**Instituto Andaluz de Ciencias de la Tierra (CSIC – UGR)**

# SISTEMAS DISPERSOS DE ARCILLAS ESPECIALES EN AGUAS MINEROMEDICINALES PARA SU EMPLEO EN TERAPÉUTICA BALNEARIA

# SUSPENSIONS OF SPECIAL CLAYS IN MINEROMEDICINAL WATERS TO BE USED IN THERAPEUTICS

RITA Mª SÁNCHEZ ESPEJO

PROGRAMA DE DOCTORADO EN MEDICINA CLÍNICA Y SALUD PÚBLICA

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* * *

# SUSPENSIONS OF SPECIAL CLAYS IN MINEROMEDICINAL WATERS TO BE USED IN THERAPEUTICS

Memoria de Tesis presentada por la Licenciada en Farmacia Rita Mª Sánchez Espejo para optar al grado de Doctor por la Universidad de Granada, con mención de Doctorado Internacional.

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# SUMMARY

# SUMMARY

Clays are frequently used in complementary and alternative medicine, including balneotherapy. Therapeutic muds are used for topical administration of mineral medicinal waters in the treatment of illnesses of the locomotor apparatus, including osteoarthritis and rheumatologic diseases. Clays are used in muds as vehicles of the mineral medicinal water, to obtain inorganic gels with rheological and thermal properties suitable to be topically applied. Despite their demonstrated longevity, the use of clays in therapeutic muds greatly requires comprehension of the attributes that sustain their use. Whit these premises, clay samples currently used in several spa centers of southern European/Mediterranean countries were studied to asses their identity, purity, richness and safety. Two of the studied samples were selected for posterior studies because of their superior quality.

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. As a first step in the comprehension of this procedure, the influence of “maturation” on the structure and properties of concentrated suspensions prepared with pharmaceutical grade clays (selected as model materials because of their known quality attributes) was studied. Rheological properties of the studied systems were related to aggregation states and networking mechanisms. These behaviour patterns found during maturation were used in subsequent steps of the thesis. In particular, the two selected clays from spa centers were used to prepare thermal muds with mineral medicinal water from 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 and possible mechanisms of action. It is accepted that 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 muds. In the studied case, maturation increased the release of cations from therapeutic muds but did not improve their thermal properties.

Accordingly, the therapeutic effects associated with thermophysical mechanisms would not require mud maturation. Maturation would be only relevant to improve chemical effects of the muds.

# CAPÍTULO I

## Introducción

# I.1. TERAPÉUTICA CON AGUA

La Terapéutica es la parte de la Medicina que enseña los preceptos y remedios para el tratamiento de las enfermedades, o dicho de otra forma, que se ocupa del tratamiento de las enfermedades mediante distintos medios, incluidos los farmacológicos (Farmacoterapia) o quirúrgicos (Cirugía). La Hidroterapia agrupa a distintas modalidades de uso terapéutico del agua. Cuando el agua usada es mineromedicinal, se emplean los términos Hidrología Médica (países mediterráneos y latinoamericanos, Balneoterapia (Alemania, Austria,…) o Crenoterapia (Francia) que son sinónimos, mientras que si se trata del empleo terapéutico del agua de mar o lago salado, se denomina Talasoterapia. Dicho esto, mientras que el agua potable, o incluso el agua de mar, son medios de tratamiento que actúan a través de mecanismos exclusivamente físicos, el agua mineromedicinal presentará, en razón de su composición, propiedades que le confieren una actividad terapéutica específica y distinta a las exclusivamente derivadas de las acciones físicas (Armijo, 1968; Armijo y San Martín, 1994; San Martín, 2006).

Con estas premisas, las aguas mineromedicinales han sido consideradas **medicamentos químicos**, dado que se trata de soluciones acuosas de diversas sales minerales (Armijo y San Martin, 1994). Estos “medicamentos” han sido usados desde la antigüedad, no obstante la aparición de numerosos principios activos sintéticos de indudable importancia en el tratamiento de numerosas patologías, hizo que durante el siglo XX el empleo de medicamentos “naturales”, como sucede con las aguas mineromedicinales, quedase en un segundo plano dentro del arsenal terapéutico. No por ello han dejado de ser objeto de interés farmacéutico y médico. En este sentido, hace casi medio siglo, el Prof. Lucas Gallego introducía la primera de una larga lista de monografías de la Real Academia Nacional de Farmacia dedicada a las Aguas Mineromedicinales, indicando que “no obstante la riqueza y eficacia de los tratamientos terapéuticos farmacológicos y físicos de fundamento científico, la cura termal persiste y se mantiene desde los tiempos más remotos, en la mayoría de los países, como un medio que ninguno de los tratamientos ha podido desterrar”, considerando las aguas mineromedicinales como “agentes farmacodinámicos de gran poder” (Gallego, 1968). Las aguas mineromedicinales son agentes terapéuticos o sustancias con actividad farmacológica. No obstante, dadas las características peculiares de las aguas mineromedicinales y la posibilidad de su empleo directo en los pacientes, se las considera también por algunos expertos como medicamentos. Así lo hace la Real Academia Nacional de Farmacia en base al informe emitido por los académicos Dres. González y Casares que indicaban que "Las aguas minero-medicinales deben siempre ser consideradas como medicamentos, ya se utilicen al pie del manantial o se prescriban después de haber sido debidamente captadas y conservadas adecuadamente" (Dictamen aprobado en la sesión de la Real Academia Nacional de Farmacia en la sesión del 28 de Julio de 1956). En este sentido, y más allá de su consideración legal actual, son indudablemente sustancias activas que convenientemente administradas y en virtud de una serie de efectos bioquímicos y/o biofísicos, se emplean para el tratamiento de distintas patologías, definición que, entendemos se ajusta a la de medicamento del actual marco legal (Ley 29/2006, de 26 de julio, de garantías y uso racional de los medicamentos y productos sanitarios). El artículo octavo de la citada ley recoge las definiciones fundamentales, incluyendo aquellas que, en razón de la importancia que tienen para nuestro estudio, transcribimos a continuación:

- *"Medicamento de uso humano: toda sustancia o combinación de sustancias que se presente como poseedora de propiedades para el tratamiento o prevención de enfermedades en seres humanos o que pueda usarse en seres humanos o administrarse a seres humanos con el fin de restaurar, corregir o modificar las funciones fisiológicas ejerciendo una acción farmacológica, inmunológica o metabólica, o de establecer un diagnóstico médico.*
- *Principio activo o sustancia activa: toda sustancia o mezcla de sustancias destinadas a la fabricación de un medicamento y que, al ser utilizadas en su producción, se convierten en un componente activo de dicho medicamento destinado a ejercer una acción farmacológica, inmunológica o metabólica con el fin de restaurar, corregir o modificar las funciones fisiológicas, o de establecer un diagnóstico".*
- *Excipiente: todo componente de un medicamento distinto del principio activo y del material de acondicionamiento.*
- *Materia prima: toda sustancia -activa o inactiva- empleada en la fabricación de un medicamento, ya permanezca inalterada, se modifique o desaparezca en el transcurso del proceso.*

- *Forma galénica o forma farmacéutica: la disposición a que se adaptan los principios activos y excipientes para constituir un medicamento. Se define por la combinación de la forma en la que el producto farmacéutico es presentado por el fabricante y la forma en la que es administrada".*

A la vista de las definiciones, las aguas mineromedicinales podrían ser consideradas materias primas y de entre ellas, sustancias activas. Cuando se administran tal cual deberían ser consideradas medicamentos, dado que se prescriben y dosifican, pero es que en el caso particular que centra esta tesis, su administración requiere del concurso de excipientes y se administran con una forma galénica o farmacéutica.

