 conductivity and density of clay pastes at various water contents for pelotherapy use.
363 Applied Clay Science 93–94, 23–27.

364 Carretero, M.I., Pozo, M., Sánchez, C., García, F.J., Medina, J.A., Bernabé, J.M., 2007.
365 Comparison of saponite and montmorillonite behaviour during static and stirring
366 maturation with seawater for pelotherapy. Applied Clay Science 36, 161-173.

367 Casás, L.M., Legido, J.L., Pozo, M., Mourelle, L., Plantier, F., Bessières, D., 2011.
368 Specific heat of mixtures of bentonitic clay with sea water or distilled water for their
369 use in thermotherapy. Applied Clay Science 524, 68-73.
370 Casás, L.M, Pozo, M., Gómez, C.P., Pozo, E., Bessiéres, L.D., Plantier, F., Legido, J.L.,
371 2013. Thermal behaviour of mixtures of bentonitic clay and saline solutions. Applied
372 Clay Science 72, 18-25.
373 Cerezo, P., Aguzzi, C., Viseras, C., 2014. Desarrollo galénico de peloides terapéuticos.
374 In: Hernández Torres, A (Ed.), Peloterapia: Aplicaciones médicas y cosméticas de
375 fangos termales, Agencia de Evaluación de Tecnologías Sanitarias (AETS)-Instituto
376 de Salud Carlos III, Madrid, pp. 141-146.
377 Decarreau, A., 1985. Partitioning of divalent transition elements between octahedral
378 sheets of trioctahedral smectites and wáter. Geochimica et Cosmochimica Acta 49,
379 1537-1544.
380 Espejo-Antúnez, L., Cardero-Durán, M.A., Garrido-Ardila, E.M., Torres-Piles, S.,
381 Caro-Puértolas, B., 2013. Clinical effectiveness of mud pack therapy in knee
382 osteoarthritis. Rheumatology 52, 659-668.
383 Evcik, D., Kavuncu, V., Yeter, A., Yigit, I., 2007. The efficacy of balneotherapy and
384 mud-pack therapy in patients with knee osteoarthritis. Joint Bone Spine. 74, 60-65.
385 Fernández-González, M.V., Martín-García, J.M., Delgado, G., Párraga, J., Delgado, R.,
386 2013. A study of the chemical, mineralogical and physicochemical properties of
387 peloids prepared with two medicinal mineral waters from Lanjarón Spa (Granada,
388 Spain). Applied Clay Science 80-81, 107-116.
389 Fraioli, A., Serio, A., Mennuni, G., Ceccarelli, F., Petraccia, L., Fontana, M., Grassi,
390 M., Valesini, G., 2011. A study on the efficacy of treatment with mud packs and baths
391 with Sillene mineral water (Chianciano Spa Italy) in patients suffering from knee
392 osteoarthritis. Rheumatology International 31(10), 1333-1340.
393 Fürrer, G., Zysset, M., Schindler, P.W., 1993. Weathering kinetics of montmorillonite
394 investigations in batch and mixed-flow reactors. In: Manning, D.A.C, Hall, P.L. and
395 Hughes (Eds.), Geochemistry of Clay-Pore Fluid Interaction, vol. 13. Chapman and
396 Hall, pp. 243-262.
397 Gámiz, E., Martín-García J.M., Fernández-González, M.V., Delgado, G., Delgado, R.,
398 2009. Influence of water type and maturation time on the properties of kaolinite–
399 saponite peloids. Applied Clay Science 46, 117-123.

