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Peloids prepared with three mineral-medicinal waters from spas in Granada. Their suitability for use in pelotherapy

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Suggested Reviewers:	the same1 the same1 the same1 thesame@thesame.es
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Response to Reviewers:	COMMENTS FROM EDITOR AND/OR REVIEWER Editor's comments: I have to thank you for the modification of the manuscript. It has been reviewed by a new reviewer and you will find the comments below. Additionally I have annotated a copy of the manuscript with 82 comments (please, use the appropriate Acrobat version to see them all), most of them minor or asking for clarification: For the general reader, please, indicate in the Introduction what a peloid is and how it is prepared. Answer: Accepted. We have modified the text following this recommendation. Lines 36-42. In the Materials section the solid:liquid ratio used to prepare the peloids has to be indicated. Also include the mineralogical characterisation of the materials used and give the reasons to use this particular kaolin/bentonite ratio. If these are published data include also a reference, but this is basic information that has to be specified in the Materials section. Answer: Accepted the suggestion. We have added in the text the mineralogical characterization of the materials and the reference of the study of these peloids (line 76-78). These raw materials are pharmaceutical and cosmetic minerals of relatively high purity (kaolin: 87% kaolinite; bentonite: 94% saponite) and they have been studied previously (Gámiz et al., 2009). Both kaolin and bentonite are the most widely used clays in spas worldwide (see Carretero, 2020). This kaolinite-esmectita mixture is empirically tested by us in various papers (Gámiz et al., 2009; Fernández-González et al., 2013, 2017): it is a solid manageable phase, has good adsorption properties, etc. In addition, it is the same mixture that we have used in others manuscripts (Fernández-González et al., 2013, 2017), to be able to compare their properties. Always using the same solid phase, prepared peloids with different liquid phases can be compared to each other. Many of the cosmetic masks for widespread use have high proportions of kaolinite (Viseras et al., 2007) and we have improved the quality of the material by introducing smectite. The methodology should be overall better detailed, including preparation of samples to apply the different techniques and number of tests performed for each determination. Answer: Accepted according to editor. We have explained how the water-clay mixture was matured (lines 78-80) and the analytical method for exchangeable cations (lines 92-93). We have performed a minimum of three repetitions on each analytical determination, but it is not usual to collect this accuracy in the text I do not understand why the authors prefer to insist on the results obtained by laser granulometry instead of those obtained by SS, which are more reliable. I agree with reviewer #1 that the laser part could have been removed. Answer: We think that laser granulometry should be included in the manuscript because it perfects it. First to compare our results with those of other papers that use SS and LD. In addition, using the two methods of granulometric determination allows to identify which is the most appropriate, when comparing the granulometric results by SS and LD with those obtained with electron microscopy and the data derived from the image analysis. This result may be useful for other researchers. The conclusive thing is that the laser method is the most used by other authors to determine the granulometry of the peloids (Pozo et al., 2019 and 2018; Armijo et al., 2016; Armijo and Maraver, 2019). In any case, it is necessary to include the granulometry of the material before maturation so that to assess the effect of maturation. Answer: The granulometry of initial mineral sample is shown in Table 2: 9,4% sand, 43,8% fine silt and 46,8% clay (determined by sieving-sedimentation, SS) and that of the matured peloid is in Table 2. Past tense should be used when reporting your investigation or that of others, this is a journal guideline. Another journal guideline is that personal expressions (we, our) should not be used. Since I have annotated the manuscript correcting typos, I have also added corrections in this sense. Reviewer #7: Here are some comments about this paper that could improve the scientific content of the work. Line 83: Description of the analytical method for exchangeable cations. Answer: Accepted according to ref. 7 (lines 92-93)

	Line 115: To have a real understanding of the processes that have taken place during the three months of maturation you should have a reference target. Answer. The processes that have taken place during the three months of maturation are explained in the text: the ionic change (lines 22, 24, 139, 249), the decrease in particle size (line 159, 172) and the dispersion process of particles in peloids fabric (lines 207, 210). Blanks are obtained by subjecting the peloid to the same treatment but with distilled water, so that the dissolution processes that take place between the solid material and the water are detected. These results should be considered when interpreting the data of the interstitial water of the peloids after maturation with MMW. Answer: Respect to blanks, we do not understand the question, because in the Technical Note there are no mixtures of the peloid with distilled water as the Reviewer refers to. Line 116: Classification of waters: A0: Ca-Mg-SO4 type water A3: Na-SO4 type water Z0: Na-SO4-Cl type water Z3: Na-SO4-Cl type water G0: Ca-SO4 type water G3: Na-SO4 type water A3 and G3 have changed the classification as the initial MMW but Z3 maintain the same classification. Answer: Revised according to ref. 7. Lines 129-130. In all waters after three months of maturation the concentrations Ca and Mg decrease and Na concentrations increase. The largest increase is observed in G3. Answer: Revised according to ref. 7. Lines 133-134. Line 121: Water from Zújar have the highest total dissolved solids (TDS) of all the waters (3726 mg/L in Z0 and 4172 mg/L in Z3), but the largest increase between initial waters and peloids waters is in G3 water (TDS of 2108 mg/L in G0 to 3222 mg/L in G3). Answer: Revised according to ref. 7. Lines 133-134.

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Peloids prepared with three mineral-medicinal waters from spas in Granada. Their suitability for use in pelotherapy

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Abstract

Three peloids were studied, prepared with three mineral-medicinal water (MMW) from the spas at Alicún (sulphate, rich in magnesium and calcium, highly mineralized), Zújar (sulphate, rich in sodium chloride, highly mineralized) and Graena (sulphate, rich in magnesium and calcium, highly mineralized) (province of Granada, Spain) and matured for three months, in order to determine their properties and suitability for use in pelotherapy. Their solid phase ~~were~~ was prepared by mixing kaolin and bentonite (9:1, w:w). In the peloids the following ~~were~~ was studied: composition of interstitial liquid, granulometry, physicochemical properties (specific surface area -SSA-, cation exchange capacity -CEC- and exchangable bases), crystallinity index of the minerals, thermal behaviour and ultramicroscopic fabric using scanning electron microscopy (SEM) and image analysis (IA). A modification of the ionic concentration of the interstitial liquid was observed with regard to the initial MMW, namely, an increase in the concentration of Na^+ and K^+, and a decrease in Ca^{2+} and Mg^{2+}, due to ~~a cationic~~ exchange between the exchangable cations of the solid phase and the ions of the MMW. Increases in the proportion of fraction <2 µm, SSA and CEC were also observed. The crystallinity index

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Key words

Peloids, mineral-medicinal waters, kaolin, bentonite, maturation, SEM-fabric, Spanish spa

1. Introduction

The use of pelotherapy in spas is becoming increasingly popular. Nonetheless, few spas employ peloids. A peloid is a maturated mud or muddy dispersion with healing and/or cosmetic properties, composed of a complex mixture of fine-grained natural materials of geological and/or biological origins, mineral water or sea water, and commonly organic compounds from biological metabolic activity" (Gomes et al., 2013). Peloids require a certain period of contact between the liquid and solid phases, known as "maturation", during which the components mix, interact and biological activity may occur. Currently there are only four Spanish spas that use peloids therapeutically: El Raposo, Arnedillo, Caldas de Boi and Archena. From these spas, mineralogical and chemical composition, and ~~others~~ other properties of the peloids ~~has~~ have been studied (Carretero et al., 2010; Pozo et al., 2013), or in El Raposo spa, the effectiveness of the pelotherapy has been studied (Gálvez et al., 2019). New peloids need to be produced to ~~counter~~ counteract the lack of these in spas, and, consequently, the study and evaluation of the quality of the raw materials suitable for their production is also required (Pozo et al., 2019; amongst others). Studies are usually carried out by mixing the raw materials (clays) with mineral-medicinal waters (MMW) or seawater, varying the maturation time

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and studying the suitability of each peloid for use in pelotherapy (Veniale et al., 2004; Carretero et al., 2007; Gámiz et al., 2009; Fernández-González et al., 2017; among others). Some of these authors (Veniale et al., 2004; Fernández-González et al., 2017) ~~report~~ reported that modifications in the clays used for peloid preparation, with different maturation times, ~~vary~~ varied according to the MMW used. For a complete review see Carretero (2020a, 2020b).

The aim of this study ~~is~~ was to evaluate the properties of the peloids prepared with three MMW from three spas (Alicún, Zújar and Graena) in the province of Granada (Spain) with very different saline concentration and the same solid phase and maturation time with regard to their potential use in pelotherapy. This evaluation ~~will be~~ was based on the modification of mineral crystallinity, chemical, physical and physicochemical properties of the peloids matured for three months. Previously, studies of peloids made with other MMW from spas in Granada were carried out (Gámiz et al., 2009; Fernández-González et al., 2013 and 2017).

The waters selected have historically been in use from 2000 years ~~ago~~. However, these waters have never been studied as the liquid phase for peloid preparation with the exception of those from Graena, studied by ~~some authors (~~Sánchez-Espejo et al.~~,~~ (2015).

The results of this research will serve as a basis for the spas whose waters are used in this work, to make their own peloids, and to implement the technique of pelotherapy in their establishments.

2. Materials and methods.

~~The~~ MMW of Alicún (A0), Zújar (Z0) and Graena (G0) were sampled. The following parameters were *in situ* measured: temperature (with a digital thermometer), pH and electrical conductivity (potentiometric method).

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The solid phase of the peloid (sample P) was prepared by mixing kaolin and bentonite in the ratio 9:1 (w:w) and were mixed with the MMW in the ratio 2:1 (liquid:solid, w:w): 1000 ml of liquid phase and 500 g of solid phase. These raw materials are pharmaceutical and cosmetic minerals of relatively high purity (kaolin: 87% kaolinite; bentonite: 94% saponite) and they have been studied previously (Gámiz et al., 2009). All samples were kept at a temperature of around 20°C. The water-clay mixture was matured during three months by periodically stirring the peloid mass while humidity control was achieved by weighing the containers in order to maintain their initial humidity conditions (Fernández-González et al., 2013, 2017); after ~~which~~ this time, the peloid samples (samples P_{A3}, P_{Z3}, P_{G3}) were obtained for determination of the fabric and cooling kinetics. The interstitial liquid (Liq_{int}) (samples A3, Z3, G3) was extracted by suction (approx. 100 kPa) and separated from the peloids while the remaining solid part (samples A3p, Z3p, G3p) was employed for the study of granulometry, mineral crystallinity and physicochemical properties.

The chemical and physicochemical properties of MMW (A0, Z0, G0) and Liq_{int} (A3, Z3, G3) ~~was carried out~~ were analyzed (Fernández-González et al., 2013).

To estimate the granulometry, two methods ~~have been~~ were used: sieving-sedimentation (SS granulometry) (Soil Survey Staff, 2014) and laser (LD granulometry) (Fernández-González et al., 2017).

The specific surface area (SSA) was determined using ethylene glycol monomethyl ether (EGME) (Carter et al., 1986); also, cation exchange capacity (CEC) and the exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) were determined by atomic absorption (MAPA, 1994).

Crystallinity was determined in the kaolinite and saponite of the initial sample and in the solid phases of the peloids using X-ray diffraction (XRD) diagrams. The Hinckley

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index (HI) (Hinckley, 1963) of the kaolinite was determined in disoriented powder diagrams, and the crystallinity of the saponite (Integral Breadth, IB) (Ehrmann et al., 2005) was determined in diagrams of clay samples solvated with ethylene glycol.

To study the peloid fabric, the methodology described by Fernández-González et al. (2017) was followed. The samples ~~has been~~ were studied using scanning electron microscopy (SEM) (Hitachi S-510) and an energy-dispersive X-ray (EDX) spectrometer. The microphotographs were analyzed with the IMAGE J software (National Institute of Health, 2008).

The cooling kinetics was studied following Ferrand and Yvon (1991) and Cara et al. (2000). Using data of temperature with time-cooling ~~we can calculate~~ ΔT was calculated: the accumulated decreases in temperature (ΔT=T0−Tn, being T0=65 °C). ΔT and time fit a logarithmic function (y=aLn(x)+b; y=ΔT, x=time) with, in all cases, R^2>0.9. The time necessary for the decrease of 22.5 °C ($\Delta t_{-22.5\ ^\circ C}$; 22.5 being 75% of the total decrease in temperature in the experiment) (Gámiz et al., 2009) was calculated for each peloid.