Todo lo dicho es, en todo caso, un interesante y controvertido aspecto del empleo terapéutico de las aguas mineromedicinales, dado que hasta la fecha no les es de aplicación la referida normativa, no obstante la Ley de 2006 indique en su artículo 7 titulado "medicamentos legalmente reconocidos", que **"En caso de duda, cuando un producto pueda responder a la definición de medicamento se le aplicará esta Ley, incluso si a dicho producto se le pudiera aplicar la definición contemplada en otra norma".**

Por razones que escapan a esta memoria, la preparación de las formas farmacéuticas de administración de las aguas mineromedicinales, su control y dispensación no recaen en un farmacéutico, salvo contadísimas excepciones. Esto, incluso cuando es manifiesto que las aguas mineromedicinales no son ajenas a lo que sucede con la mayoría de las sustancias con actividad farmacológica, en lo que se refiere a la necesidad de una correcta administración. En palabras del Profesor Vila Jato, "los principios activos son en general inadecuados para ser trasladados al organismo sin un vector, por lo que la Farmacia ha desarrollado las llamadas formas galénicas, en un claro homenaje a Galeno, adaptadas a las diferentes vías de administración" (Vila Jato, 2006), o en palabras del Prof. Cadórniga, "es preciso adecuarlo a la vía de administración o, lo que es lo mismo, conferir a la sustancia activa una forma farmacéutica cuyas propiedades deben estar en armonía con el objetivo que se desea alcanzar" (Cadórniga, 1983).

# I.2. PELOTERAPIA

Dicho lo anterior, cuando el agua mineromedicinal se aplica por vía tópica mezclada con otros componentes de distinta naturaleza, la terapia recibe el nombre de "peloterapia", y el preparado aplicado "peloide". Los peloides deben ser considerados medicamentos, por todo lo anteriormente dicho, en los que el agua mineromedicinal es la sustancia con actividad farmacológica (sustancia activa) y el resto de componentes los excipientes que sirven de vehículo del agua, posibilitando su preparación, asegurando su estabilidad y permitiendo su aplicación (Cerezo et al., 2014).

Desde un punto de vista etimológico, el término peloide procede del griego "Pelòs" (πελδς), que significa fango. En 1933 el *International Standard Measurements Committe* (I.S.N.C.) propone su uso como término general para los barros de uso medicinal y en 1937 la *International Society of Medical Hydrology* (ISMH) lo incluye con el mismo significado, como recogen distintos autores (Maraver, 2006; García et al., 2014). La definición se establece años más tarde, en la IV Conferencia Científica de la ISMH, celebrada en la ciudad de Dax (Francia), en la que quedan definidos los peloides como "*productos naturales consistentes en la mezcla de un agua mineral, comprendidas el agua de mar y la de lagos salados, con materias orgánicas o inorgánicas, resultantes de procesos geológicos o biológicos o a la vez geológicos y biológicos, utilizados con una finalidad terapéutica en forma de envoltura o baños*" (ISMH, 1949). Del término peloide deriva el concepto de peloterapia (del griego "Pelòs" (πελδς) = fango y "terapeia" = curación o remedio), entendiéndose como la aplicación local o generalizada de barros termales para tratar afecciones reumáticas, artritis y daños osteomusculares (Ferrand e Yvon, 1991; Veniale et al., 2004).

A partir de la primera definición de peloide, antes citada, han sido muchas las discusiones y controversias científicas, y diferentes autores han propuesto definiciones alternativas que han sido recogidas en el trabajo de Gomes et al. (2013).

Veniale et al. (2004) definen el barro termal como *aquel barro hipertermal o hipertermalizado producido por una mezcla primaria o secundaria de un componente sólido (normalmente un geomaterial arcilloso) con agua minero-termal. Después de una adecuada preparación ("maduración"), la mezcla da lugar a la producción de un barro terapéutico ("peloide") que es usado como un cataplasma en la práctica* *médica"*. Destacar de esta definición, la existencia de una formulación, preparación y administración.

Viseras et al. (2006a) definen los peloides como *"productos medicinales naturales de consistencia semisólida, constituidos por la interposición de sólidos orgánicos y/o inorgánicos en agua mineromedicinal que preparados convenientemente y administrados por vía tópica, en forma de aplicaciones locales o baños y en virtud de una serie de acciones biofísicas y/o bioquímicas se emplean en terapéutica para el tratamiento o prevención de ciertas patologías, o bien, para corregir sus efectos en el organismo".*

A la vista de estas y otras definiciones, Gomes et al. (2013) proponen la siguiente definición: "*lodo o dispersión de lodo con propiedades curativas y/o cosméticas, compuesto de una mezcla compleja de materiales naturales de grano fino de origen geológico y/o biológico, agua mineral o agua de mar, y comúnmente compuestos orgánicos procedentes de la actividad metabólica biológica*". Resulta llamativo que los autores incluyan propiedades curativas y cosméticas en el mismo producto. Efectivamente existen peloides de empleo cosmético y otros de uso terapéutico, pero difícilmente son intercambiables. Asimismo, obvian la necesidad de administrar o de aplicar estos lodos.

De acuerdo con las distintas definiciones, es evidente que el término peloide, al igual que la expresión fango termal, tienen un significado médico y no geológico o naturalista, como apuntaba hace años el Profesor Veniale (Veniale, 1997). De esta premisa surge la necesidad de plantear su estudio en términos farmacéuticos.

La principal clasificación de peloides es la propuesta por la ISMH en 1949 (ISMH, 1949), que los divide usando como criterios el origen, la composición y las condiciones de obtención (Tabla I.I).

1. Fangos o lodos, aquellos peloides en los que el componente sólido es un mineral (predominantemente arcilloso), disperso en agua mineral sulfurada, sulfatada o clorurada; se maduran en aguas hipertermales, mesotermales o hipotermales y el proceso se realiza *in situ* en tanques.
2. Limos, aquellos cuyo componente sólido es mineral (arcilla, cuarzo y caliza) y el componente líquido es agua de mar o lago salado. Se maduran en aguas hipotermales y el proceso se realiza *in situ*. En estos el componente orgánico es bastante más elevado que en los fangos.