400 Gavini, E., Rassu, G., Muzzarelli, C., Cossu, M., Giunchedi, P., 2008. Spray-dried
401 microspheres based on methylpyrrolidinone chitosan as new carrier for nasal
402 administration of metoclopramide. European Journal of Pharmaceutics 68, 245-252.
403 Giacomino, M.I., De Michele, D.F., 2007. Is mud an anti-inflammatory? Annals of
404 Internal Medicine 24(7), 352-353.
405 Goluvev, S.V., Bauer, A., Pokrovsky, O.S., 2006. Effect of pH and organic ligands on
406 the kinetics of smectite dissolution at 25.8C. Geochimica et Cosmochimica Acta 70,
407 4436-4451.
408 Gomes, C. and Silva, J.B., 2007. Minerals and clay minerals in medical geology.
409 Applied Clay Science 36,4-21.
410 Gomes, C, Carretero, M.I., Pozo, M., Maraver, F., Cantista, P., Armijo, F., Legido, J.
411 L., Teixeirag, F., Rautureauh, M., Delgado, R., 2013. Peloids and pelotherapy:
412 Historical evolution, classification and glossary. Applied Clay Science 75-76, 28–38.
413 Grassi, M., Lucchetta, M.C., Rini, G.B., Raffa, S., 2003. Fangotherapy in chronic
414 degenerative rheumatic diseases. Clinica Terapeutica 154, 45-48.
415 Khiari, I., Mefteh, S., Sánchez-Espejo, R.,Cerezo, P.,Aguzzi, C.,López-Galindo, A.,
416 Jamoussi, F., Viseras, C., 2014. Study of traditional Tunisian medina clays used in
417 therapeutic and cosmetic mud-packs. Applied Clay Science 101, 141-148.
418 Lagaly, G., 2006. Colloid clay science. In: Bergaya, F., Theng, B.K.G., Lagaly, G.
419 (Eds.), Handbook of Clay Science, Developments in Clay Science, vol. 1., Elsevier,
420 Amsterdam, pp. 325-349.
421 Legido, J.L., Medina, C., Mourelle, M.L., Carretero, M.I., Pozo, M., 2007. Comparative
422 study of the cooling rates of bentonite, sepiolite and common clays for their use in
423 pelotherapy. Applied Clay Science 36, 148–160.
424 López-Galindo, A., Torres-Ruiz, J., González-López, J.M., 1996. Mineral quantification
425 in sepiolite-palygorskite deposits using X-ray diffraction and chemical data. Clay
426 Minerals 31, 217-224.
427 Martín-Ramos, J.D. (Ed.), 2004. Using XPowder©, a software package for powder X-
428 Ray diffraction analysis. D.L.GR-1001/04, Spain, 1-105.
429 Pozo, M., Carretero, M.I., Maraver, F., Pozo, E., Gómez, I., Armijo, F., Martín Rubí,
430 J.A., 2013. Composition and physico-chemical properties of peloids used in Spanish
431 spas: A comparative study. Applied Clay Science 83-84, 270-279.

Sánchez, C.J., Parras, J., Carretero, M.I., 2002. The effect of maturation upon the mineralogical and physicochemical properties of illite-smectite clays for pelotherapy. Clay Minerals 37, 457-464.

Sánchez-Espejo, R., Aguzzi, C., Cerezo, P., Salcedo, I., López-Galindo, A., Viseras, C., 2014. Folk pharmaceutical formulations in western Mediterranean: Identification and safety of clays used in pelotherapy. Journal of Ethnopharmacology 155, 810-814.

Schott, H., 1995. Reología. In: Gennaro, A.R. (Ed.), Remington Farmacia, vol. I., Medica Panamericana, Buenos Aires, pp. 426-455.

Sondi, I., Tomašic, V., Filipović-Vinceković, N., 2008. Release of silicon and aluminum from montmorillonite surfaces in aqueous systems. Croatica Chemica Acta 81, 623-629.

Sondi, I., Tomašic, V., Filipović-Vinceković, N., 2008. Release of silicon and aluminum from montmorillonite surfaces in aqueous systems. Croat. Chem. Acta 81, 623–629.

Suárez Muñoz, M., Melián Rodríguez, C., Gelen Rudnikas, A., Díaz Rizo, O., Martínez- Santos, M., Ruiz-Romera, E., Fagundo Castillo, J.R., Pérez-Gramatges, A., Martínez- Villegas, N.V., Blanco Padilla, D., Hernández Díaz, R., González-Hernández, P., 2015. Physicochemical characterization, elemental speciation and hydrogeochemical modeling of river and peloid sediments used for therapeutic uses. Appl. Clay Sci. 104, 36–47.

Tateo, F., Agnini, C., Carraro, A., Giannossi, M.L., Margiotta, S., Medici, L., Finizio, F.E., Summa, V., 2010. Short-term and long-term maturation of different clays for pelotherapy in an alkaline-sulphate mineral water (Rapolla, Italy). Applied Clay Science 50, 503-511.

Veniale, F., Barberis, E., Carcangiu, G., Morandi, N., Setti, M., Tamanini, M., Tessier, D., 2004. Formulation of muds for pelotherapy: effects of "maturation" by different mineral waters. Applied Clay Science 25, 135-148.