3. Results and discussion.

3.1. Analysis of the waters (MMW and Liq_{int})

The values of dry residue obtained for the samples of MMW (Table 1), were greater than 2 g ~~l~~ L^{-1}, demonstrating that these are highly mineralized waters, corroborated by electrical conductivity values (>3 mS cm^{-1}). The temperature of the MMW during sampling was 35.0, 39.0 and 41.7 ºC for A0, Z0 and G0, respectively and in agreement with Maraver and Armijo (2010) measures. Thus, these MMW could be classified (in balneological terms) as “mesothermal”, A0, and “hyperthermal”, Z0 and G0. The pH values obtained in all the samples (MMW and Liq_{int}) were very similar, ranging around 7.5- 8. These values ~~are~~ were related to the presence of bicarbonates in

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the three MMW, probably resulting from the geological origin of these waters, which emerge in areas rich in limestones and dolomites. According to the chemical composition of the MMW, Alicún and Graena ~~was~~ were classified as calcium- and magnesium-rich sulphate waters, and Zújar was sodium chloride-rich sulphate water, in agreement with Maraver and Armijo (2010).

After three months' maturation, Na^+ concentration ~~had~~ increased (in Liq_{int}), particularly in the peloids from Alicun and Graena (the largest increase is observed in G3). In fact, A3 ~~is~~ was classified as ~~calcium-, magnesium- and~~ sodium-rich sulphate waters, differently from A0. The same occurred in the Z3 and G3, ~~although maintaining the same classification as the initial MMW,~~ which are classified as sodium chloride-rich sulphate waters and sodium rich sulphate waters, respectively. In all cases, the concentration of calcium and magnesium decreased in the Liq_{int} (in some samples, almost threefold after maturation: i.e., for magnesium, from 150 mg l^{-1} in A0 to 60 mg l^{-1} in A3). In addition, there was a higher ionic concentration in Liq_{int} of Graena than in that of Zújar (whose MMW ~~has~~ had the driest residue) ~~than in that of Graena~~, resulting in the peloids prepared with MMW from Alicún (with the lowest residue) presented the lowest ionic content. On the other hand, the concentration of SO_4^{2-}, Cl^-, CO_3^{2-} present in the Liq_{int} ~~increase~~ increased in relation to that of the MMW. Thus, the decrease observed in the ions Ca^{2+} and Mg^{2+} in Liq_{int} must be related to the retention of these ions in the clay used for the solid phase, resulting in cationic exchange of K^+ and Na^+ from the solid phase with Ca^{2+} and Mg^{2+}. This is supported by the observed increase in Ca^{2+} and Mg^{2+} and decrease in Na^+ and K^+, on determining the exchangeable cations in the solid phase of the peloids (see Table 3 and section 3.3). All these evidences ~~are indicating~~ indicate that during maturation, there ~~is~~ was an exchange of cations from the mineral phase to the liquid phase of the peloids and vice-versa. Changes in the

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Commented [P38]: Revised according to Ref. 7. Samples Z0 and G0, are also calcium and magnesium rich waters.

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composition of the interstitial liquid of peloids, compared to MMW, were registered by some authors (Fernández-González et al., 2013; Spilioti et al., 2017; Yücesoy et al., 2019). There ~~are~~ were differences detected in the ionic concentration of the interstitial liquid with respect to the initial MMW, so that therapeutic applications of the peloids ~~will~~ would not have exactly the same effect as the initial MMW have.

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3.2. Granulometry

Granulometry´s results (for LD or SS) showed great homogeneity. In LD (Figure 1), the peloids consist of fine silt (>50%), clay fraction not even attaining 20%. In SS, fine silt reach around 40% and clay fraction is 53%. The difference of results between the SS and LD is attributed to that they are different techniques. Taubner et al. (2009) have developed equations to transform data between the two methods. When applied to our case, these equations provided coherent results, particularly for the clay fraction. E.g., the clay content measured with LD in A3p is 18,1% (Table 2) turns into 53,01% by using Taubner et al. (2009) equations, a value very similar to 53,8% (SS-measured).

The results SS show that a small increase in the fraction <2 µm occurs during maturation, when compared to sample P. The decrease in particle size has also been reported by other authors (da Silva et al., 2015).

3.3. Physicochemical properties

The external SSA of the peloids (Table 3) increased with respect to sample P, most evident in Z3p. It may be due both to the composition of the MMW (sodium acts as a dispersing cation) (Table 1) and to the peloid fabric (~~greater~~ higher porosity) that implies greater surface area (Figures 2, 3 and 4) (Table 4). A similar tendency was observed for total SSA, which presents values higher than external SSA, sometimes duplicating them; this may be due to the method used to determine total SSA, involving the use of ethylene glycol monoethyl ether (EGME), a polar molecule.

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The CEC of the peloids ranged from 10.28 $cmol_+kg^{-1}$ to 11.12 $cmol_+kg^{-1}$, and increased slightly in all the matured peloids compared to sample P. This is due to the increase in the fraction <2 μm and SSA.

For the exchangeable cations, the sequence of quantities in the sample P was Na>>K~Mg>Ca. In the peloids, this sequence was substantially modified: Ca>Mg>Na>K. These modifications are important, as they affect to the ionic concentration of the interstitial liquid. The exchangeable cations can potencially be transferred to human sweat (Carretero et al., 2010) and then to the skin.

3.4. Mineral crystallinity

The crystallinity index of the kaolinite (HI) in the peloids ranged between 0.55 and 0.63 (Table 3), the range of medium values, according to Hinckley (1963). HI decreased in the three peloids (Table 3) when compared to sample P. The increase in defects in the kaolinite structure during maturation is due to the larger particles in vermicular stacks or pseudohexagonal crystals, with high cristallinity (Delgado et al., 1994), when preparing and maturing the peloids, they break down into individual smaller particles with more defects (Gámiz et al., 2009; Fernández-González et al., 2017). SEM study detected kaolinite stacks in sample P but not in the peloids, which were always composed of laminar particles (Figures 2, 3 and 4).

The Integral Breath (IB) values of the smectite (saponite) were around 1.18; according to Ehrmann et al. (2005), these would be considered "well crystalline". The crystallinity was slightly higher in the peloids than in the original mineral. This may be due to the potassium, which enters the interlaminar zone of the saponite and is sequestered, thereby stabilizing the structure and increasing the perfection of crystallinity, making the saponite closer to mica (Velde and Barré, 2010). This hypothesis is supported by the considerable decrease in the concentration of

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exchangeable potassium in the peloids, compared to the initial sample. Sánchez et al. (2002), in contrast to the present study, reported a decrease in the crystallinity of saponites during maturation. However, in this case the composition of the MMW (iron-rich, bicarbonate and sulphate) was different to our MMW. Carretero et al. (2007), using seawater for the maturation, also reported a decrease in the crystallinity of the smectites with maturation time.

3.5. Fabric

The SEM fabric of the three peloids revealed (Figures 2, 3, 4) to be ordered hierarchically by size in primary particles of similar size (around 1.10 µm), which create clusters of varying size (5 µm - P_{G3}, 10 µm- P_{A3}, 15 µm- P_{Z3}) (Table 4). There were also large laminar particles with generally joints face-face with some face-edge. Porosity was significant in all three samples, although there were some differences with regard to the MMW employed ($P_{Z3} >> P_{G3} > P_{A3}$). Thus, the peloid prepared with MMW from Zújar showed the most porous fabric. The disperse and reticulated appearance of P_{A3} fabric (Figure 2a) ~~is~~ was more evident in P_{G3} (Figure 4a), where there ~~has been~~ was a dispersion process (individualization of particles). In case of P_{Z3} (Figure 3c), small "clusters" with a tactoid-like appearance were also observed, forming an open fabric, also porous and reticulated which even classified as "house of cards/honeycomb".

The EDX microanalysis (Figures 2c, 3c and 4c) ~~corroborate~~ corroborated the kaolinitic nature, with peaks of silica and aluminum of similar heights. There were also peaks of magnesium, iron and a small one of potassium, which can be attributed to the disperse smectite between the kaolinite laminae (particularly notable in P_{G3}). In no case was a microanalysis clearly attributable to saponite detected.

Possible relationships between the IA fabric parameters (Table 4) and the properties of the peloids were also considered. The granulometry (Table 2), crystallinity

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index of the smectite and the exchangeable cations (principally Na^+) (Table 3) ~~are~~ were clearly related to the fabric parameters. Thus, the Feret diameter of the pores ~~is~~ was positively related to the percentage of fine silt measured by LD (r = 0.998; P<0.05). The mean area of the pores ~~is~~ was negatively and strongly correlated with the IB (r = -0.997; P<0.05). The sodium of the exchange complex ($cmol_+kg^{-1}$) is negatively related with the size of the primary particles (r = 0.999; P<0.01) so that the higher the concentration of sodium the smaller the primary particles (they disperse). The fabric is thus highly dependent on, and informative of, the properties of the solid phase and the concentration and nature of the cations present in the MMW. This fabric of peloids is of great interest and, as yet, relatively undeveloped.

3.6 Cooling kinetics.

The descending sequence of cooling times $\Delta t_{-22,5ºC}$ (in minutes) (Figure 5) would be from slowest to fastest: P_{G3} (12.32) > P_{A3} (11.75) > P_{Z3} (11.37). The difference was only one minute and ~~are~~ were therefore thermally similar, as they ~~have~~ had a similar water content, particle size and mineral composition of the initial clay (Ferrand and Yvon, 1991; Armijo et al., 2016). Millero (2001) reported that salinity inversely affected the thermal conductivity of water so that less saline waters conducted heat better and cooled more rapidly. In the present study, albeit with small differences, the quantities of solid residue in the Liq_{int} followed the sequence Z3 > G3 > A3 and the expected cooling sequence would be P_{A3}< P_{G3}< P_{Z3}, the opposite of what actually occurs. Therefore, the characteristics of water do not fully explain the behavior of these peloids. In this way, a correlation was observed between the time taken $\Delta t_{-22,5ºC}$ (min) and Feret diameter of the pores (r = -0.997; P<0.05), and also the Feret diameter of the clusters. Consequently, a fabric with smaller clusters will cool more slowly ($\Delta t_{-22,5ºC}$ higher) according to Gámiz et al. (2009).

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4. Final considerations on the potential applications of peloids.

~~As we have found~~ The differences between MMW and Liq_{int}, ~~this assumes~~ mean that the peloid will act not exactly like MMW at the skin level. This fact ~~has already~~ was detected by Fernández-González et al. (2013). The differences ~~are~~ were due to some ions of MMW ~~are~~ retained by the clay during maturation (mainly calcium and magnesium) while others ~~are~~ were released to the liquid phase (through ionic exchange processes). The pH values of the Liq_{int} (around pH = 8, Table 1) confer a slight alkalinity on the peloids, which could be used to treat a variety of skin conditions (Tateo et al., 2010). Topical application of these peloids would thus produce a change in the chemical reactions on the skin, on changing the acid equilibrium of this tissue (Takigawa et al., 2005).

The granulometry of ~~our~~ the peloids (silty-clayey) coincides with that found by other authors for peloids for use in spas (Karakaya et al., 2010; Pozo et al., 2013). With regard to their suitability, this granulometry renders them suitable for use in spas (Carretero et al., 2010, 2014).

The values of both external and total SSA were relatively high (50 and 100 m^2 per gram, respectively) rendering this material suitable for the adsorption and release of active principles, skin cleansing, etc. A high SSA results in a greater capacity for absorption and adsorption (Carretero et al., 2014). In terms of CEC, according to Matike et al. (2011), the peloids in the present study (with values of CEC <15 $cmol_+kg^{-1}$) would behave as ion sources.

In all the samples, the crystalline perfection of the kaolinite (HI) decreased with maturation, although some small differences in HI were observed, depending on the MMW employed. This phenomenon could improve the ability to delay the release of

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drugs adsorbed to the peloid (Delgado et al., 1994), rendering ~~our~~ the peloids ~~being~~ suitable for therapeutic application.

The degree of cooling of the three peloids ~~is~~ was similar. The values of $\Delta t_{22,5^{\circ}C}$ ~~are~~ were within the range of peloids prepared with other MMW from spas in Granada (Gámiz et al., 2009; Fernández-González et al., 2017).

The type of dispersed-, porous- and reticulated-fabric observed in the peloids are considered of interest for pelotherapy. Even P_{Z3} is "house of cards/honeycomb", the best of the three. According to Vali and Bachmann (1988), the fabric of the colloidal dispersions of clay is related to the rheological properties relevant to peloid application. Fabrics such as "house of cards/honeycomb" or reticulated increase the viscosity or elasticity of the mud, both of which are favourable properties for handling and applying to the skin. The mechanisms for the release and adsorption of ions and polar molecules are also affected by the microstructure.

~~We can thus conclude~~ It can be concluded that the three peloids studied have similar properties, which make them potentially suitable for use in pelotherapy. Thus, the MMW from the Alicún, Zújar and Graena spas are a good raw material for the preparation of peloids.

Dedication

This study is dedicated to the memory of the eminent professor and researcher Emilio Galán, our friend, who dedicated his life and work to the study of minerals and their applications.

Acknowledgements

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408

Figure captions

Figure 1 Laser granulometry of peloid samples A3p, Z3p and G3p.