3. Turbas, constituidas por un componente sólido orgánico y un componente líquido que puede ser agua mineral alcalina, sulfurada o agua de mar. Se maduran en aguas hipertemales, mesotermales o hipotermales y este proceso se realiza al aire libre o en recintos cerrados.
4. Biogleas, cuyo componente sólido es orgánico (algas y bacterias) y el componente líquido, de ordinario agua mineral sulfurada. Se maduran en aguas hipertermales, *in situ*.
5. Sapropelli, el componente sólido es de naturaleza orgánica-inorgánica y el componente líquido es agua sulfurada o alcalina, hipotermal. El proceso de maduración es *in situ*.
6. Gyttja, al igual que el anterior, el componente sólido es de naturaleza orgánica-inorgánica, pero la fase líquida es agua de mar.

**Tabla I.I.** Clasificación de los peloides de acuerdo con la ISMH (1949)

| DENOMINACIÓN | COMPONENTES DE LAS FASES | | CONDICIONES | |
|---|---|---|---|---|
| | SÓLIDO | AGUA | TEMPERATURA | MADURACIÓN |
| Fangos o lodos | Inorgánico (mineral) | Sulfuradas<br>Sulfatadas<br>Cloruradas<br>Bromuradas<br>Ioduradas | Hipertermal<br>Mesotermal<br>Hipotermal | *In situ*<br>En tanque |
| Limos | Inorgánico (mineral) | Agua del mar<br>Lago salado | Hipotermal | *In situ* |
| Turbas | Orgánico | Alcalinas<br>Sulfuradas<br>Carbonatadas<br>Ferruginosas<br>Agua del mar | Hipertermal<br>Mesotermal<br>Hipotermal | Aire libre<br>Recinto cerrado |
| Biogleas (tipo baregina) | Orgánico | Sulfuradas | Hipertermal | *In situ* |
| Otras Biogleas | Orgánico | No Sulfuradas | Hipertermal<br>Mesotermal<br>Hipotermal | |
| Sapropelli | Mixto | Alcalinas<br>Sulfuradas | Hipotermal | |
| Gyttja | Mixto | Agua de mar | Hipotermal | |

Con gran diferencia, los peloides más empleados son los fangos o lodos, por lo que a partir de este punto, centraremos nuestra atención en ellos y usaremos el término peloide o fango terapéutico de forma indistinta para referirnos a los sistemas constituidos por una fase sólida fundamentalmente inorgánica dispersa en agua mineromedicinal.

Los fangos terapéuticos que aparecen por el depósito de sedimentos procedentes de aguas de río, de lago salado o principalmente como sedimento de aguas mineromedicinales, se denominan peloides o fangos naturales, a diferencia de aquellos elaborados *ex profeso* para su utilización mezclando los componentes de acuerdo a un procedimiento que varía dependiendo del establecimiento balneario, y que se han venido denominando fangos o peloides artificiales (Veniale et al., 2007). Por tanto, los peloides naturales no requieren de ninguna preparación especial antes de su uso, mientras que los artificiales deben ser convenientemente elaborados/procesados antes de ser utilizados. En todo caso, ambos requieren o deberían requerir de controles de estabilidad, seguridad y eficacia antes de su aplicación.

Puesto que el empleo de peloides naturales está limitado en cuanto a la cantidad de material disponible (los peloides son recursos minerales no renovables y escasos) y a la imposibilidad de establecer una pauta común en las condiciones de elaboración, la mayoría de centros termales utilizan peloides artificiales. La elaboración de peloides artificiales se hace siguiendo procedimientos que, a priori, intentan simular las condiciones en las que aparecen los peloides naturales (Veniale, 2004). Dependiendo de la forma en que se realiza, cabe distinguir distintos tipos de peloides, como veremos al tratar los aspectos de elaboración de los fangos terapéuticos. Por ejemplo, son diferentes los peloides "madurados" de los "extemporáneos" (Armijo et al., 2010; Teixeira, 2010), entendidos como aquellos que son preparados en el momento de su uso y que, a diferencia de la mayoría de las veces, no son sometidos a ningún proceso de "maduración".

Paralelamente, la incorporación de otros excipientes a los peloides, conduce a plantear la necesidad de considerar otros tipos de peloides artificiales complejos, en los que junto con la sustancia activa y el excipiente habitual, se incluyan otros destinados a mejorar las propiedades (Cerezo et al., 2005). Evidentemente, un objetivo irrenunciable de la preparación debería ser mejorar, en la medida de lo posible, las propiedades de los peloides naturales, disminuyendo los inconvenientes que puedan presentar, como el contenido de elementos tóxicos, impurezas y otros. Los aspectos formulativos, así como aquellos derivados de la preparación son, sin duda, argumentos que justifican la necesidad de abordar el estudio de estos sistemas y preparados desde un punto de vista farmacéutico.

# I.3. FANGOS TERAPÉUTICOS

En las últimas décadas, distintos autores han estudiado la composición y propiedades de los fangos terapéuticos, tanto naturales como artificiales, usados en diversos centros termales, poniendo de manifiesto una elevada variabilidad composicional y con ella de sus propiedades. Estos estudios incluyen centros termales de Argentina (Baschini et al., 2010), Brasil (Untura et al., 2010), Cuba (Suarez et al., 2011), Croacia (Mihelčić et al., 2012), España (Maraver et al., 2005; Armijo-Suárez, 2007; Armijo et al., 2010; Carretero et al., 2010; Fernández-González et al., 2013; Pozo et al., 2013), Georgia (Masiukovichi et al., 2013), Italia (Veniale et al., 2004), Mongolia (Tserenpil et al., 2010), Portugal (Terroso et al., 2006; Rebelo et al., 2011b), Rusia (Zavgorud´ko et al., 2013), Túnez (Khiari et al., 2014), Turquia (Karakaya et al., 2010), entre otros. En todos los casos, hay que distinguir entre el componente activo; el agua mineromedicinal, el vehículo; el componente sólido rico en arcilla, y las impurezas inorgánicas u orgánicas que pueden aparecer como resultado de la elaboración (metales, sales, microorganismos, etc.). Una vez conocidos los dos componentes fundamentales del sistema disperso sólido/líquido, será necesario estudiar en profundidad la metodología de preparación de los sistemas y en definitiva las propiedades del preparado obtenido.

## I.3.1. COMPONENTE ACTIVO: AGUA MINEROMEDICINAL

El agua termal se ha utilizado desde la antigüedad, para prevenir o curar enfermedades, y en todo caso como medio para mejorar la calidad de vida. Su empleo entronca con el uso cultural y tradicional de recursos naturales para la mejora de la calidad de vida de las comunidades próximas a la fuente termal. Puede decirse que el empleo de aguas mineromedicinales para el tratamiento de diversas patologías constituye un importante proceder terapéutico, respaldado por siglos de utilización. Casi todos los pueblos de la antigüedad han tenido conocimiento de las virtudes terapéuticas y curativas de determinadas aguas y de los beneficios que se desprendían del uso regular del baño y del uso del agua como remedio natural (Armijo, 1968; Armijo y San Martín, 1994; Maraver, 2004). Entre los griegos este tipo de cura alcanzó enorme difusión, y la mayor parte de sus centros médicos disponían de manantiales cuyas aguas eran usadas con fines curativos. Pero fue en la época de los romanos cuando el uso del agua como remedio para la enfermedad alcanzó su auge con los *balnea* y los *thermae*. Esta tradición fue recogida posteriormente por los árabes, que fomentaron el empleo de los baños, así como la utilización de las aguas minerales (Armijo y San Martín, 1994). El uso de las instalaciones termales no se limitaba a acciones terapéuticas, sino que en la mayoría de los casos se empleaban con fines preventivos, de acuerdo con la dicotomía propia de la medicina griega y romana. En el centro termal quedaban así, agrupadas las funciones médicas asociadas a la mitología romana, que a través de los miembros de la familia de Escolapio, Dios Romano de la Medicina, incluían el tratamiento del dolor (bajo la protección de Epióne, esposa de Escolapio), la prevención y tratamiento de la enfermedad (dones de sus dos hijas; Higéa y Panacea), así como la convalecencia de enfermos (bajo la protección de su hijo Telesforo). No es de extrañar que los antiguos mirasen como sagrados todos los manantiales de aguas termales, dado su empleo en el tratamiento de un gran número de enfermedades.