Veniale, F., Bettero, A., Jobstraibizer, P., Setti, M., 2007. Thermal muds: Perspectives of innovations. Applied Clay Science 36, 141-147.

Viseras, C., Aguzzi, C., Cerezo, P., Lopez-Galindo, A., 2007. Uses of clay minerals in semisolid health care and therapeutic products. Applied Clay Science 36, 37-50.

Wieland, E. and Stumm, W., 1992. Dissolution kinetics of kaolinite in acidic aqueous solutions at 25ºC. Geochimica et Cosmochimica Acta 56, 3339-3355.

Table 1. Major-element content (w/w %) of the studied samples.

	I[a]	I_0	I_1	I_2	I_3	II[a]	II_0	II_1	II_2	II_3
SiO_2	48.64	48.46	47.65	47.86	48.16	45.83	47.45	48.84	48.78	49.80
Al_2O_3	17.23	17.74	17.55	17.56	17.42	17.23	18.94	19.66	19.75	19.93
Fe_2O_3	6.52	5.98	6.26	6.11	6.32	6.67	8.11	7.97	7.76	8.10
MnO	0.18	0.18	0.18	0.19	0.20	0.03	0.02	0.02	0.02	0.02
MgO	2.35	2.10	2.27	2.28	2.29	2.03	1.68	1.74	1.73	1.72
CaO	7.97	7.27	7.82	7.71	8.01	6.32	1.66	1.63	1.55	1.71
Na_2O	1.26	0.97	1.32	1.32	1.29	3.14	2.48	1.91	2.02	2.06
K_2O	2.46	2.11	2.41	2.37	2.41	1.72	1.85	1.84	1.83	1.85
TiO_2	0.76	0.69	0.73	0.72	0.74	0.96	1.00	1.00	0.97	1.04
P_2O_5	0.15	0.11	0.15	0.14	0.14	0.29	0.22	0.23	0.22	0.23
SO_3	0.57	0.76	0.77	0.74	0.84	5.77	1.72	1.72	1.62	1.73
Cl	0.04	0.03	0.03	0.03	0.03	1.65	1.20	0.71	0.75	0.82
LOI	10.90	13.20	12.70	12.80	12.00	8.56	13.80	12.70	13.10	11.10

LOI (loss on ignition)

[a]Taken from Sánchez-Espejo et al., 2014.

Table 2. Water content (w/w %) and pH (25 ºC) of the samples (mean values ± s.d.; n = 3).

	Water content (w/w %)	pH (25 ºC)
I_0	67.95 ± 1.813	8.04 ± 0.047
I_1	65.65 ± 0.191	7.83 ± 0.039
I_2	65.55 ± 0.233	7.92 ± 0.019
I_3	65.36 ± 0.536	8.09 ± 0.036
II_0	64.31 ± 0.108	7.45 ± 0.020
II_1	66.45 ± 0.306	7.27 ± 0.008
II_2	66.11 ± 0.786	7.37 ± 0.024
II_3	66.45 ± 0.306	7.43 ± 0.014

Table 3. Statistical diameters and SPAN factor of the samples (mean values ± s.d.; n=3).

	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	SPAN factor
I_0	1.32 ± 0.017	4.71 ± 0.093	18.45 ± 0.532	3.63
I_1	1.31 ± 0.029	4.66 ± 0.098	16.84 ± 1.499	3.34
I_2	1.33 ± 0.019	4.41 ± 0.092	15.89 ± 0.422	3.31
I_3	1.40 ± 0.021	4.65 ± 0.081	16.63 ± 1.170	3.27
II_0	1.24 ± 0.122	3.66 ± 0.198	9.16 ± 0.609	2.16
II_1	1.25 ± 0.044	3.52 ± 0.158	8.53 ± 0.384	2.07
II_2	1.31 ± 0.051	3.76 ± 0.215	8.82 ± 0.744	2.00
II_3	1.39 ± 0.083	3.84 ± 0.288	8.73 ± 0.561	2.01

Table 4. Apparent viscosities (200 s^{-1}, 25ºC) and yield values of the samples (mean values ± s.d.; n=6).