Figure 2 SEM-EDX and IA of sample P_{A3}. a) Image; b) Binary pore mask of a); c) Image, detail of a); d) Pore mask of c)

Figure 3 SEM-EDX and IA of the sample P_{Z3}. a) Image; b) Binary pore mask of a); c) Image, detail; d) Pore mask of c)

Figure 4 SEM-EDX and IA of the sample P_{G3}. a) Image; b) Pore mask of a); c) Image, detail; d) Pore mask of c)

Figure 5 Cooling kinetics of peloids P_{A3}, P_{Z3} and P_{G3}. Variation of temperature, ΔT, with time ($\Delta T = T_0 - T_n$; $T_0 = 65$ ºC).

421 Table 1 Parameters of the mineral-medicinal waters (MMW: A0, Z0, G0) and the liquid phases of peloids (Liq_{int}: A3, Z3, G3).

	Samples					
	A0[a]	A3	Z0	Z3	G0[b]	G3
Electrical conductivity (20 ºC) (mS cm^{-1})	3.02 [c]	2.86	6.72 [c]	5.75	3.35[c]	3.20
pH	7.98 [c]	7.87	7.42[c]	8.18	7.68[c]	7.88
Temperature (ºC)	35.0 [c]	-	39.0 [c]	-	41.7 [c]	-
Solid residue (110 ºC) (g L^{-1})	2.05	2.34	3.85	4.40	2.47	2.91
Chloride (mg L^{-1})	81.14	113.30	804.66	994.60	9.35	53.25
Sulphate (mg L^{-1})	1296.10	1303.70	1581.65	1603.20	1423.45	2084.10
Carbonate (mg L^{-1})	0.00	150.00	0.00	120.00	0.00	60.00
Bicarbonate (mg L^{-1})	242.40	122.00	203.60	91.50	120.00	113.02
Calcium (mg L^{-1})	335.00	107.00	321.00	170.00	417.45	134.75
Magnesium (mg L^{-1})	150.00	60.00	160.00	100.00	99.20	50.00
Sodium (mg L^{-1})	100.00	648.73	640.00	1078.50	32.50	714.86
Potassium (mg L^{-1})	5.00	9.00	15.00	14.00	6.00	12.00
Iron (mg L^{-1})	0.01	ND	0.02	0.01	0.03	0.03

422 Abbreviations: ND= not detected

423 [a] Trace anions: NO_3^- = 0,445 mg N l^{-1}; NH_4^+ < 0,04 mg N l^{-1}

424 [b] Trace anions: NO_3^- < 0,002 mg N l^{-1}; NH_4^+ = 0,14 mg N l^{-1}

425 [c] Measured at the sampling point

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Table 2 Granulometry of the solid phase (%) of the peloids (A3p, Z3p, G3p). Sieving-sedimentation and laser methods.

		Sand				Silt			Clay
Sample[a]	Technique	Total sand (USDA) (2 – 0.05 mm)	Coarse sand (USDA) (2 – 0.2 mm)	Fine sand (USDA) (0.2 – 0.05 mm)	Total sand (Internacional system) (2 – 0.02 mm)	Total silt (USDA) (0.05 – 0.002 mm)	Coarse silt (USDA) (0.05 – 0.02 mm)	Fine silt (USDA) (0.02 – 0.002 mm)	Clay (USDA) (<0.002 mm)
A3p	SS	1.6	0.1	1.5	10.8	44.6	6.8	37.8	53.8
	LD	7.6	0.0	7.7	28.5	74.3	20.9	53.4	18.1
Z3p	SS	1.9	0.1	1.8	9.8	44.5	1.3	43.2	53.7
	LD	7.3	0.0	7.3	27.9	74.5	20.6	53.9	18.2
G3p	SS	2.2	0.1	2.1	7.2	44.2	1.9	42.3	53.6
	LD	10.5	0.0	10.5	30.3	72.4	19.8	52.6	17.1

Abbreviations: USDA= United States Department of Agriculture; SS= Sieving-sedimentation; LD= Laser

[a]P (initial mineral sample): 9,4% sand, 43,8% fine silt and 46,8% clay (determined by sieving-sedimentation, SS)

Table 3 Physicochemical parameters and mineral crystallinity indices of the initial mineral sample (P) and solid phase of peloids (A3p, Z3p, G3p).

	Samples			
	P	A3p	Z3p	G3p
External SSA ($m^2 g^{-1}$)	42.5	53.9	63.7	57.9
Total SSA ($m^2 g^{-1}$)	100.9	120.1	113.9	116.5
CEC ($cmol_+ kg^{-1}$)	9.4	11.12	10.83	10.28
Ca^{2+} ($cmol_+ kg^{-1}$)	5.1	13.11	7.75	8.96
Mg^{2+} ($cmol_+ kg^{-1}$)	5.5	7.63	6.69	6.38
Na^+ ($cmol_+ kg^{-1}$)	8.4	4.78	5.79	4.29
K^+ ($cmol_+ kg^{-1}$)	5.6	0.41	0.38	0.32
HI	0.71	0.66	0.63	0.55
IB	1.24	1.18	1.17	1.19

Abbreviations: SSA= specific surface area; CEC= cation exchange capacity; HI= Hincley's Index (for kaolinite); IB= Integral breadth (for saponite)

438 Table 4 Fabric parameters of the peloids (P_{A3}, P_{Z3}, P_{G3}) measured with SEM-IA.

	Samples		
	P_{A3}	P_{Z3}	P_{G3}
Feret diameter of primary particles			
Mean (µm)	1.14	1.09	1.16
Max – min (µm)	2.3 - 0.49	1.88 - 0.52	2.66 - 0.52
n	50	50	50
Feret diameter of particle clusters			
Mean (µm)	9.94	14.94	5.52
Max – min (µm)	15.48 - 4.59	24.84 - 8.98	9.26 - 2.94
n	50	50	50
Voids			
Total area occupied (%)	11.34	24.87	14.35
Mean area (µm^2)	6.42	10.92	2.99
Feret diameter (µm)	4.20	4.80	3.00
n	355	455	921

439

Peloids prepared with three mineral-medicinal waters from spas in Granada. Their suitability for use in pelotherapy

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Abstract

Three peloids were studied, prepared with three mineral-medicinal water (MMW) from the spas at Alicún (sulphate, rich in magnesium and calcium, highly mineralized), Zújar (sulphate, rich in sodium chloride, highly mineralized) and Graena (sulphate, rich in magnesium and calcium, highly mineralized) (province of Granada, Spain) and matured for three months, in order to determine their properties and suitability for use in pelotherapy. Their solid phase ~~were~~ was prepared by mixing kaolin and bentonite (9:1, w:w). In the peloids the following ~~were~~ was studied: composition of interstitial liquid, granulometry, physicochemical properties (specific surface area -SSA-, cation exchange capacity -CEC- and exchangable bases), crystallinity index of the minerals, thermal behaviour and ultramicroscopic fabric using scanning electron microscopy (SEM) and image analysis (IA). A modification of the ionic concentration of the interstitial liquid was observed with regard to the initial MMW, namely, an increase in the concentration of Na^+ and K^+, and a decrease in Ca^{2+} and Mg^{2+}, due to ~~a cationic~~ exchange between the exchangable cations of the solid phase and the ions of the MMW. Increases in the proportion of fraction <2 µm, SSA and CEC were also observed. The crystallinity index

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of the kaolinite ~~had~~ decreased after three months' maturation, as compared to the initial mineral sample. The fabric developed during maturation was porous and reticulated and the fabric parameters ~~are~~ were related to the thermal properties of the peloid. The properties of the three peloids studied make them potentially suitable for use in pelotherapy.

Key words

Peloids, mineral-medicinal waters, kaolin, bentonite, maturation, SEM-fabric, Spanish spa

1. Introduction

The use of pelotherapy in spas is becoming increasingly popular. Nonetheless, few spas employ peloids. A peloid is a maturated mud or muddy dispersion with healing and/or cosmetic properties, composed of a complex mixture of fine-grained natural materials of geological and/or biological origins, mineral water or sea water, and commonly organic compounds from biological metabolic activity" (Gomes et al., 2013). Peloids require a certain period of contact between the liquid and solid phases, known as "maturation", during which the components mix, interact and biological activity may occur. Currently there are only four Spanish spas that use peloids therapeutically: El Raposo, Arnedillo, Caldas de Boi and Archena. From these spas, mineralogical and chemical composition, and ~~others~~ other properties of the peloids ~~has~~ have been studied (Carretero et al., 2010; Pozo et al., 2013), or in El Raposo spa, the effectiveness of the pelotherapy has been studied (Gálvez et al., 2019). New peloids need to be produced to ~~counter~~ counteract the lack of these in spas, and, consequently, the study and evaluation of the quality of the raw materials suitable for their production is also required (Pozo et al., 2019; amongst others). Studies are usually carried out by mixing the raw materials (clays) with mineral-medicinal waters (MMW) or seawater, varying the maturation time

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and studying the suitability of each peloid for use in pelotherapy (Veniale et al., 2004; Carretero et al., 2007; Gámiz et al., 2009; Fernández-González et al., 2017; among others). Some of these authors (Veniale et al., 2004; Fernández-González et al., 2017) ~~report~~ reported that modifications in the clays used for peloid preparation, with different maturation times, ~~vary~~ varied according to the MMW used. For a complete review see Carretero (2020a, 2020b).

The aim of this study ~~is~~ was to evaluate the properties of the peloids prepared with three MMW from three spas (Alicún, Zújar and Graena) in the province of Granada (Spain) with very different saline concentration and the same solid phase and maturation time with regard to their potential use in pelotherapy. This evaluation ~~will be~~ was based on the modification of mineral crystallinity, chemical, physical and physicochemical properties of the peloids matured for three months. Previously, studies of peloids made with other MMW from spas in Granada were carried out (Gámiz et al., 2009; Fernández-González et al., 2013 and 2017).

The waters selected have historically been in use from 2000 years ~~ago~~. However, these waters have never been studied as the liquid phase for peloid preparation with the exception of those from Graena, studied by ~~some authors (~~Sánchez-Espejo et al.~~,~~ (2015).

The results of this research will serve as a basis for the spas whose waters are used in this work, to make their own peloids, and to implement the technique of pelotherapy in their establishments.

2. Materials and methods.

~~The~~ MMW of Alicún (A0), Zújar (Z0) and Graena (G0) were sampled. The following parameters were *in situ* measured: temperature (with a digital thermometer), pH and electrical conductivity (potentiometric method).

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The solid phase of the peloid (sample P) was prepared by mixing kaolin and bentonite in the ratio 9:1 (w:w) and were mixed with the MMW in the ratio 2:1 (liquid:solid, w:w): 1000 ml of liquid phase and 500 g of solid phase. These raw materials are pharmaceutical and cosmetic minerals of relatively high purity (kaolin: 87% kaolinite; bentonite: 94% saponite) and they have been studied previously (Gámiz et al., 2009). All samples were kept at a temperature of around 20°C. The water-clay mixture was matured during three months by periodically stirring the peloid mass while humidity control was achieved by weighing the containers in order to maintain their initial humidity conditions (Fernández-González et al., 2013, 2017); after ~~which~~ this time, the peloid samples (samples P_{A3}, P_{Z3}, P_{G3}) were obtained for determination of the fabric and cooling kinetics. The interstitial liquid (Liq_{int}) (samples A3, Z3, G3) was extracted by suction (approx. 100 kPa) and separated from the peloids while the remaining solid part (samples A3p, Z3p, G3p) was employed for the study of granulometry, mineral crystallinity and physicochemical properties.

The chemical and physicochemical properties of MMW (A0, Z0, G0) and Liq_{int} (A3, Z3, G3) ~~was carried out~~ were analyzed (Fernández-González et al., 2013).

To estimate the granulometry, two methods ~~have been~~ were used: sieving-sedimentation (SS granulometry) (Soil Survey Staff, 2014) and laser (LD granulometry) (Fernández-González et al., 2017).

The specific surface area (SSA) was determined using ethylene glycol monomethyl ether (EGME) (Carter et al., 1986); also, cation exchange capacity (CEC) and the exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) were determined by atomic absorption (MAPA, 1994).

Crystallinity was determined in the kaolinite and saponite of the initial sample and in the solid phases of the peloids using X-ray diffraction (XRD) diagrams. The Hinckley

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index (HI) (Hinckley, 1963) of the kaolinite was determined in disoriented powder diagrams, and the crystallinity of the saponite (Integral Breadth, IB) (Ehrmann et al., 2005) was determined in diagrams of clay samples solvated with ethylene glycol.

To study the peloid fabric, the methodology described by Fernández-González et al. (2017) was followed. The samples ~~has been~~ were studied using scanning electron microscopy (SEM) (Hitachi S-510) and an energy-dispersive X-ray (EDX) spectrometer. The microphotographs were analyzed with the IMAGE J software (National Institute of Health, 2008).