Las aguas minerales han sido definidas como "soluciones naturales formadas bajo condiciones geológicas específicas y caracterizadas por un dinamismo químico-físico" (Lotti y Ghersetich, 1995). Aparecen en superficie en forma de manantiales y sus efectos terapéuticos están determinados por el contenido en cationes y aniones y la presencia de sales y oligoelementos, pero también están relacionados con la presión osmótica y la temperatura. La Sociedad Española de Hidrología Médica las define como "*aquellas aguas que, por su composición química, física y físico-química, tienen propiedades terapéuticas cuya utilidad terapéutica está avalada por el Estado al declararlas Utilidad Pública*" (http://www.hidromed.org/hm/index.php/conceptos-basicos/aguas-minero-medicinales).

La clasificación de las aguas mineromedicinales ha variado a lo largo de la historia en cuanto a terminología, pero no lo ha hecho en cuanto a las indicaciones, que se han mantenido fielmente hasta la actualidad. Las aguas minerales pueden ser clasificadas de acuerdo a su composición química y propiedades físicas (temperatura). Así, lo frecuente es clasificarlas, en función del anión predominante, en aguas mineromedicinales sulfurosas, sulfatadas, bicarbonatadas, carbónicas, ferruginosas o cloruradas. También pueden clasificarse dependiendo de la temperatura de emergencia en aguas frías (< 20 ºC), hipotermales (20 - 30 ºC), termales (30 - 40 ºC) o hipertermales (> 40 ºC) (Armijo y San Martín, 1994; Maraver, 2004).

En cuanto a su aplicación, se administran por distintas vías (oral, tópica e inhalatoria), en forma de baños, duchas, vapores, bebida, etc. La posología y duración del tratamiento dependerá en gran medida del estado del enfermo y de la patología a tratar. Los protocolos usados por el personal médico para el empleo de las aguas mineromedicinales son básicamente empíricos, de forma que en palabras de dos reconocidos expertos “se ha ido aprendiendo la mejor manera de aplicar estas aguas como tratamiento de diferentes enfermedades” (Armijo y San Martín, 1994).

Muchos de los estudios científicos realizados se han centrado en estudiar la composición de las aguas en vista a establecer relaciones con las acciones terapéuticas observadas, sin que hasta la fecha existan estudios que hayan abordado en profundidad los perfiles farmacocinéticos resultado de la administración de las aguas, así como los parámetros asociados a su comportamiento biofarmacéutico. Estos aspectos ya los apuntaban como críticos los citados expertos (Armijo y San Martín, 1994), y son sin duda uno de los campos científicos que deberían ser financiados para el avance del conocimiento en esta materia.

En esencia, la composición de las aguas mineromedicinales es distinta en cada centro termal, aunque su empleo sea similar. Las razones que explican esta paradoja están lejos de ser explicadas por la ciencia médica. No obstante, a efectos de su administración, no resulta necesario explicar este punto, por lo que en esta memoria, no parece razonable seguir profundizando en este aspecto. Es un problema farmacológico y no farmacéutico.

### I.3.2. EXCIPIENTES: ARCILLAS

En razón de la importancia que tienen los excipientes usados para la administración tópica del agua mineromedicinal para los objetivos de esta tesis, intentaremos profundizar de forma lo más breve posible en el concepto, estructura, propiedades y empleo de las arcillas como excipiente de formas farmacéuticas semisólidas.

### I.3.2.1. Concepto de arcilla

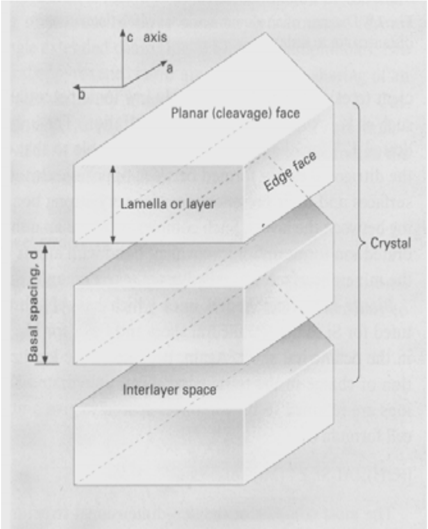
El término “arcilla” deriva del griego “argilos”, cuya raíz (“argos”) significa blanco. El diccionario de la Real Academia de la Lengua Española (RAE, 2001), define arcilla como: “Tierra finamente dividida, constituida por agregados de silicatos de aluminio hidratados, que procede de la descomposición de minerales de aluminio, blanca cuando es pura y con coloraciones diversas según las impurezas que contiene”. La comisión de nomenclatura de la Asociación Internacional para el estudio de arcillas (AIPEA) y de la “Clay Mineral Society” (CMS) define arcilla como “un material natural, compuesto principalmente de minerales de pequeño tamaño de partícula (la mayor parte filosilicatos), que es generalmente plástico en presencia de una cantidad apropiada de agua, y que se endurece al secarse” (Guggenheim y Martin, 1995; Guggenheim et al., 2006).

Profundizando en su concepto, son dos los criterios a tener en cuenta; uno granulométrico o textural, que define las arcillas como “partículas de dimensiones inferiores a 2 µm” y otro mineralógico que las define como “minerales estratificados o semi-estratificados hidratados” (Rautureau et al., 2004).

Puesto que las arcillas están compuestas principalmente por un grupo de minerales denominados filosilicatos, muchos autores utilizan la denominación de “minerales de la arcilla” para referirse a los filosilicatos (Hurbult y Klein, 1991) y no resulta de extrañar que se solapen ambos términos. El Comité de Nomenclatura de la AIPEA utiliza el término de “minerales de la arcilla” tanto para referirse a los filosilicatos como a otros materiales presentes en fases minoritarias y coparticipes en las propiedades fisicoquímicas de la arcilla. Tales minerales se denominan “fases asociadas a los minerales de la arcilla” e incluyen el cuarzo, feldespato, calcita y dolomita.