	Viscosity (Pa.s)	Yield value (Pa)
I_0	0.29 ± 0.005	53.89 ± 0.586
I_1	0.38 ± 0.006	71.88 ± 1.665
I_2	0.42 ± 0.006	82.10 ± 0.382
I_3	0.57 ± 0.017	108.89 ± 0.311
II_0	0.55 ± 0.013	96.19 ± 0.598
II_1	0.73 ± 0.027	137.1 ± 1.435

II_2	0.78 ± 0.021	143.61 ± 0.905
II_3	0.96 ± 0.012	181.05 ± 1.075

Table 5. Thermal parameters of the studied samples (mean values ± s.d.; n=3).

	C_p (J/g K)	t_{32^oC} (min)	$T_{20\ min}$ (ºC)
I_0	3.21 ± 0.051	30.95 ± 0.056	35.9 ± 0.025
I_1	3.07 ± 0.139	29.16 ± 0.130	35.4 ± 0.038
I_2	3.09 ± 0.307	29.46 ± 0.157	35.5 ± 0.042
I_3	3.08 ± 0.059	29.61 ± 1.333	35.4 ± 0.198
II_0	2.98 ± 0.004	29.53 ± 0.178	35.5 ± 0.051
II_1	3.24 ± 0.233	30.14 ± 0.024	35.7 ± 0.003
II_2	3.15 ± 0.089	28.45 ± 1.561	35.2 ± 0.490
II_3	3.32 ± 0.397	30.80 ± 0.221	35.9 ± 0.040

Table 6. Cation exchange capacity and amounts of individual cations (meq/100g) (mean values ± s.d.; n=3).

	I_0	I_1	I_2	I_3
Na	22.11 ± 0.100	21.31 ± 2.038	20.46 ± 0.631	19.65 ± 0.038
K	1.96 ± 0.160	2.06 ± 0.186	1.92 ± 0.107	1.97 ± 0.005
Mg	3.12 ± 0.017	2.98 ± 0.249	2.89 ± 0.095	2.71 ± 0.004
Ca	8.62 ± 0.068	7.81 ± 0.694	7.29 ± 0.333	7.42 ± 0.228
CEC	35.80 ± 0.345	34.16 ± 3.167	32.56 ± 1.165	31.74 ± 0.257

	II_0	II_1	II_2	II_3
Na	44.45 ± 0.503	48.05 ± 0.355	48.21 ± 0.315	48.14 ± 0.178
K	4.04 ± 0.255	4.35 ± 0.466	4.58 ± 0.381	4.22 ± 0.728
Mg	8.46 ± 0.305	9.19 ± 0.691	9.09 ± 0.573	8.13 ± 1.152
Ca	22.23 ± 1.147	23.39 ± 1.941	23.48 ± 1.808	22.79 ± 3.749
CEC	79.17 ± 2.211	84.99 ± 3.453	85.37 ± 3.078	83.28 ± 5.808

Table 7. Amount of cations released (meq/100g) from the therapeutic muds (mean values ± s.d.; n = 3).

	I_0	I_1	I_2	I_3
Na	0.00 ± 0.000	0.10 ± 0.111	12.76 ± 1.577	8.10 ± 1.058
K	0.59 ± 0.102	0.75 ± 0.254	1.47 ± 0.518	1.88 ± 0.294
Mg	0.53 ± 0.140	1.02 ± 0.007	0.72 ± 0.175	0.90 ± 0.080
Ca	1.11 ± 0.330	2.35 ± 0.083	2.21 ± 0.422	2.34 ± 0.102

	II_0	II_1	II_2	II_3
Na	0.06 ± 0.007	0.10 ± 0.008	21.62 ± 0.730	14.26 ± 4.603
K	1.14 ± 0.080	1.13 ± 0.066	1.87 ± 0.078	2.35 ± 1.027
Mg	1.94 ± 0.129	2.37 ± 0.242	2.95 ± 0.170	2.82 ± 0.990
Ca	3.93 ± 0.211	4.67 ± 0.398	6.07 ± 0.605	5.97 ± 1.573