The cooling kinetics was studied following Ferrand and Yvon (1991) and Cara et al. (2000). Using data of temperature with time-cooling ~~we can calculate~~ ΔT was calculated: the accumulated decreases in temperature (ΔT=T0−Tn, being T0=65 °C). ΔT and time fit a logarithmic function (y=aLn(x)+b; y=ΔT, x=time) with, in all cases, R^2>0.9. The time necessary for the decrease of 22.5 °C ($\Delta t_{-22.5\ ^\circ C}$; 22.5 being 75% of the total decrease in temperature in the experiment) (Gámiz et al., 2009) was calculated for each peloid.

3. Results and discussion.

3.1. Analysis of the waters (MMW and Liq_{int})

The values of dry residue obtained for the samples of MMW (Table 1), were greater than 2 g ~~l~~ L^{-1}, demonstrating that these are highly mineralized waters, corroborated by electrical conductivity values (>3 mS cm^{-1}). The temperature of the MMW during sampling was 35.0, 39.0 and 41.7 ºC for A0, Z0 and G0, respectively and in agreement with Maraver and Armijo (2010) measures. Thus, these MMW could be classified (in balneological terms) as “mesothermal”, A0, and “hyperthermal”, Z0 and G0. The pH values obtained in all the samples (MMW and Liq_{int}) were very similar, ranging around 7.5- 8. These values ~~are~~ were related to the presence of bicarbonates in

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the three MMW, probably resulting from the geological origin of these waters, which emerge in areas rich in limestones and dolomites. According to the chemical composition of the MMW, Alicún and Graena ~~was~~ were classified as calcium- and magnesium-rich sulphate waters, and Zújar was sodium chloride-rich sulphate water, in agreement with Maraver and Armijo (2010).

After three months' maturation, Na^+ concentration ~~had~~ increased (in Liq_{int}), particularly in the peloids from Alicun and Graena (the largest increase is observed in G3). In fact, A3 ~~is~~ was classified as ~~calcium-, magnesium- and~~ sodium-rich sulphate waters, differently from A0. The same occurred in the Z3 and G3, ~~although maintaining the same classification as the initial MMW,~~ which are classified as sodium chloride-rich sulphate waters and sodium rich sulphate waters, respectively. In all cases, the concentration of calcium and magnesium decreased in the Liq_{int} (in some samples, almost threefold after maturation: i.e., for magnesium, from 150 mg l^{-1} in A0 to 60 mg l^{-1} in A3). In addition, there was a higher ionic concentration in Liq_{int} of Graena than in that of Zújar (whose MMW ~~has~~ had the driest residue) ~~than in that of Graena~~, resulting in the peloids prepared with MMW from Alicún (with the lowest residue) presented the lowest ionic content. On the other hand, the concentration of SO_4^{2-}, Cl^-, CO_3^{2-} present in the Liq_{int} ~~increase~~ increased in relation to that of the MMW. Thus, the decrease observed in the ions Ca^{2+} and Mg^{2+} in Liq_{int} must be related to the retention of these ions in the clay used for the solid phase, resulting in cationic exchange of K^+ and Na^+ from the solid phase with Ca^{2+} and Mg^{2+}. This is supported by the observed increase in Ca^{2+} and Mg^{2+} and decrease in Na^+ and K^+, on determining the exchangeable cations in the solid phase of the peloids (see Table 3 and section 3.3). All these evidences ~~are indicating~~ indicate that during maturation, there ~~is~~ was an exchange of cations from the mineral phase to the liquid phase of the peloids and vice-versa. Changes in the

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composition of the interstitial liquid of peloids, compared to MMW, were registered by some authors (Fernández-González et al., 2013; Spilioti et al., 2017; Yücesoy et al., 2019). There ~~are~~ were differences detected in the ionic concentration of the interstitial liquid with respect to the initial MMW, so that therapeutic applications of the peloids ~~will~~ would not have exactly the same effect as the initial MMW have.

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3.2. Granulometry

Granulometry´s results (for LD or SS) showed great homogeneity. In LD (Figure 1), the peloids consist of fine silt (>50%), clay fraction not even attaining 20%. In SS, fine silt reach around 40% and clay fraction is 53%. The difference of results between the SS and LD is attributed to that they are different techniques. Taubner et al. (2009) have developed equations to transform data between the two methods. When applied to our case, these equations provided coherent results, particularly for the clay fraction. E.g., the clay content measured with LD in A3p is 18,1% (Table 2) turns into 53,01% by using Taubner et al. (2009) equations, a value very similar to 53,8% (SS-measured).

The results SS show that a small increase in the fraction <2 µm occurs during maturation, when compared to sample P. The decrease in particle size has also been reported by other authors (da Silva et al., 2015).

3.3. Physicochemical properties

The external SSA of the peloids (Table 3) increased with respect to sample P, most evident in Z3p. It may be due both to the composition of the MMW (sodium acts as a dispersing cation) (Table 1) and to the peloid fabric (~~greater~~ higher porosity) that implies greater surface area (Figures 2, 3 and 4) (Table 4). A similar tendency was observed for total SSA, which presents values higher than external SSA, sometimes duplicating them; this may be due to the method used to determine total SSA, involving the use of ethylene glycol monoethyl ether (EGME), a polar molecule.

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The CEC of the peloids ranged from 10.28 $cmol_+kg^{-1}$ to 11.12 $cmol_+kg^{-1}$, and increased slightly in all the matured peloids compared to sample P. This is due to the increase in the fraction <2 μm and SSA.

For the exchangeable cations, the sequence of quantities in the sample P was Na>>K~Mg>Ca. In the peloids, this sequence was substantially modified: Ca>Mg>Na>K. These modifications are important, as they affect to the ionic concentration of the interstitial liquid. The exchangeable cations can potencially be transferred to human sweat (Carretero et al., 2010) and then to the skin.

3.4. Mineral crystallinity

The crystallinity index of the kaolinite (HI) in the peloids ranged between 0.55 and 0.63 (Table 3), the range of medium values, according to Hinckley (1963). HI decreased in the three peloids (Table 3) when compared to sample P. The increase in defects in the kaolinite structure during maturation is due to the larger particles in vermicular stacks or pseudohexagonal crystals, with high cristallinity (Delgado et al., 1994), when preparing and maturing the peloids, they break down into individual smaller particles with more defects (Gámiz et al., 2009; Fernández-González et al., 2017). SEM study detected kaolinite stacks in sample P but not in the peloids, which were always composed of laminar particles (Figures 2, 3 and 4).

The Integral Breath (IB) values of the smectite (saponite) were around 1.18; according to Ehrmann et al. (2005), these would be considered "well crystalline". The crystallinity was slightly higher in the peloids than in the original mineral. This may be due to the potassium, which enters the interlaminar zone of the saponite and is sequestered, thereby stabilizing the structure and increasing the perfection of crystallinity, making the saponite closer to mica (Velde and Barré, 2010). This hypothesis is supported by the considerable decrease in the concentration of

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exchangeable potassium in the peloids, compared to the initial sample. Sánchez et al. (2002), in contrast to the present study, reported a decrease in the crystallinity of saponites during maturation. However, in this case the composition of the MMW (iron-rich, bicarbonate and sulphate) was different to our MMW. Carretero et al. (2007), using seawater for the maturation, also reported a decrease in the crystallinity of the smectites with maturation time.

3.5. Fabric

The SEM fabric of the three peloids revealed (Figures 2, 3, 4) to be ordered hierarchically by size in primary particles of similar size (around 1.10 µm), which create clusters of varying size (5 µm - P_{G3}, 10 µm- P_{A3}, 15 µm- P_{Z3}) (Table 4). There were also large laminar particles with generally joints face-face with some face-edge. Porosity was significant in all three samples, although there were some differences with regard to the MMW employed ($P_{Z3} >> P_{G3} > P_{A3}$). Thus, the peloid prepared with MMW from Zújar showed the most porous fabric. The disperse and reticulated appearance of P_{A3} fabric (Figure 2a) ~~is~~ was more evident in P_{G3} (Figure 4a), where there ~~has been~~ was a dispersion process (individualization of particles). In case of P_{Z3} (Figure 3c), small "clusters" with a tactoid-like appearance were also observed, forming an open fabric, also porous and reticulated which even classified as "house of cards/honeycomb".

The EDX microanalysis (Figures 2c, 3c and 4c) ~~corroborate~~ corroborated the kaolinitic nature, with peaks of silica and aluminum of similar heights. There were also peaks of magnesium, iron and a small one of potassium, which can be attributed to the disperse smectite between the kaolinite laminae (particularly notable in P_{G3}). In no case was a microanalysis clearly attributable to saponite detected.

Possible relationships between the IA fabric parameters (Table 4) and the properties of the peloids were also considered. The granulometry (Table 2), crystallinity

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index of the smectite and the exchangeable cations (principally Na^+) (Table 3) ~~are~~ were clearly related to the fabric parameters. Thus, the Feret diameter of the pores ~~is~~ was positively related to the percentage of fine silt measured by LD (r = 0.998; P<0.05). The mean area of the pores ~~is~~ was negatively and strongly correlated with the IB (r = -0.997; P<0.05). The sodium of the exchange complex ($cmol_+kg^{-1}$) is negatively related with the size of the primary particles (r = 0.999; P<0.01) so that the higher the concentration of sodium the smaller the primary particles (they disperse). The fabric is thus highly dependent on, and informative of, the properties of the solid phase and the concentration and nature of the cations present in the MMW. This fabric of peloids is of great interest and, as yet, relatively undeveloped.

3.6 Cooling kinetics.

The descending sequence of cooling times $\Delta t_{-22,5ºC}$ (in minutes) (Figure 5) would be from slowest to fastest: P_{G3} (12.32) > P_{A3} (11.75) > P_{Z3} (11.37). The difference was only one minute and ~~are~~ were therefore thermally similar, as they ~~have~~ had a similar water content, particle size and mineral composition of the initial clay (Ferrand and Yvon, 1991; Armijo et al., 2016). Millero (2001) reported that salinity inversely affected the thermal conductivity of water so that less saline waters conducted heat better and cooled more rapidly. In the present study, albeit with small differences, the quantities of solid residue in the Liq_{int} followed the sequence Z3 > G3 > A3 and the expected cooling sequence would be P_{A3}< P_{G3}< P_{Z3}, the opposite of what actually occurs. Therefore, the characteristics of water do not fully explain the behavior of these peloids. In this way, a correlation was observed between the time taken $\Delta t_{-22,5ºC}$ (min) and Feret diameter of the pores (r = -0.997; P<0.05), and also the Feret diameter of the clusters. Consequently, a fabric with smaller clusters will cool more slowly ($\Delta t_{-22,5ºC}$ higher) according to Gámiz et al. (2009).

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4. Final considerations on the potential applications of peloids.

~~As we have found~~ The differences between MMW and Liq_{int}, ~~this assumes~~ mean that the peloid will act not exactly like MMW at the skin level. This fact ~~has already~~ was detected by Fernández-González et al. (2013). The differences ~~are~~ were due to some ions of MMW ~~are~~ retained by the clay during maturation (mainly calcium and magnesium) while others ~~are~~ were released to the liquid phase (through ionic exchange processes). The pH values of the Liq_{int} (around pH = 8, Table 1) confer a slight alkalinity on the peloids, which could be used to treat a variety of skin conditions (Tateo et al., 2010). Topical application of these peloids would thus produce a change in the chemical reactions on the skin, on changing the acid equilibrium of this tissue (Takigawa et al., 2005).

The granulometry of ~~our~~ the peloids (silty-clayey) coincides with that found by other authors for peloids for use in spas (Karakaya et al., 2010; Pozo et al., 2013). With regard to their suitability, this granulometry renders them suitable for use in spas (Carretero et al., 2010, 2014).

The values of both external and total SSA were relatively high (50 and 100 m^2 per gram, respectively) rendering this material suitable for the adsorption and release of active principles, skin cleansing, etc. A high SSA results in a greater capacity for absorption and adsorption (Carretero et al., 2014). In terms of CEC, according to Matike et al. (2011), the peloids in the present study (with values of CEC <15 $cmol_+kg^{-1}$) would behave as ion sources.

In all the samples, the crystalline perfection of the kaolinite (HI) decreased with maturation, although some small differences in HI were observed, depending on the MMW employed. This phenomenon could improve the ability to delay the release of

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drugs adsorbed to the peloid (Delgado et al., 1994), rendering ~~our~~ the peloids ~~being~~ suitable for therapeutic application.

The degree of cooling of the three peloids ~~is~~ was similar. The values of $\Delta t_{22,5^{\circ}C}$ ~~are~~ were within the range of peloids prepared with other MMW from spas in Granada (Gámiz et al., 2009; Fernández-González et al., 2017).

The type of dispersed-, porous- and reticulated-fabric observed in the peloids are considered of interest for pelotherapy. Even P_{Z3} is "house of cards/honeycomb", the best of the three. According to Vali and Bachmann (1988), the fabric of the colloidal dispersions of clay is related to the rheological properties relevant to peloid application. Fabrics such as "house of cards/honeycomb" or reticulated increase the viscosity or elasticity of the mud, both of which are favourable properties for handling and applying to the skin. The mechanisms for the release and adsorption of ions and polar molecules are also affected by the microstructure.