### I.3.2.2. Estructura de los minerales de la arcilla

La característica estructural fundamental de los filosilicatos es su disposición en capas apiladas, de forma que su densidad es baja, así como su dureza, en función del tipo de apilamiento (Baronnet, 1988; Milovski y Kononov, 1988; Battey, 1990; Hurlbut y Klein, 1991; Prothero y Shwab, 1996). Estan constituidos esencialmente por dos tipos de estratos, dispuestos regularmente el uno del otro a lo largo del eje *c* (Fig. I.1):



Terminología:

- **Plano de átomos** (O, OH, Si).
- **Estrato**: combinación de planos individuales de átomos (ej. estrato tetraédrico).
- **Lámina o Paquete**: combinación de estratos (ej. lamina TO, estrato tetraédrico y octaédrico consecutivos).
- **Cristal**: combinación de paquetes o láminas.
- **Interlámina**: espacio entre dos láminas sucesivas. Puede ser ocupado por agua, iones, moléculas orgánicas.
- **Espacio basal (d)**: parámetro característico de los fillosilicatos que deriva de la repetición de los planos de átomos a distancia regular en los cristales. Se considera constituido por la suma del paquete y la interlámina.

**Fig. I.1.** Estructura general de los filosilicatos

Estratos tetraédricos: constituidos por el conjunto de tetraedros ($SiO_4^{-4}$) en disposición hexagonal con tres oxígenos compartidos y el cuarto enlace Si-O en dirección perpendicular al plano, dando lugar a agrupaciones $Si_4O_{10}^{-4}$. Pueden darse sustituciones de modo que el Si puede estar parcialmente sustituido por Al, apareciendo unidades tales como, $AlSi_3O_{10}^{-5}$ ó $Al_2Si_2O_{10}^{-6}$ (Fig. I.2).

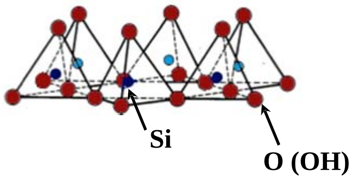


**Fig. I.2.** Estrato tetraédrico

Estratos octaédricos: constituidos por la coordinación hexaédrica de una serie de cationes (Mg, Al, Fe (II), Fe (III), Li, Ti, V, Cr, Mn, Co, Ni, Cu, Zn) con oxígenos, grupos OH-, e incluso F-, ubicados en las capas tetraédricas. La unidad estructural básica estará constituida por 4 tetraedros y 3 octaedros; en función de si están ocupados los 3 octaedros o sólo 2, por un catión, podemos hablar de estratos octaédricos trioctaédricos (formados por cationes divalentes en todas las posiciones octaédricas, como por ejemplo el $Mg^{2+}$) y estratos octaédricos dioctaédricos (formados por cationes trivalentes que ocupan 2 de cada 3 posiciones octaédricas, como por ejemplo el $Al^{3+}$). (Fig. I.3).

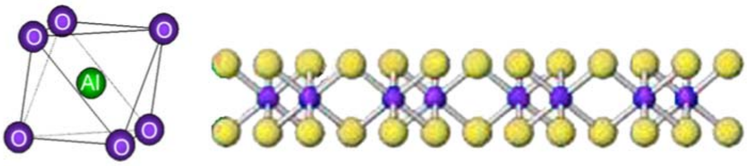


**Fig. I.3.** Estrato octaédrico

Los estratos tetraédricos (T) y octaédricos (O) se asocian para formar paquetes estructurales, que pueden ser eléctricamente neutros o presentar una carga negativa, compensada por cationes no coordinados que se localizan en el espacio interlaminar.

La alternancia de estratos da lugar a distintos tipos de filosilicatos (Tabla I.II):

- Filosilicatos tipo 1:1. La estructura básica estará formada por un estrato tetraédrico (T) y un octaédrico (O), siendo este último dioctaédrico o trioctaédrico. Se trataría de la capa T-O.
- Filosilicatos tipo 2:1. Siendo su estructura básica dos estratos tetraédricos (T) y uno octaédrico (O), el cual puede ser dioctaédrico o trioctaédrico. Se trataría de la capa T-O-T, ó, también conocida como estructura de “sandwich”. Cuando el Si está sustituido por otros elementos, tales como Al (III) o Fe (III) se genera la aparición de carga, puesto que se pierde la neutralidad. El balance de carga se mantiene por la presencia en el espacio interlaminar, o espacio existente entre dos láminas consecutivas, de cationes (como por ejemplo en el grupo de las micas), cationes hidratados (como por ejemplo en las vermiculitas y esmectitas) o grupos hidroxilos coordinados octaédricamente, similares a los estratos octaédricos, como sucede en las cloritas (por lo que también se les denomina T-O-T-O ó 2:1:1). Los cationes interlaminares normalmente son alcalinos ($Na^{+}$ y $K^{+}$) o alcalinotérreos ($Mg^{2+}$ y $Ca^{2+}$). Se considera que la unidad formada por una lámina (T-O ó T-O-T) más el espacio interlaminar, es la unidad estructural.

**Tabla I.II.** Clasificación de los filosilicatos de acuerdo con la AIPEA

| **Tipo** | **Material interlaminar** | **Grupo** | **Subgrupo** | **Mineral (ejemplos)** | **Formula mineralógica** |
|---|---|---|---|---|---|
| **1:1** | Ninguno o agua | Serpentina-Kaolinita ($x \equiv 0$) | Serpentinas<br>Kaolin | Crisotilo<br>Kaolinita<br>Halloysita | $Al_2Si_2O_5(OH)_4$<br>$Mg_3Si_2O_5(OH)_4$ |
| | Ninguno | Talco-Pirofilita ($x \equiv 0$) | Talco<br>Pirofilita | Talco<br>Pirofilita | $Mg_3Si_4O_{10}(OH)_2$<br>$Al_2Si_4O_{10}(OH)_2$ |
| **2:1** | Cationes hidratados intercambiables | Esmectita ($x \equiv 0.2\text{-}0.6$) | Saponita<br>Montmorillonita | Saponita<br>Hectorita<br>Montmorillonita<br>Nontronita | $Ca_{0.25}Si_4(Mg_{2.5})Li_{0.5})O_{10}(OH)_2nH_2O$<br>$(Na,Ca)(Al,Mg)_2(Si_4O_{10})(OH)_2nH_2O$ |
| | | Vermiculita ($x \equiv 0.6\text{-}0.9$) | Vermiculitas dioctaédricas | Vermiculitas dioctaédricas | |
| | | | Vermiculitas trioctaédricas | Vermiculitas trioctaédricas | $Ca_{0.65}(Si_{2.86}Al_{1.14})(Mg_{2.83}Al_{0.15}Fe_{0.01})O_{10}(OH)_2nH_2O$ |
| | Cationes no hidratados | Mica ($x \equiv 0.5\text{-}1$) | Micas dioctaédricas | Moscovita, Paragonita | $KAl_2(Al, Si_3O_{10})(OH)_2$ |
| | | | Micas trioctaédricas | Biotita, Lepiolita | $K(Fe,Mg)_3(Al,Si_3)O_{10}(OH)_2$ |
| | | Mica frágil ($x \equiv 2$) | Micas frágiles dioctaédricas | Margarita | $Ca(Si_2Al_2)Al_2O_{10}(OH)_2$ |
| | | | Micas frágiles trioctaédricas | Clintonita | $Ca(Mg,Al)_3(Al_3Si)O_{10}(OH)_2$ |
| | Hidróxidos | Clorita<br>x variable | Cloritas Dioactaédricas | Donbassita | $Al_2(Al_{2.33})(Si_3AlO_{10})_4(OH)_8$ |
| | | | Cloritas Di, Trioctaedricas | Cookeita | $Li, Al_4(Si_3Al)O_{10}(OH)_8$ |
| | | | Cloritas Trioctaedricas | Clinochloro | $(Fe,Mg,Al)_6(Si, Al)_4O_{10}(OH)_8Si_{12}O_{30}$ |
| | Ninguno | Sepiolita-Palygorskita (x variable) | Sepiolitas<br>Palygorskitas | Sepiolita<br>Palygorskita | $Mg_8(OH)_4(OH_2)8H_2O$<br>$(Mg,Fe,Al)_5Si_8O_{20}(OH)_2(OH_2)4H_2O$ |