Figures

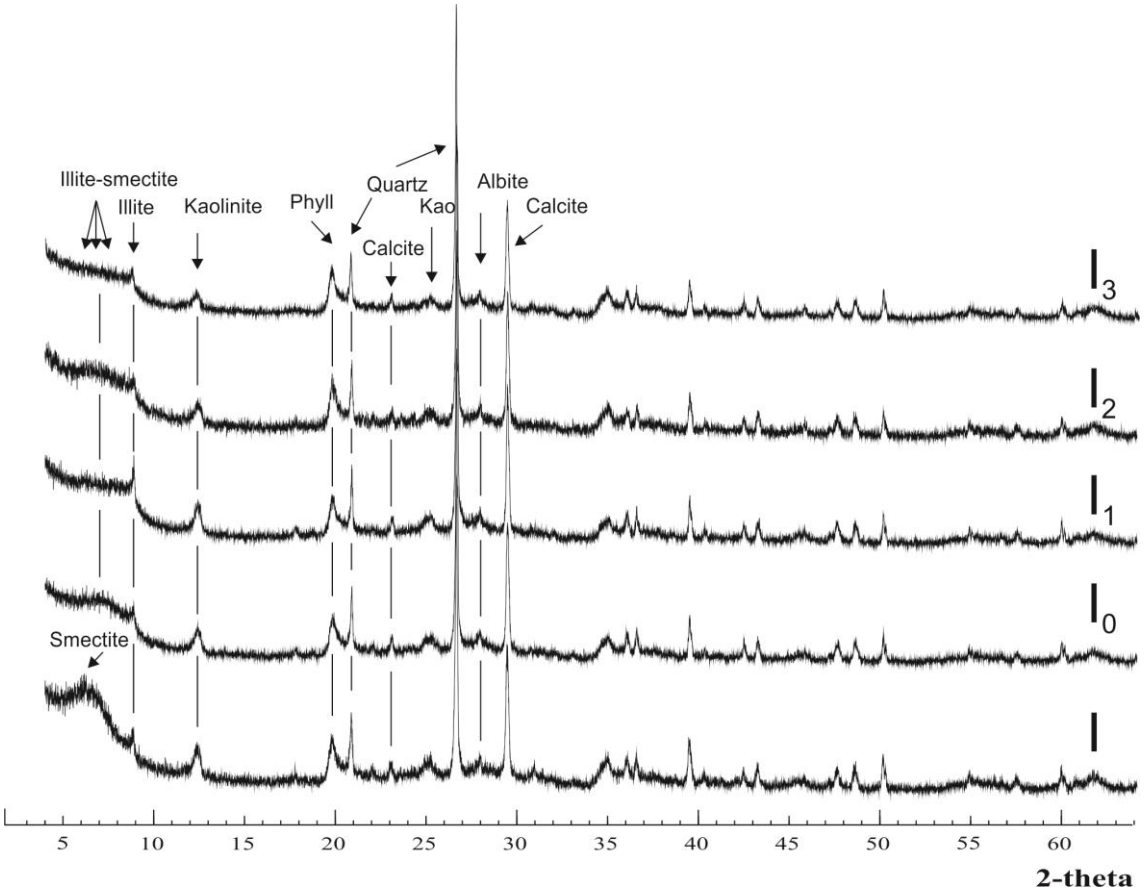

Figure 1. XRD patterns of clay I and its corresponding muds.