~~We can thus conclude~~ It can be concluded that the three peloids studied have similar properties, which make them potentially suitable for use in pelotherapy. Thus, the MMW from the Alicún, Zújar and Graena spas are a good raw material for the preparation of peloids.

Dedication

This study is dedicated to the memory of the eminent professor and researcher Emilio Galán, our friend, who dedicated his life and work to the study of minerals and their applications.

Acknowledgements

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Figure captions

Figure 1 Laser granulometry of peloid samples A3p, Z3p and G3p.

Figure 2 SEM-EDX and IA of sample P_{A3}. a) Image; b) Binary pore mask of a); c) Image, detail of a); d) Pore mask of c)

Figure 3 SEM-EDX and IA of the sample P_{Z3}. a) Image; b) Binary pore mask of a); c) Image, detail; d) Pore mask of c)

Figure 4 SEM-EDX and IA of the sample P_{G3}. a) Image; b) Pore mask of a); c) Image, detail; d) Pore mask of c)

Figure 5 Cooling kinetics of peloids P_{A3}, P_{Z3} and P_{G3}. Variation of temperature, ΔT, with time ($\Delta T = T_0 - T_n$; $T_0 = 65$ ºC).

421 Table 1 Parameters of the mineral-medicinal waters (MMW: A0, Z0, G0) and the liquid phases of peloids (Liq_{int}: A3, Z3, G3).

	Samples					
	A0[a]	A3	Z0	Z3	G0[b]	G3
Electrical conductivity (20 ºC) (mS cm^{-1})	3.02 [c]	2.86	6.72 [c]	5.75	3.35[c]	3.20
pH	7.98 [c]	7.87	7.42[c]	8.18	7.68[c]	7.88
Temperature (ºC)	35.0 [c]	-	39.0 [c]	-	41.7 [c]	-
Solid residue (110 ºC) (g L^{-1})	2.05	2.34	3.85	4.40	2.47	2.91
Chloride (mg L^{-1})	81.14	113.30	804.66	994.60	9.35	53.25
Sulphate (mg L^{-1})	1296.10	1303.70	1581.65	1603.20	1423.45	2084.10
Carbonate (mg L^{-1})	0.00	150.00	0.00	120.00	0.00	60.00
Bicarbonate (mg L^{-1})	242.40	122.00	203.60	91.50	120.00	113.02
Calcium (mg L^{-1})	335.00	107.00	321.00	170.00	417.45	134.75
Magnesium (mg L^{-1})	150.00	60.00	160.00	100.00	99.20	50.00
Sodium (mg L^{-1})	100.00	648.73	640.00	1078.50	32.50	714.86
Potassium (mg L^{-1})	5.00	9.00	15.00	14.00	6.00	12.00
Iron (mg L^{-1})	0.01	ND	0.02	0.01	0.03	0.03

422 Abbreviations: ND= not detected

423 [a] Trace anions: NO_3^- = 0,445 mg N l^{-1}; NH_4^+ < 0,04 mg N l^{-1}

424 [b] Trace anions: NO_3^- < 0,002 mg N l^{-1}; NH_4^+ = 0,14 mg N l^{-1}

425 [c] Measured at the sampling point

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Table 2 Granulometry of the solid phase (%) of the peloids (A3p, Z3p, G3p). Sieving-sedimentation and laser methods.

		Sand				Silt			Clay
Sample[a]	Technique	Total sand (USDA) (2 – 0.05 mm)	Coarse sand (USDA) (2 – 0.2 mm)	Fine sand (USDA) (0.2 – 0.05 mm)	Total sand (Internacional system) (2 – 0.02 mm)	Total silt (USDA) (0.05 – 0.002 mm)	Coarse silt (USDA) (0.05 – 0.02 mm)	Fine silt (USDA) (0.02 – 0.002 mm)	Clay (USDA) (<0.002 mm)
A3p	SS	1.6	0.1	1.5	10.8	44.6	6.8	37.8	53.8
	LD	7.6	0.0	7.7	28.5	74.3	20.9	53.4	18.1
Z3p	SS	1.9	0.1	1.8	9.8	44.5	1.3	43.2	53.7
	LD	7.3	0.0	7.3	27.9	74.5	20.6	53.9	18.2
G3p	SS	2.2	0.1	2.1	7.2	44.2	1.9	42.3	53.6
	LD	10.5	0.0	10.5	30.3	72.4	19.8	52.6	17.1

Abbreviations: USDA= United States Department of Agriculture; SS= Sieving-sedimentation; LD= Laser

[a]P (initial mineral sample): 9,4% sand, 43,8% fine silt and 46,8% clay (determined by sieving-sedimentation, SS)

Table 3 Physicochemical parameters and mineral crystallinity indices of the initial mineral sample (P) and solid phase of peloids (A3p, Z3p, G3p).

	Samples			
	P	A3p	Z3p	G3p
External SSA ($m^2 g^{-1}$)	42.5	53.9	63.7	57.9
Total SSA ($m^2 g^{-1}$)	100.9	120.1	113.9	116.5
CEC ($cmol_+ kg^{-1}$)	9.4	11.12	10.83	10.28
Ca^{2+} ($cmol_+ kg^{-1}$)	5.1	13.11	7.75	8.96
Mg^{2+} ($cmol_+ kg^{-1}$)	5.5	7.63	6.69	6.38
Na^+ ($cmol_+ kg^{-1}$)	8.4	4.78	5.79	4.29
K^+ ($cmol_+ kg^{-1}$)	5.6	0.41	0.38	0.32
HI	0.71	0.66	0.63	0.55
IB	1.24	1.18	1.17	1.19

Abbreviations: SSA= specific surface area; CEC= cation exchange capacity; HI= Hincley's Index (for kaolinite); IB= Integral breadth (for saponite)

438 Table 4 Fabric parameters of the peloids (P_{A3}, P_{Z3}, P_{G3}) measured with SEM-IA.

	Samples		
	P_{A3}	P_{Z3}	P_{G3}
Feret diameter of primary particles			
Mean (µm)	1.14	1.09	1.16
Max – min (µm)	2.3 - 0.49	1.88 - 0.52	2.66 - 0.52
n	50	50	50
Feret diameter of particle clusters			
Mean (µm)	9.94	14.94	5.52
Max – min (µm)	15.48 - 4.59	24.84 - 8.98	9.26 - 2.94
n	50	50	50
Voids			
Total area occupied (%)	11.34	24.87	14.35
Mean area (µm^2)	6.42	10.92	2.99
Feret diameter (µm)	4.20	4.80	3.00
n	355	455	921

439

Peloids prepared with three mineral-medicinal waters from spas in Granada. Their suitability for use in pelotherapy

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Abstract

Three peloids were studied, prepared with three mineral-medicinal water (MMW) from the spas at Alicún (sulphate, rich in magnesium and calcium, highly mineralized), Zújar (sulphate, rich in sodium chloride, highly mineralized) and Graena (sulphate, rich in magnesium and calcium, highly mineralized) (province of Granada, Spain) and matured for three months, in order to determine their properties and suitability for use in pelotherapy. Their solid phase was prepared by mixing kaolin and bentonite (9:1, w:w). In the peloids the following was studied: composition of interstitial liquid, granulometry, physicochemical properties (specific surface area -SSA-, cation exchange capacity -CEC- and exchangable bases), crystallinity index of the minerals, thermal behaviour and ultramicroscopic fabric using scanning electron microscopy (SEM) and image analysis (IA). A modification of the ionic concentration of the interstitial liquid was observed with regard to the initial MMW, namely, an increase in the concentration of Na^+ and K^+, and a decrease in Ca^{2+} and Mg^{2+}, due to exchange between the exchangable cations of the solid phase and the ions of the MMW. Increases in the proportion of fraction <2 µm, SSA and CEC were also observed. The crystallinity index

of the kaolinite decreased after three months' maturation, as compared to the initial mineral sample. The fabric developed during maturation was porous and reticulated and the fabric parameters were related to the thermal properties of the peloid. The properties of the three peloids studied make them potentially suitable for use in pelotherapy.

Key words

Peloids, mineral-medicinal waters, kaolin, bentonite, maturation, SEM-fabric, Spanish spa

1. Introduction

The use of pelotherapy in spas is becoming increasingly popular. Nonetheless, few spas employ peloids. A peloid is a maturated mud or muddy dispersion with healing and/or cosmetic properties, composed of a complex mixture of fine-grained natural materials of geological and/or biological origins, mineral water or sea water, and commonly organic compounds from biological metabolic activity" (Gomes et al., 2013). Peloids require a certain period of contact between the liquid and solid phases, known as "maturation", during which the components mix, interact and biological activity may occur. Currently there are only four Spanish spas that use peloids therapeutically: El Raposo, Arnedillo, Caldas de Boi and Archena. From these spas, mineralogical and chemical composition, and other properties of the peloids have been studied (Carretero et al., 2010; Pozo et al., 2013), or in El Raposo spa, the effectiveness of the pelotherapy has been studied (Gálvez et al., 2019). New peloids need to be produced to counteract the lack of these in spas, and, consequently, the study and evaluation of the quality of the raw materials suitable for their production is also required (Pozo et al., 2019; amongst others). Studies are usually carried out by mixing the raw materials (clays) with mineral-medicinal waters (MMW) or seawater, varying the maturation time and studying the suitability of each peloid for use in pelotherapy (Veniale et al., 2004;

Carretero et al., 2007; Gámiz et al., 2009; Fernández-González et al., 2017; among others). Some of these authors (Veniale et al., 2004; Fernández-González et al., 2017) reported that modifications in the clays used for peloid preparation, with different maturation times, varied according to the MMW used. For a complete review see Carretero (2020a, 2020b).

The aim of this study was to evaluate the properties of the peloids prepared with three MMW from three spas (Alicún, Zújar and Graena) in the province of Granada (Spain) with very different saline concentration and the same solid phase and maturation time with regard to their potential use in pelotherapy. This evaluation was based on the modification of mineral crystallinity, chemical, physical and physicochemical properties of the peloids matured for three months. Previously, studies of peloids made with other MMW from spas in Granada were carried out (Gámiz et al., 2009; Fernández-González et al., 2013 and 2017).

The waters selected have historically been in use from 2000 years. However, these waters have never been studied as the liquid phase for peloid preparation with the exception of those from Graena, studied by Sánchez-Espejo et al. (2015).

The results of this research will serve as a basis for the spas whose waters are used in this work, to make their own peloids, and to implement the technique of pelotherapy in their establishments.

2. Materials and methods.

MMW of Alicún (A0), Zújar (Z0) and Graena (G0) were sampled. The following parameters were *in situ* measured: temperature (with a digital thermometer), pH and electrical conductivity (potentiometric method).

The solid phase of the peloid (sample P) was prepared by mixing kaolin and bentonite in the ratio 9:1 (w:w) and were mixed with the MMW in the ratio 2:1

77 (liquid:solid, w:w): 1000 ml of liquid phase and 500 g of solid phase. These raw
78 materials are pharmaceutical and cosmetic minerals of relatively high purity (kaolin:
79 87% kaolinite; bentonite: 94% saponite) and they have been studied previously (Gámiz
80 et al., 2009). All samples were kept at a temperature of around 20°C. The water-clay
81 mixture was matured during three months by periodically stirring the peloid mass while
82 humidity control was achieved by weighing the containers in order to maintain their
83 initial humidity conditions (Fernández-González et al., 2013, 2017); after this time, the
84 peloid samples (samples P_{A3}, P_{Z3}, P_{G3}) were obtained for determination of the fabric and
85 cooling kinetics. The interstitial liquid (Liq_{int}) (samples A3, Z3, G3) was extracted by
86 suction (approx. 100 kPa) and separated from the peloids while the remaining solid part
87 (samples A3p, Z3p, G3p) was employed for the study of granulometry, mineral
88 crystallinity and physicochemical properties.
89 The chemical and physicochemical properties of MMW (A0, Z0, G0) and Liq_{int} (A3,
90 Z3, G3) were analyzed (Fernández-González et al., 2013).
91 To estimate the granulometry, two methods were used: sieving-sedimentation (SS
92 granulometry) (Soil Survey Staff, 2014) and laser (LD granulometry) (Fernández-
93 González et al., 2017).
94 The specific surface area (SSA) was determined using ethylene glycol monomethyl
95 ether (EGME) (Carter et al., 1986); also, cation exchange capacity (CEC) and the
96 exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) were determined by atomic absorption
97 (MAPA, 1994).
98 Crystallinity was determined in the kaolinite and saponite of the initial sample and in
99 the solid phases of the peloids using X-ray diffraction (XRD) diagrams. The Hinckley
100 index (HI) (Hinckley, 1963) of the kaolinite was determined in disoriented powder

diagrams, and the crystallinity of the saponite (Integral Breadth, IB) (Ehrmann et al., 2005) was determined in diagrams of clay samples solvated with ethylene glycol.