X = carga por unidad de fórmula

Dentro del amplio grupo de los filosilicatos, el grupo de las esmectitas es el de mayor interés en cuanto a uso farmacéutico, debido principalmente a su estructura (filosilicato tipo 2:1) y a los cationes intercambiables presentes en la interlámina. Las suspensiones acuosas de los minerales de este grupo originan sistemas altamente estructurados, con propiedades reológicas muy interesantes. La familia de las esmectitas está formada por todas aquellas arcillas que presentan una estructura de "sandwich", es decir, que está constituida por dos hojas de tipo T y por otra intermedia de tipo O (T-O-T). Como resultado de las sustituciones isomórficas del silicio parcialmente en la capa T, o bien el magnesio o el hierro sustituyendo al aluminio en la capa O, e incluso de flúor (F) en sustitución del oxígeno de la capa O, aparecen distintos minerales (Tabla I.III).

**Tabla I.III.** Algunos tipos de esmectitas con sus fórmulas moleculares

| | |
|---|---|
| ***Dioctaédricas*** | |
| Montmorillonita | $(OH)_4Si_8(Al_{3.34}Mg_{0.66})O_{20}$ |
| Beidellita | $(OH)_4Si_8(Al_{6.34}Mg_{1.66})Al_{4.34}O_{20}$ |
| Nontronita | $(OH)_4(Si_{7.34}Al_{0.66})Fe^{3+}_{4.34}O_{20}$ |
| ***Trioctaédricas*** | |
| Hectorita | $(OH)_4Si_8(Mg_{5.34}Li_{0.66})O_{20}$ |
| Saponita | $(OH)_4(Si_{7.34}Al_{0.66})Mg_6O_{20}$ |

Entre los minerales del grupo de las esmectitas destaca la montmorillonita, cuyo nombre deriva del francés Montmorillon, lugar de Francia en que se encontró en 1847 (Damour y Salvetat, 1847). Cuando una roca tiene una elevada proporción de este mineral se denomina Bentonita. Este nombre aparece asimismo como monografía de las principales farmacopeas asociado a la elevada pureza en montmorillonita o a minerales del grupo de las esmectitas que confieren propiedades similares a los preparados, por lo general comercializados bajo nombres registrados entre los que destaca el Veegum$^{©}$ en sus distintas variedades (López-Galindo et al., 2007; Viseras et al., 2007).

### *I.3.2.3.Propiedades de las arcillas*

Por definición, las arcillas son materiales que al incorporarles agua presentan un comportamiento plástico. Esta propiedad es, sin duda, referente de las arcillas, pero junto a la plasticidad, otras propiedades las hacen interesantes en el ámbito de la farmacia.

Capacidad de intercambio catiónico (C.I.C.)

Como consecuencia de las distintas sustituciones de cationes estructurales por otros cationes de diferente valencia se origina una carga residual en la superficie de las arcillas (sustitución isomórfica). Esta carga superficial se compensa con la adsorción química de cationes intercambiables y por tanto es responsable directa de la capacidad de intercambio catiónico. Por ejemplo, cuando se sustituye un átomo de silicio ($Si^{4+}$) por uno de aluminio ($Al^{3+}$) en la capa tetraédrica, o uno de $Al^{3+}$ por uno de $Mg^{2+}$ en la octaédrica, aparece una carga residual negativa en la superficie, la cual puede ser compensada mediante la adsorción química de cationes de cambio como $Na^{+}$, $K^{+}$, o $Mg^{2+}$. La sustitución en la capa octaédrica se pone de manifiesto más levemente a nivel superficial, dado que se trata de una capa más interna de la estructura. Por tanto la capacidad de intercambio catiónico de una arcilla será directamente proporcional a la densidad de carga superficial existente. También la acidez superficial está relacionada directamente con este hecho, observándose que a mayor número de sustituciones isomórficas en la capa tetraédrica, mayor es su acidez (Castaing, 1998). Las arcillas con mayor C.I.C. son las esmectitas, diferenciándose en tipo cálcica, cuando la carga superficial se compensa con cationes $Ca^{2+}$ y tipo sódica cuando se compensa con cationes $Na^{+}$, teniendo estas últimas mayor C.I.C. (200 y 100 respectivamente).

Superficie específica

La superficie específica ($m^2/g$) permite tener una idea relativa del área externa accesible; cuanto mayor sea, mayor cantidad de sustancias podrán ser distribuidas de manera homogénea sobre ella. Pero siendo así resulta esencial que dicha superficie tenga muy baja actividad química para minimizar y/o evitar las posibles interacciones negativas con sustancias de alto valor nutritivo o farmacológico.

Hidratación

La hidratación de las arcillas implica la adsorción de moléculas de agua a través de la hidratación de los iones interlaminares y la interacción de la superficie del mineral con las moléculas de agua y con los iones (cationes y/o aniones), pero también interviene la actividad (contenido) de agua presente. En cuanto a la capacidad de hinchamiento en agua podemos decir que sirve de base para la clasificación industrial más aceptada de las bentonitas. Según este criterio Patterson y Murray (1983) establecieron los siguientes tipos: Bentonitas altamente hinchables o sódicas, Bentonitas poco hinchables o cálcicas y Bentonitas moderadamente hinchables o intermedias. Posteriormente Odom (1984), siguiendo los mismos criterios las divide en: Bentonitas sódicas, Bentonitas cálcio-magnésicas y Tierras de Fuller o tierras ácidas. En esta última clasificación se incorporan como materiales bentoníticos las Tierras de Fuller (conocidas en España como tierras de batán) mas, a manera de aclaración, indicamos que bajo la denominación de Tierras de Fuller se comercializa gran variedad de minerales de la arcilla que van desde esmectitas cálcicas y/o paligorskita, a montmorillonita asociada a ópalo como constituyente mayoritario, e incluso haloisita y caolinita.