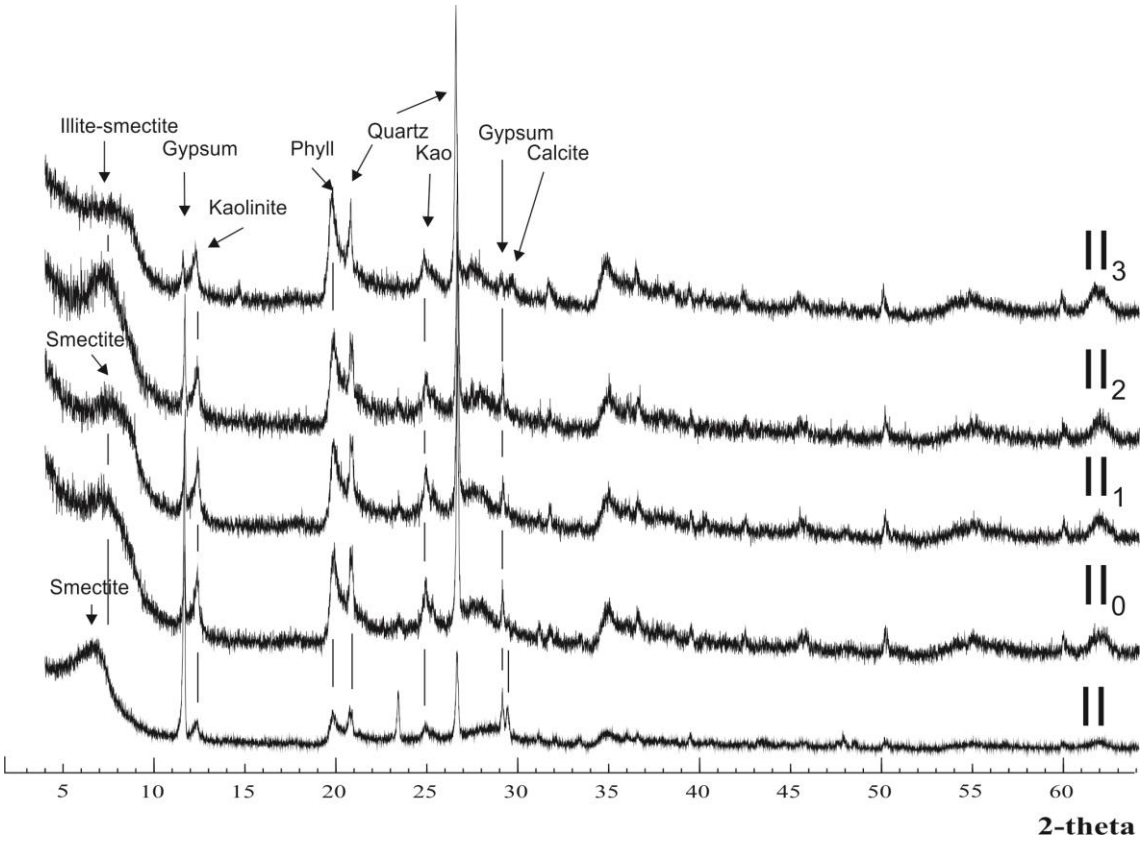

Figure 2. XRD patterns of clay II and its corresponding muds.

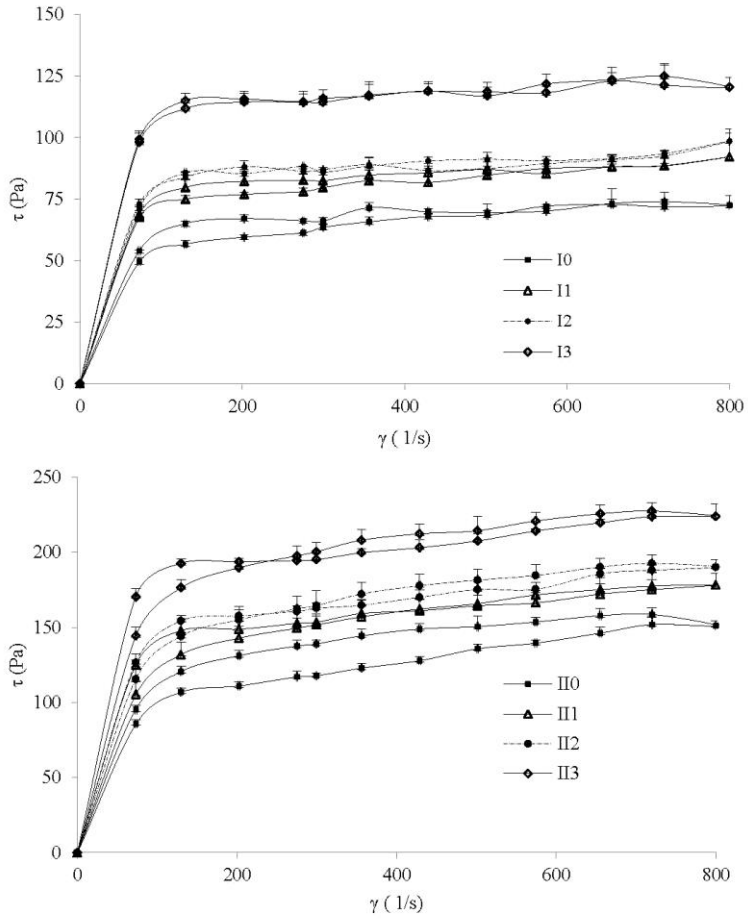

Figure 3. Flow curves of the studied samples. Up (muds prepared with clay I) and down (muds prepared with clay II).