To study the peloid fabric, the methodology described by Fernández-González et al. (2017) was followed. The samples were studied using scanning electron microscopy (SEM) (Hitachi S-510) and an energy-dispersive X-ray (EDX) spectrometer. The microphotographs were analyzed with the IMAGE J software (National Institute of Health, 2008).

The cooling kinetics was studied following Ferrand and Yvon (1991) and Cara et al. (2000). Using data of temperature with time-cooling ΔT was calculated: the accumulated decreases in temperature (ΔT=T0−Tn, being T0=65 °C). ΔT and time fit a logarithmic function (y=aLn(x)+b; y=ΔT, x=time) with, in all cases, R^2>0.9. The time necessary for the decrease of 22.5 °C ($\Delta t_{-22.5\ ^\circ C}$; 22.5 being 75% of the total decrease in temperature in the experiment) (Gámiz et al., 2009) was calculated for each peloid.

3. Results and discussion.

3.1. Analysis of the waters (MMW and Liq_{int})

The values of dry residue obtained for the samples of MMW (Table 1), were greater than 2 g L^{-1}, demonstrating that these are highly mineralized waters, corroborated by electrical conductivity values (>3 mS cm^{-1}). The temperature of the MMW during sampling was 35.0, 39.0 and 41.7 ºC for A0, Z0 and G0, respectively and in agreement with Maraver and Armijo (2010) measures. Thus, these MMW could be classified (in balneological terms) as "mesothermal", A0, and "hyperthermal", Z0 and G0. The pH values obtained in all the samples (MMW and Liq_{int}) were very similar, ranging around 7.5- 8. These values were related to the presence of bicarbonates in the three MMW, probably resulting from the geological origin of these waters, which emerge in areas rich in limestones and dolomites. According to the chemical

126 composition of the MMW, Alicún and Graena were classified as calcium- and
127 magnesium-rich sulphate waters, and Zújar was sodium chloride-rich sulphate water, in
128 agreement with Maraver and Armijo (2010).

129 After three months' maturation, Na^+ concentration increased (in Liq_{int}),
130 particularly in the peloids from Alicun and Graena (the largest increase is observed in
131 G3). In fact, A3 was classified as sodium-rich sulphate waters, differently from A0. The
132 same occurred in the Z3 and G3, which are classified as sodium chloride-rich sulphate
133 waters and sodium rich sulphate waters, respectively. In all cases, the concentration of
134 calcium and magnesium decreased in the Liq_{int} (in some samples, almost threefold after
135 maturation: i.e., for magnesium, from 150 mg l^{-1} in A0 to 60 mg l^{-1} in A3). In addition,
136 there was a higher ionic concentration in Liq_{int} of Graena than in that of Zújar (whose
137 MMW ~~has~~ had the driest residue), resulting in the peloids prepared with MMW from
138 Alicún (with the lowest residue) presented the lowest ionic content. On the other hand,
139 the concentration of SO_4^{2-}, Cl^-, CO_3^{2-} present in the Liq_{int} increased in relation to that of
140 the MMW. Thus, the decrease observed in the ions Ca^{2+} and Mg^{2+} in Liq_{int} must be
141 related to the retention of these ions in the clay used for the solid phase, resulting in
142 cationic exchange of K^+ and Na^+ from the solid phase with Ca^{2+} and Mg^{2+}. This is
143 supported by the observed increase in Ca^{2+} and Mg^{2+} and decrease in Na^+ and K^+, on
144 determining the exchangeable cations in the solid phase of the peloids (see Table 3 and
145 section 3.3). All these evidences indicate that during maturation, there was an exchange
146 of cations from the mineral phase to the liquid phase of the peloids and vice-versa.
147 Changes in the composition of the interstitial liquid of peloids, compared to MMW,
148 were registered by some authors (Fernández-González et al., 2013; Spilioti et al., 2017;
149 Yücesoy et al., 2019). There were differences detected in the ionic concentration of the

150 interstitial liquid with respect to the initial MMW, so that therapeutic applications of the
151 peloids would not have exactly the same effect as the initial MMW have.

152 *3.2. Granulometry*

153 Granulometry´s results (for LD or SS) showed great homogeneity. In LD (Figure
154 1), the peloids consist of fine silt (>50%), clay fraction not even attaining 20%. In SS,
155 fine silt reach around 40% and clay fraction is 53%. The difference of results between
156 the SS and LD is attributed to that they are different techniques. Taubner et al. (2009)
157 have developed equations to transform data between the two methods. When applied to
158 our case, these equations provided coherent results, particularly for the clay fraction.
159 E.g., the clay content measured with LD in A3p is 18,1% (Table 2) turns into 53,01%
160 by using Taubner et al. (2009) equations, a value very similar to 53,8% (SS-measured).

161 The results SS show that a small increase in the fraction <2 μm occurs during
162 maturation, when compared to sample P. The decrease in particle size has also been
163 reported by other authors (da Silva et al., 2015).

164 *3.3. Physicochemical properties*

165 The external SSA of the peloids (Table 3) increased with respect to sample P,
166 most evident in Z3p. It may be due both to the composition of the MMW (sodium acts
167 as a dispersing cation) (Table 1) and to the peloid fabric (higher porosity) that implies
168 greater surface area (Figures 2, 3 and 4) (Table 4). A similar tendency was observed for
169 total SSA, which presents values higher than external SSA, sometimes duplicating
170 them; this may be due to the method used to determine total SSA, involving the use of
171 ethylene glycol monoethyl ether (EGME), a polar molecule.

172 The CEC of the peloids ranged from 10.28 $cmol_{+}kg^{-1}$ to 11.12 $cmol_{+}kg^{-1}$, and
173 increased slightly in all the matured peloids compared to sample P. This is due to the
174 increase in the fraction <2 μm and SSA.

For the exchangeable cations, the sequence of quantities in the sample P was Na>>K~Mg>Ca. In the peloids, this sequence was substantially modified: Ca>Mg>Na>K. These modifications are important, as they affect to the ionic concentration of the interstitial liquid. The exchangeable cations can potencially be transferred to human sweat (Carretero et al., 2010) and then to the skin.

3.4. Mineral crystallinity

The crystallinity index of the kaolinite (HI) in the peloids ranged between 0.55 and 0.63 (Table 3), the range of medium values, according to Hinckley (1963). HI decreased in the three peloids (Table 3) when compared to sample P. The increase in defects in the kaolinite structure during maturation is due to the larger particles in vermicular stacks or pseudohexagonal crystals, with high cristallinity (Delgado et al., 1994), when preparing and maturing the peloids, they break down into individual smaller particles with more defects (Gámiz et al., 2009; Fernández-González et al., 2017). SEM study detected kaolinite stacks in sample P but not in the peloids, which were always composed of laminar particles (Figures 2, 3 and 4).

The Integral Breath (IB) values of the smectite (saponite) were around 1.18; according to Ehrmann et al. (2005), these would be considered “well crystalline”. The crystallinity was slightly higher in the peloids than in the original mineral. This may be due to the potassium, which enters the interlaminar zone of the saponite and is sequestered, thereby stabilizing the structure and increasing the perfection of crystallinity, making the saponite closer to mica (Velde and Barré, 2010). This hypothesis is supported by the considerable decrease in the concentration of exchangeable potassium in the peloids, compared to the initial sample. Sánchez et al. (2002), in contrast to the present study, reported a decrease in the crystallinity of saponites during maturation. However, in this case the composition of the MMW (iron-

rich, bicarbonate and sulphate) was different to our MMW. Carretero et al. (2007), using seawater for the maturation, also reported a decrease in the crystallinity of the smectites with maturation time.

3.5. Fabric

The SEM fabric of the three peloids revealed (Figures 2, 3, 4) to be ordered hierarchically by size in primary particles of similar size (around 1.10 µm), which create clusters of varying size (5 µm - P_{G3}, 10 µm- P_{A3}, 15 µm- P_{Z3}) (Table 4). There were also large laminar particles with generally joints face-face with some face-edge. Porosity was significant in all three samples, although there were some differences with regard to the MMW employed ($P_{Z3} >> P_{G3} > P_{A3)}$. Thus, the peloid prepared with MMW from Zújar showed the most porous fabric. The disperse and reticulated appearance of P_{A3} fabric (Figure 2a) was more evident in P_{G3} (Figure 4a), where there was a dispersion process (individualization of particles). In case of P_{Z3} (Figure 3c), small "clusters" with a tactoid-like appearance were also observed, forming an open fabric, also porous and reticulated which even classified as "house of cards/honeycomb".

The EDX microanalysis (Figures 2c, 3c and 4c) corroborated the kaolinitic nature, with peaks of silica and aluminum of similar heights. There were also peaks of magnesium, iron and a small one of potassium, which can be attributed to the disperse smectite between the kaolinite laminae (particularly notable in P_{G3}). In no case was a microanalysis clearly attributable to saponite detected.

Possible relationships between the IA fabric parameters (Table 4) and the properties of the peloids were also considered. The granulometry (Table 2), crystallinity index of the smectite and the exchangeable cations (principally Na^+) (Table 3) were clearly related to the fabric parameters. Thus, the Feret diameter of the pores was positively related to the percentage of fine silt measured by LD (r = 0.998; $P<0.05$). The

mean area of the pores was negatively and strongly correlated with the IB (r = -0.997; P<0.05). The sodium of the exchange complex ($cmol_+kg^{-1}$) is negatively related with the size of the primary particles (r = 0.999; P<0.01) so that the higher the concentration of sodium the smaller the primary particles (they disperse). The fabric is thus highly dependent on, and informative of, the properties of the solid phase and the concentration and nature of the cations present in the MMW. This fabric of peloids is of great interest and, as yet, relatively undeveloped.

3.6 Cooling kinetics.

The descending sequence of cooling times $\Delta t_{-22,5^oC}$ (in minutes) (Figure 5) would be from slowest to fastest: P_{G3} (12.32) > P_{A3} (11.75) > P_{Z3} (11.37). The difference was only one minute and were therefore thermally similar, as they had a similar water content, particle size and mineral composition of the initial clay (Ferrand and Yvon, 1991; Armijo et al., 2016). Millero (2001) reported that salinity inversely affected the thermal conductivity of water so that less saline waters conducted heat better and cooled more rapidly. In the present study, albeit with small differences, the quantities of solid residue in the Liq_{int} followed the sequence Z3 > G3 > A3 and the expected cooling sequence would be P_{A3}< P_{G3}< P_{Z3}, the opposite of what actually occurs. Therefore, the characteristics of water do not fully explain the behavior of these peloids. In this way, a correlation was observed between the time taken $\Delta t_{-22,5^oC}$ (min) and Feret diameter of the pores (r = -0.997; P<0.05), and also the Feret diameter of the clusters. Consequently, a fabric with smaller clusters will cool more slowly ($\Delta t_{-22,5^oC}$ higher) according to Gámiz et al. (2009).

4. Final considerations on the potential applications of peloids.

The differences between MMW and Liq_{int}, mean that the peloid will act not exactly like MMW at the skin level. This fact was detected by Fernández-González et

al. (2013). The differences were due to some ions of MMW retained by the clay during maturation (mainly calcium and magnesium) while others were released to the liquid phase (through ionic exchange processes). The pH values of the Liq_{int} (around pH = 8, Table 1) confer a slight alkalinity on the peloids, which could be used to treat a variety of skin conditions (Tateo et al., 2010). Topical application of these peloids would thus produce a change in the chemical reactions on the skin, on changing the acid equilibrium of this tissue (Takigawa et al., 2005).

The granulometry of the peloids (silty-clayey) coincides with that found by other authors for peloids for use in spas (Karakaya et al., 2010; Pozo et al., 2013). With regard to their suitability, this granulometry renders them suitable for use in spas (Carretero et al., 2010, 2014).

The values of both external and total SSA were relatively high (50 and 100 m^2 per gram, respectively) rendering this material suitable for the adsorption and release of active principles, skin cleansing, etc. A high SSA results in a greater capacity for absorption and adsorption (Carretero et al., 2014). In terms of CEC, according to Matike et al. (2011), the peloids in the present study (with values of CEC <15 $cmol_+kg^{-1}$) would behave as ion sources.

In all the samples, the crystalline perfection of the kaolinite (HI) decreased with maturation, although some small differences in HI were observed, depending on the MMW employed. This phenomenon could improve the ability to delay the release of drugs adsorbed to the peloid (Delgado et al., 1994), rendering the peloids suitable for therapeutic application.

The degree of cooling of the three peloids was similar. The values of $\Delta t_{-22,5^oC}$ were within the range of peloids prepared with other MMW from spas in Granada (Gámiz et al., 2009; Fernández-González et al., 2017).