### *I.3.2.4. Arcillas Comerciales*

Las arcillas comerciales son aquellas que sirven como materia prima industrial, de las cuales un 90 % de la producción se dedica preferentemente a la fabricación de materiales de construcción y agregados, y sólo un 10 % se emplea en otras industrias (fabricación de papel, caucho, pinturas, adsorbentes, decolorantes, arenas de moldeo, productos químicos y farmacéuticos, agricultura, etc.) En general al primer tipo (las que se utilizan en construcción) se les denomina arcillas cerámicas y son arcillas compuestas por dos o más minerales de la arcilla, generalmente ilita y esmectita, con importantes cantidades de otros minerales que no son filosilicatos (carbonatos, cuarzo...).

Al segundo tipo se les denomina arcillas especiales, constituidas fundamentalmente por un sólo tipo de mineral de la arcilla, y sus propiedades dependen esencialmente de las características de ese mineral. Estas se pueden dividir en: Caolines y arcillas caoliníferas, Bentonitas y arcillas fibrosas (Sepiolita y Paligorskita).

### *I.3.2.5. Aplicaciones terapéuticas y cosméticas de las arcillas*

El hombre ha utilizado desde tiempos prehistóricos los recursos naturales con fines terapéuticos, principalmente plantas y partes de plantas, pero también ha usado materiales inorgánicos, y en particular las arcillas, debido a su abundancia, accesibilidad y propiedades especiales (López Galindo et al., 2006; Gomes y Silva, 2007). La primera evidencia histórica del uso de la arcilla con finalidad terapéutica aparece en una tablilla sumerio-mesopotámica, datada alrededor del 2500 a. C. (Biggs, 2005). La mayor parte de las civilizaciones antiguas emplearon arcillas para curar heridas, aliviar irritaciones cutáneas o tratar trastornos gastrointestinales, y son muchos los textos antiguos mediterráneos/europeos los que evidencian estos usos (De Vos, 2010). Así, en el libro "De Materia Médica", de Dioscórides, se puede encontrar una sección dedicada a minerales y sustancias químicas usadas en farmacia (Carretero, 2002). Hay referencias del uso de las "tierras medicinales" en Mesopotamia, el Antiguo Egipto y la Antigua Grecia, y una de ellas, la "Terra sigilata", puede considerarse como el primer medicamento registrado de la historia (Finkelman, 2006; Perea, 2014). Otros ejemplos de "tierras medicinales" son la terra Lemnia (procedente de Lemnos), la terra Melitea (de Malta), la terra Armenica (de Armenia), la terra Silesiaca (de Silesia), la terra Florentina (de Italia) o la terra Hispanica (de España).

Las arcillas se han utilizado como excipientes y principios activos en formas farmacéuticas y cosméticos (López Galindo y Viseras, 2004; López Galindo et al., 2006) y como nanomateriales para la obtención de sistemas de liberación de fármacos e ingeniería de tejidos (Aguzzi et al., 2010; Viseras et al., 2010; Aguzzi et al., 2013a; Salcedo et al., 2012, 2014).

Como principios activos se han utilizado como antidiarreicos, protectores gastrointestinales, antiácidos, antiinflamatorios, antisépticos, laxativos osmóticos y protectores dermatológicos, principalmente por la capacidad que tienen para adsorber y retener agua, bacterias y algunas toxinas (López-Galindo et al., 2011; Carretero et al., 2006, 2013). Como excipientes se han incluido en las formulaciones como lubricantes, emulsionantes, espesantes, viscosizantes, agentes humectantes, agentes de suspensión, estabilizantes, agentes de relleno, abrasivos, adsorbentes, aglutinantes, agentes de recubrimiento, antiagregantes, aglutinantes, con el objetivo de facilitar la administración de los principios activos, mejorar su eficacia y asegurar la estabilidad hasta la fecha límite de su utilización (Viseras et al., 2007; Rowe et al., 2009). Tanto como principios activos, como excipientes, están presentes en formas de administración sólidas (comprimidos, cápsulas, granulados y polvos), líquidas (suspensiones y emulsiones) y semisólidas (pomadas, pastas, geles, ungüentos, cremas, espumas).

Centraremos a continuación la atención en los usos farmacéuticos como excipiente de formas farmacéuticas semisólidas, por ser este el empleo que tendrán las arcillas en la elaboración de fangos terapéuticos.

A mitad del siglo pasado, Levy (1962a, b) publicó un estudio sobre los aspectos reológicos de las dispersiones tixotrópicas de montmorillonita y los cambios producidos en su comportamiento al añadir polisorbato 80, así como la cinética de recuperación estructural (reestructuración). Tamasini y Trandafilov en 1964 investigaron el comportamiento tixotrópico de un gel de bentonita (15 %) tratado con álcali y el de sus mezclas con sustancias tanto hidrófilas como hidrófobas. Unos años antes, Jenkins et al.

(1957) citaban el magma de bentonita al 5 % como agente de suspensión indicando que se añada a la formulación un 40 % del citado magma, que es estable a pH 3-10 e incompatible con iones calcio y electrolitos di y trivalentes. La elaboración del magma de bentonita, así como el gel y emulsiones A/O y O/A, junto con la descripción del producto y ejemplos de formulaciones, aparece citado en obras de carácter general como en Denoël (1971), o en Helman (1980), quien presentaba a la bentonita como un emulgente perteneciente al tipo de "sólidos finamente divididos" considerándolo como sólido pulverulento de carácter hidrófilo (bentonita y trisilicato magnésico). Se emplea generalmente en la formulación de emulsiones O/A (Le Hir, 1995), sin embargo como opinaba Martin (1971) hay que tener en cuenta que la obtención de emulsiones A/O u O/A depende del orden de mezclado, ya que si la fase oleosa se añade al magma de bentonita se obtiene una emulsión O/A, si es el magma al aceite, una emulsión A/O. Otra aplicación de la bentonita fue descrita por Bianculli (1965), para la formulación de una pasta para electrodos en electroencefalografía, compuesta por una solución saturada de calcio (540 mL), glicerina (30 mL) y bentonita (908 g). También Barr (1964) presentaba a las arcillas entre los coloides protectores empleados como agentes estabilizadores de suspensiones, mostrando la aplicabilidad de las del grupo de la montmorillonita. De la misma manera, Tufegdzic (1965) empleaba con éxito la bentonita como estabilizador de suspensiones por la formación con el agua de un vehículo con propiedades reológicas elásticas. Estudios con los mismos propósitos fueron los de Samyn y Jung (1967) quienes hablaban de las suspensiones defloculadas de arcillas y de su tixotropía negativa; Kibbe y Araujo (1969) describen las propiedades tixotrópicas de sistemas dispersos montmorillonita/agua, aplicando para su tipificación técnicas de RMN, y Schnaare et al. (1976) investigaron un método rápido y eficaz para la obtención de curvas de flujo en fluidos no newtonianos tixotrópicos tiempo-dependientes, aplicado a una dispersión de bentonita y carbopol 940. En 1980, Ponti y Zanotti-Gerosa estudian la posible influencia de estos agentes de estabilización en suspensiones de carboximetilcelulosa. La utilización de Veegum$^{©}$ (silicato de Al y Mg) de alta viscosidad (Veegum$^{©}$ HV) presenta la ventaja de conferir a las suspensiones propiedades tixotrópicas a concentraciones inferiores a las de bentonita (5-10 %). La mezcla de Veegum$^{©}$ (2 %) y laponita en agua destilada caliente (80-90 ºC), bajo agitación intensa durante 5 minutos, origina una dispersión que se deja en reposo 24 horas (para que la hidratación sea suficiente y tenga las propiedades reológicas adecuadas). Siendo esta dispersión sensible a electrolitos y sustancias de naturaleza catiónica que la floculan, recomendándose añadir un coloide protector (preferentemente carboximetil celulosa, agente viscosizante soluble en agua, en proporción 1/4 o 1/5 en peso) o un agente peptizante, como el citrato sódico que por aumentar el potencial Z reducen el efecto floculante (Denoël et al., 1971). Primorac et al. (1986a, b) determinaron la viscosidad de geles utilizados como vehículos para anticonceptivos vaginales a base de carboximetil celulosa y Veegum© HV, así como el efecto de sus concentraciones y el tiempo de almacenamiento en la rotura del gel, llegando a concluir que los geles de Veegum© muestran un incremento de viscosidad con la concentración (especialmente a 7.5 %) y con el almacenamiento. Posteriormente, observaron la influencia de la adición a los citados geles de lauril sulfato sódico, constatando que en los geles de Veegum© la viscosidad disminuye al aumentar la concentración de laurilsulfato sódico (especialmente para valores de 5 %).