The type of dispersed-, porous- and reticulated-fabric observed in the peloids are considered of interest for pelotherapy. Even P_{Z3} is "house of cards/honeycomb", the best of the three. According to Vali and Bachmann (1988), the fabric of the colloidal dispersions of clay is related to the rheological properties relevant to peloid application. Fabrics such as "house of cards/honeycomb" or reticulated increase the viscosity or elasticity of the mud, both of which are favourable properties for handling and applying to the skin. The mechanisms for the release and adsorption of ions and polar molecules are also affected by the microstructure.

It can be concluded that the three peloids studied have similar properties, which make them potentially suitable for use in pelotherapy. Thus, the MMW from the Alicún, Zújar and Graena spas are a good raw material for the preparation of peloids.

Dedication

This study is dedicated to the memory of the eminent professor and researcher Emilio Galán, our friend, who dedicated his life and work to the study of minerals and their applications.

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401

Figure captions

Figure 1 Laser granulometry of peloid samples A3p, Z3p and G3p.

Figure 2 SEM-EDX and IA of sample P_{A3}. a) Image; b) Binary pore mask of a); c) Image, detail of a); d) Pore mask of c)

Figure 3 SEM-EDX and IA of the sample P_{Z3}. a) Image; b) Binary pore mask of a); c) Image, detail; d) Pore mask of c)

Figure 4 SEM-EDX and IA of the sample P_{G3}. a) Image; b) Pore mask of a); c) Image, detail; d) Pore mask of c)

Figure 5 Cooling kinetics of peloids P_{A3}, P_{Z3} and P_{G3}. Variation of temperature, ΔT, with time ($\Delta T = T_0 - T_n$; $T_0 = 65$ ºC).

414 Table 1 Parameters of the mineral-medicinal waters (MMW: A0, Z0, G0) and the liquid phases of peloids (Liq_{int}: A3, Z3, G3).

	Samples					
	A0[a]	A3	Z0	Z3	G0[b]	G3
Electrical conductivity (20 ºC) (mS cm^{-1})	3.02 [c]	2.86	6.72 [c]	5.75	3.35[c]	3.20
pH	7.98 [c]	7.87	7.42[c]	8.18	7.68[c]	7.88
Temperature (ºC)	35.0 [c]	-	39.0 [c]	-	41.7 [c]	-
Solid residue (110 ºC) (g L^{-1})	2.05	2.34	3.85	4.40	2.47	2.91
Chloride (mg L^{-1})	81.14	113.30	804.66	994.60	9.35	53.25
Sulphate (mg L^{-1})	1296.10	1303.70	1581.65	1603.20	1423.45	2084.10
Carbonate (mg L^{-1})	0.00	150.00	0.00	120.00	0.00	60.00
Bicarbonate (mg L^{-1})	242.40	122.00	203.60	91.50	120.00	113.02
Calcium (mg L^{-1})	335.00	107.00	321.00	170.00	417.45	134.75
Magnesium (mg L^{-1})	150.00	60.00	160.00	100.00	99.20	50.00
Sodium (mg L^{-1})	100.00	648.73	640.00	1078.50	32.50	714.86
Potassium (mg L^{-1})	5.00	9.00	15.00	14.00	6.00	12.00
Iron (mg L^{-1})	0.01	ND	0.02	0.01	0.03	0.03

415 Abbreviations: ND= not detected

416 [a] Trace anions: NO_3^- = 0,445 mg N l^{-1}; NH_4^+ < 0,04 mg N l^{-1}

417 [b] Trace anions: NO_3^- < 0,002 mg N l^{-1}; NH_4^+ = 0,14 mg N l^{-1}

418 [c] Measured at the sampling point

419

420 Table 2 Granulometry of the solid phase (%) of the peloids (A3p, Z3p, G3p). Sieving-sedimentation and laser methods.

		Sand				Silt			Clay
Sample[a]	Technique	Total sand (USDA) (2 – 0.05 mm)	Coarse sand (USDA) (2 – 0.2 mm)	Fine sand (USDA) (0.2 – 0.05 mm)	Total sand (Internacional system) (2 – 0.02 mm)	Total silt (USDA) (0.05 – 0.002 mm)	Coarse silt (USDA) (0.05 – 0.02 mm)	Fine silt (USDA) (0.02 – 0.002 mm)	Clay (USDA) (<0.002 mm)
A3p	SS	1.6	0.1	1.5	10.8	44.6	6.8	37.8	53.8
	LD	7.6	0.0	7.7	28.5	74.3	20.9	53.4	18.1
Z3p	SS	1.9	0.1	1.8	9.8	44.5	1.3	43.2	53.7
	LD	7.3	0.0	7.3	27.9	74.5	20.6	53.9	18.2
G3p	SS	2.2	0.1	2.1	7.2	44.2	1.9	42.3	53.6
	LD	10.5	0.0	10.5	30.3	72.4	19.8	52.6	17.1

421 Abbreviations: USDA= United States Department of Agriculture; SS= Sieving-sedimentation; LD= Laser

422 [a]P (initial mineral sample): 9,4% sand, 43,8% fine silt and 46,8% clay (determined by sieving-sedimentation, SS)

Table 3 Physicochemical parameters and mineral crystallinity indices of the initial mineral sample (P) and solid phase of peloids (A3p, Z3p, G3p).

	Samples			
	P	A3p	Z3p	G3p
External SSA ($m^2 g^{-1}$)	42.5	53.9	63.7	57.9
Total SSA ($m^2 g^{-1}$)	100.9	120.1	113.9	116.5
CEC ($cmol_+ kg^{-1}$)	9.4	11.12	10.83	10.28
Ca^{2+} ($cmol_+ kg^{-1}$)	5.1	13.11	7.75	8.96
Mg^{2+} ($cmol_+ kg^{-1}$)	5.5	7.63	6.69	6.38
Na^+ ($cmol_+ kg^{-1}$)	8.4	4.78	5.79	4.29
K^+ ($cmol_+ kg^{-1}$)	5.6	0.41	0.38	0.32
HI	0.71	0.66	0.63	0.55
IB	1.24	1.18	1.17	1.19

Abbreviations: SSA= specific surface area; CEC= cation exchange capacity; HI= Hincley's Index (for kaolinite); IB= Integral breadth (for saponite)

431 Table 4 Fabric parameters of the peloids (P_{A3}, P_{Z3}, P_{G3}) measured with SEM-IA.

	Samples		
	P_{A3}	P_{Z3}	P_{G3}
Feret diameter of primary particles			
Mean (µm)	1.14	1.09	1.16
Max – min (µm)	2.3 - 0.49	1.88 - 0.52	2.66 - 0.52
n	50	50	50
Feret diameter of particle clusters			
Mean (µm)	9.94	14.94	5.52
Max – min (µm)	15.48 - 4.59	24.84 - 8.98	9.26 - 2.94
n	50	50	50
Voids			
Total area occupied (%)	11.34	24.87	14.35
Mean area (µm^2)	6.42	10.92	2.99
Feret diameter (µm)	4.20	4.80	3.00
n	355	455	921

432

Figure 1

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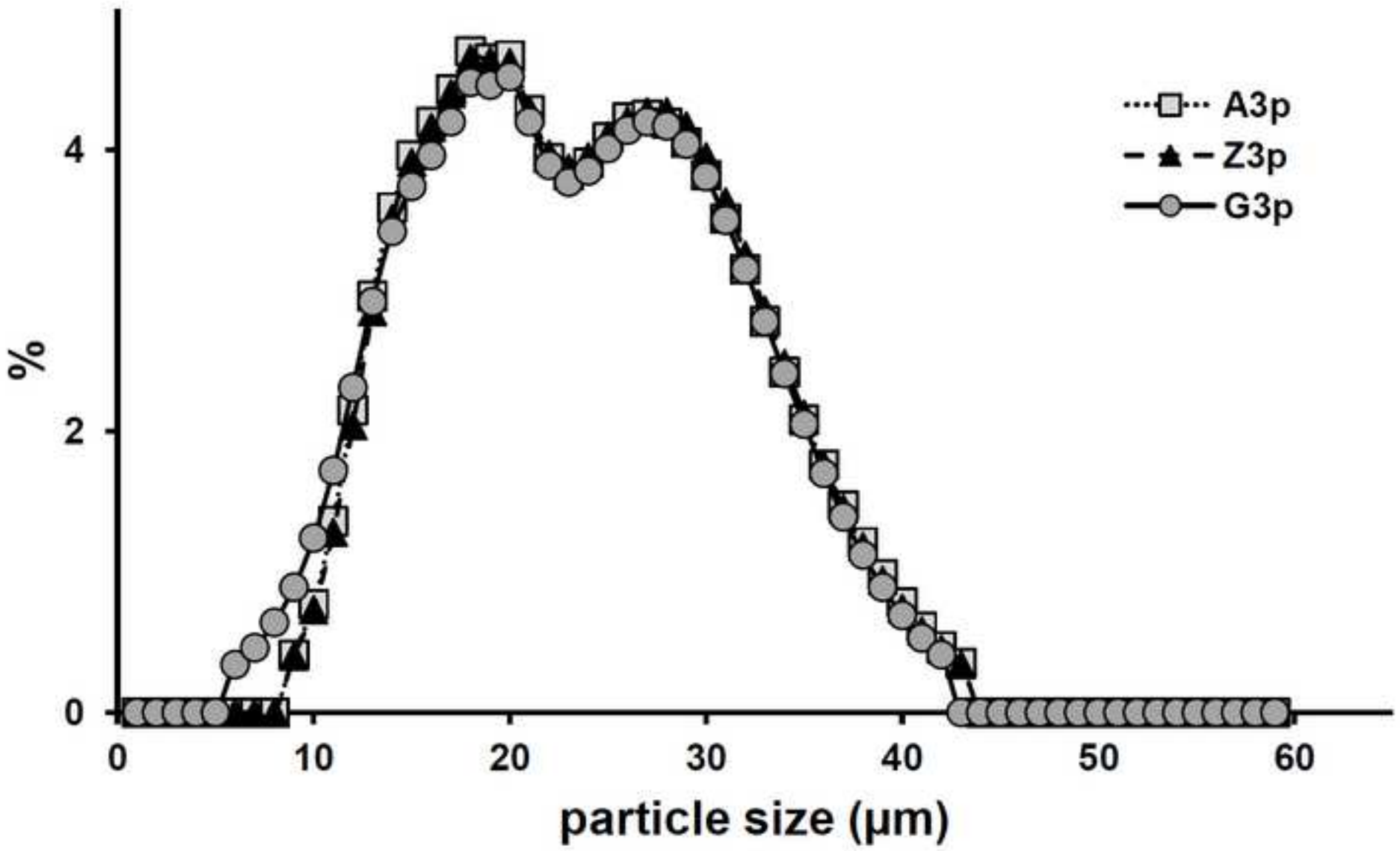

Figure 2

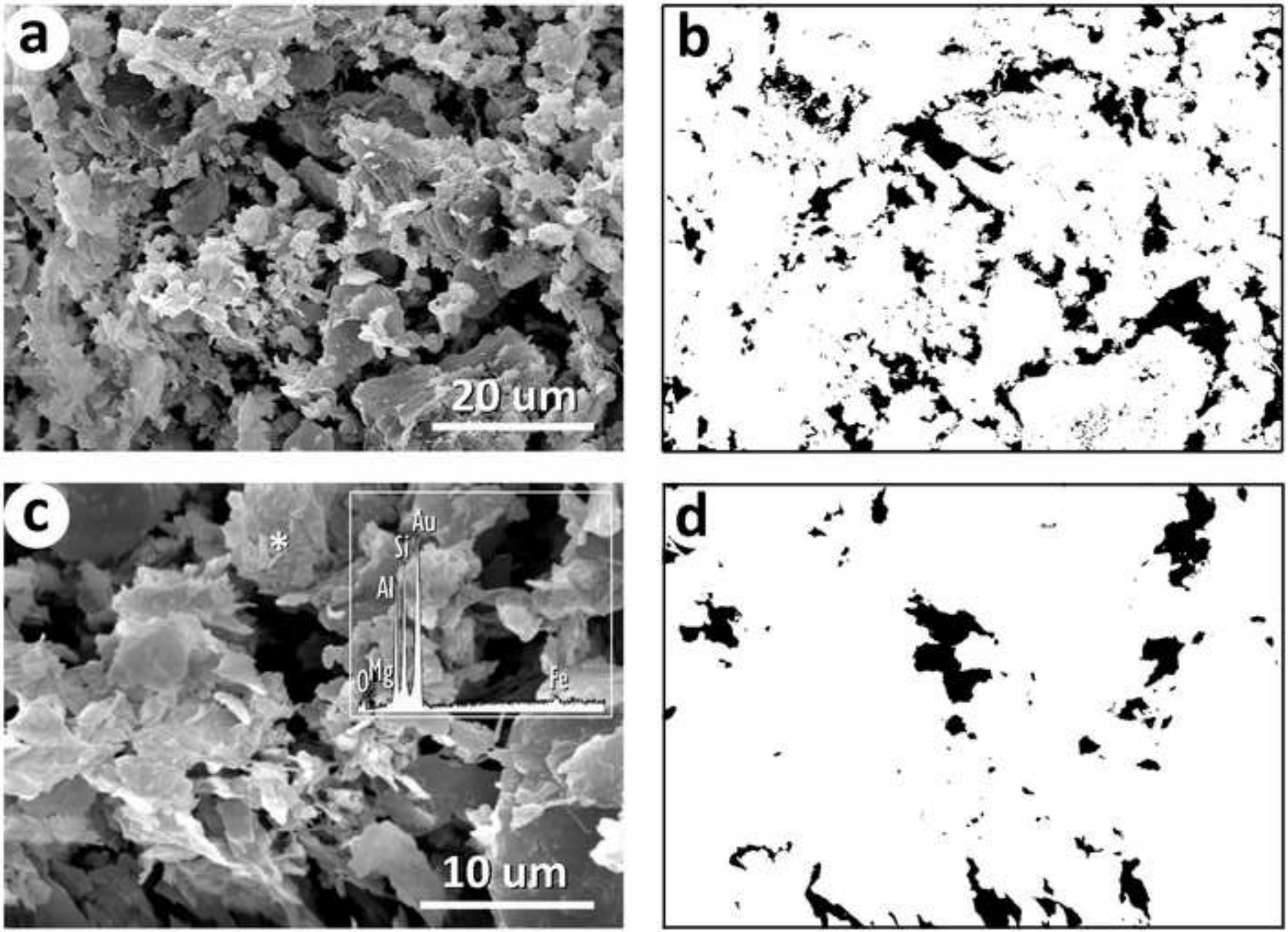

Figure 3

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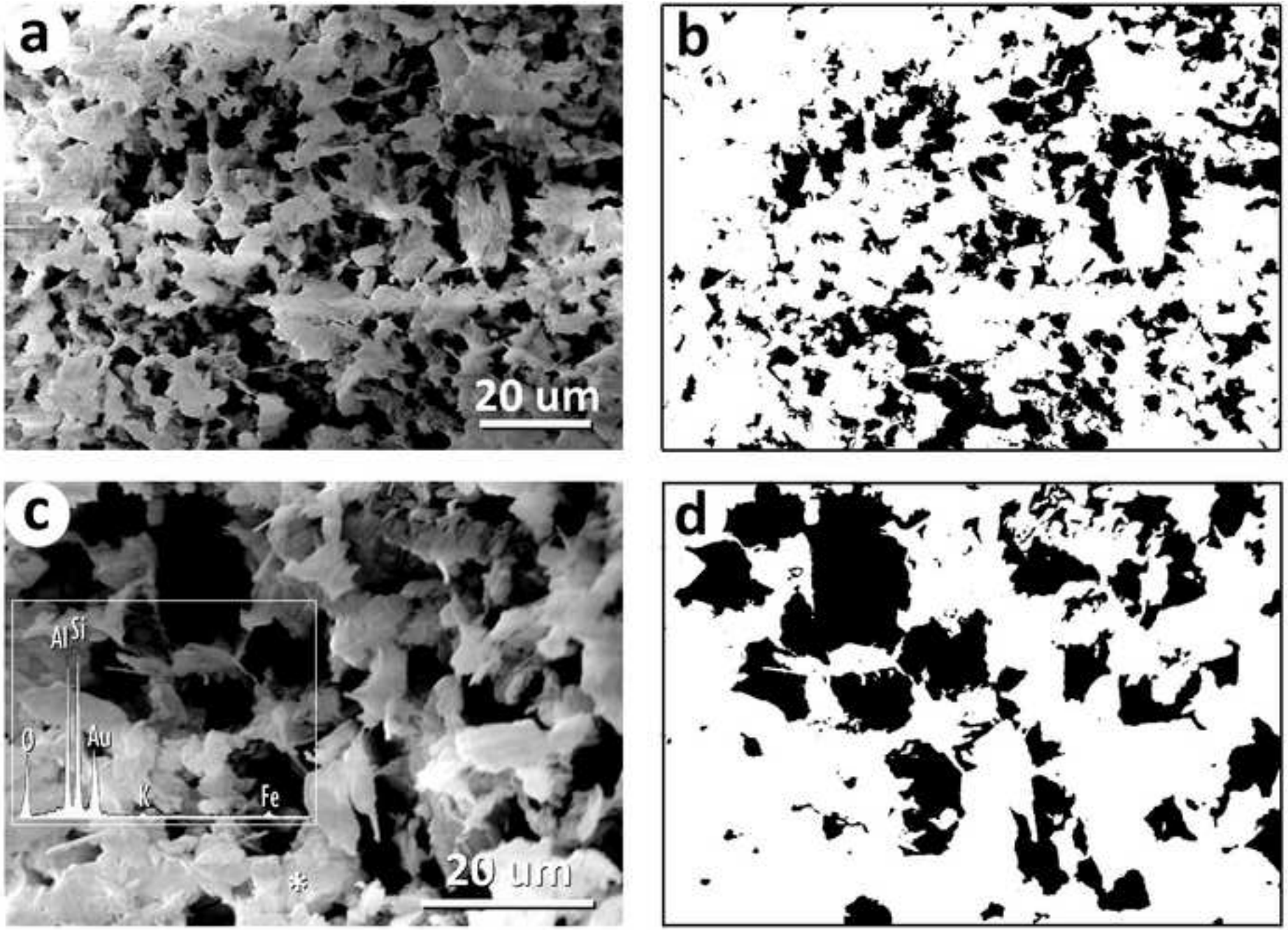

Figure 4

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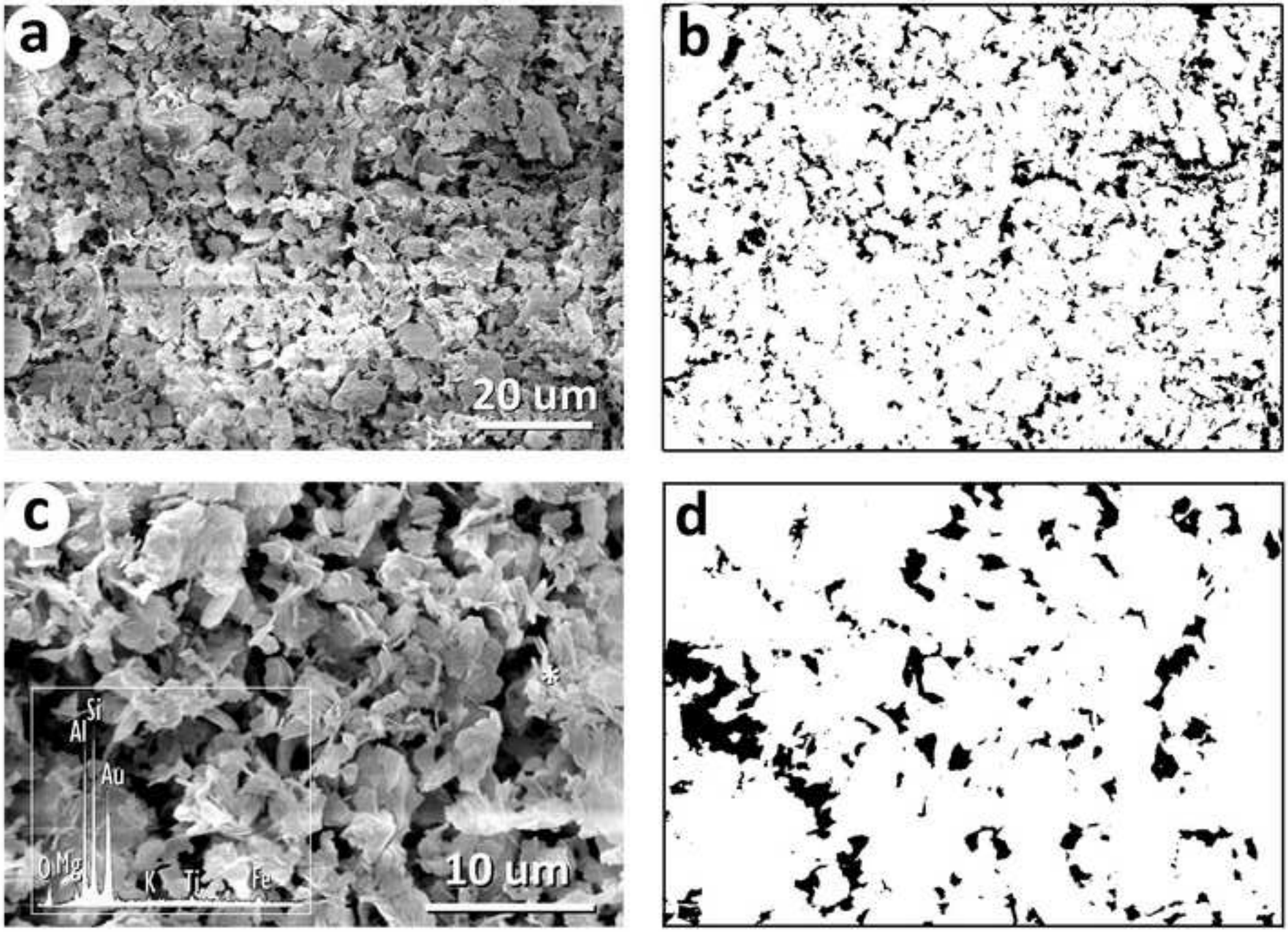
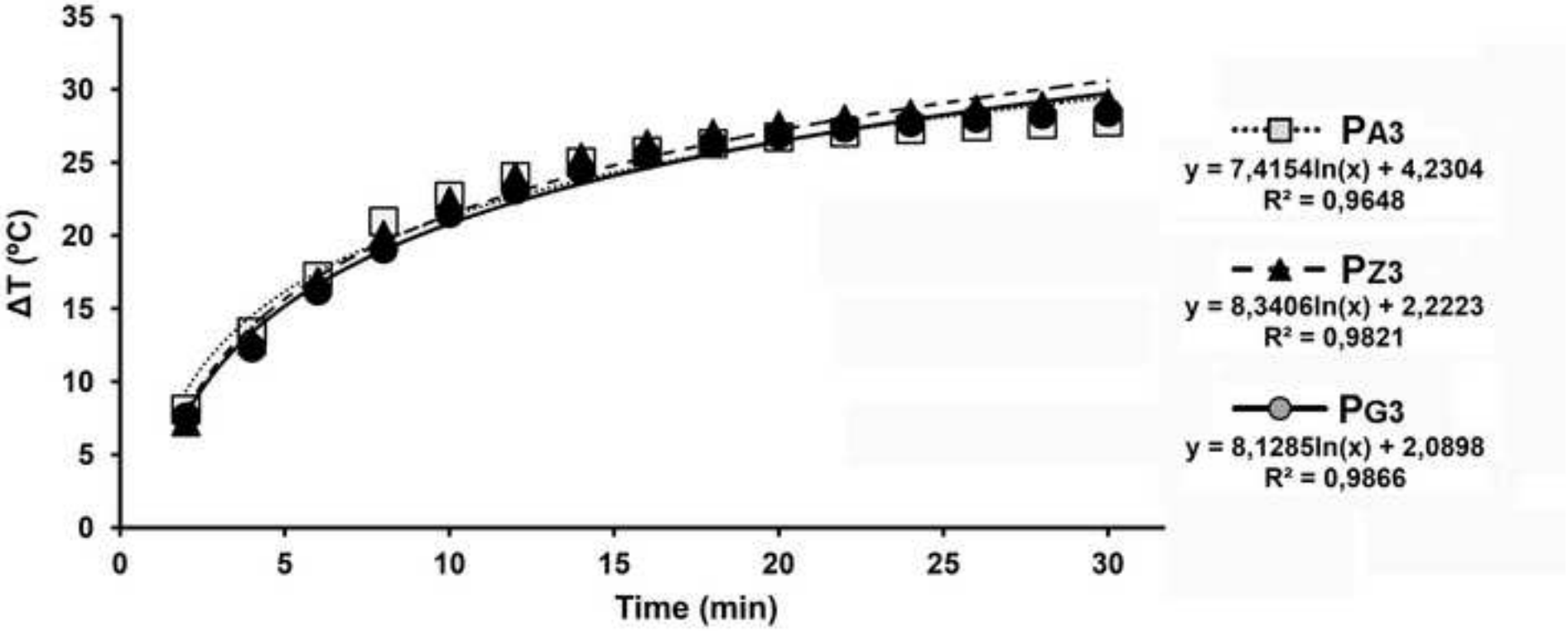


Figure 5



Conflicts of Interest Statement

Manuscript title: **Peloids prepared with three mineral-medicinal waters from spas in Granada**

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Credit Author Statement

All authors have contributed equally to the obtaining and description of laboratory data and in the drafting and discussion of the manuscript