Debemos puntualizar que dentro de los llamados hidrogeles o excipientes semiconsistentes hidratados se cita a la bentonita, que incorporada en agua al 10-15 % da lugar a geles bien tolerados, lavables y con poder de penetración nulo, por tanto apropiados para uso únicamente tópico (Le Hir, 1995). Dicho poder gelificante aumenta con la adición de sustancias alcalinas y disminuye en presencia de ácidos (Denoël et al., 1971). El principal inconveniente es que los preparados se "resecan" con facilidad, pues el agua no es retenida con firmeza, y durante el almacenamiento puede producirse pérdida de agua; el problema se minimiza añadiendo a la dispersión de bentonita vaselina, glicerina o sistemas reguladores.

Siguiendo el orden cronológico, en 1987 Serrao y Bregni, obtienen complejos insolubles con montmorillonita y compuestos orgánicos polares que utilizan en la estabilización de emulsiones de fase externa acuosa (O/A) y en 1991, Ciullo y Braun, también con emulsiones O/A, y en sistemas simples, observan el incremento de la viscosidad por efecto de la mezcla de Veegum© Ultra y carbopol (sinergismo reológico). Braun (1991), investigó también el efecto sinérgico en lociones hidratantes tópicas por mezclas de los mismos productos y observó que pequeños cambios en las concentraciones de los ingredientes producen fuertes variaciones en la reología de los sistemas simples; en las emulsiones O/A también se producía sinergismo, pero el valor del efecto era menor en relación a los sistemas simples. En 1992, son Chen y Zatz los que caracterizaron reológicamente el resultado de la interacción, en este caso en sistemas goma Xantano y Veegum$^{©}$, postulando que el Veegum$^{©}$ proporcionaba rigidez y la goma Xantano aumentaba la viscosidad de las dispersiones. Nuevamente Ciullo y Braun (1992) abordaron la cuestión presentando las ventajas de Veegum$^{©}$ frente a otras arcillas esmectíticas en la estabilización de preparados tópicos. La asociación de bentonita con otros excipientes, como el almidón, fue investigada por Besün (1997a, b), que describió tanto la estructura de los geles, como sus propiedades reológicas. Estos y otros muchos autores emplean excipientes arcillosos para elaborar formas farmacéuticas semisólidas. En el grupo de investigación en el que he realizado mi tesis doctoral son numerosos los estudios publicados en los últimos años en este sentido (Viseras y Lopez-Galindo, 1999; Viseras et al., 1999; Viseras y Lopez-Galindo, 2000; Viseras et al., 2000; Viseras et al., 2001b; López Galindo y Viseras, 2004; Viseras et al., 2007).

Paralelamente al empleo estrictamente farmacéutico, las mismas arcillas son usadas en cosmética con funcionalidades similares. Es sabido que el magma y geles de bentonita se emplean como materias primas en este campo; dichas bases no solo proporcionan viscosidad, sino que además resultan interesantes por sus propiedades humectantes y poder estabilizador de emulsiones O/A. Un gel es base de la loción de calamina, de acción superficial, empleada como calmante (Aron-Brunetière, 1985). En Dermofarmacia tenemos multitud de ejemplos de formulaciones de mascarillas faciales elaboradas con arcillas coloidales para pieles normales (pH 5-6) con distintas acciones, como por ejemplo calmante o nutritiva. Además, no se puede olvidar el empleo de sustancias opacas, como es la bentonita, como pantalla solar (actúan reflejando totalmente las radiaciones ultravioletas). Sin embargo debido a que adolece de los inconvenientes que poseen todas las sustancias utilizadas con tal fin (poder cubriente, opacidad, difícil aplicación, extensión en capas gruesas) no resultan cosméticamente aceptables; no obstante, habrá casos en los que dichos filtros "pantalla" son precisos y aconsejables, como por ejemplo para protección en la nieve, aplicación sobre cicatrices y sobre ciertas manchas de la piel (Aron-Brunetère, 1985). Se emplean también en cosmética como base para maquillajes y como agentes de suspensión en productos para el cabello. En jabones de tocador los silicatos naturales actúan como productos cubrientes, siendo los más empleados el talco, la esteatita y la bentonita (Déribère y Esme, 1952). En dentífricos también se emplea la bentonita y Veegum$^{©}$ como agentes ligantes; la bentonita coloidal retiene el agua y permite obtener una pasta bien uniforme cuando se añade a los dentífricos (en proporción 2-3 %); lo importante y a tener en cuenta, es que en el caso de esta bentonita coloidal se puede emplear en una cantidad mayor, dado que no contiene cuarzo, el cual arañaría el esmalte dental y además por su ligero efecto abrasivo favorece la limpieza de los dientes Bonadeo (1963, 1964). El mismo autor habla de su uso en depilatorios, dado que estas arcillas facilitan la eliminación de la pasta extendida sobre la piel y al mismo tiempo disminuyen el riesgo de irritación y de su utilización en lacas de uñas, entre cuyos componentes se incluyen sustancias modificadoras de la viscosidad como la bentonita y bentonas, siendo estas últimas el resultado de la combinación de bentonita con bases de amonio cuaternario, que por la existencia de un grupo lipófilo gelifican cuando se dispersan adecuadamente en disolventes orgánicos. Podemos citar otras aplicaciones de la bentonita en este campo. Como aditivo reológico, en la elaboración de distintos preparados: Ciullo (1981) presentaba unas fórmulas de buena viscosidad y flujo elaboradas con Veegum$^{©}$ y goma xantano, 9:1 y 2:1, con buenas cualidades estabilizadoras de todo tipo de suspensiones, lociones y productos de maquillaje fluidos. Alexander (1986 a, b, 1990) en una revisión dedicada a los aditivos reológicos de uso en la industria cosmética para preparados del cuidado personal, incluye la montmorillonita y derivados organofílicos. Este mismo autor, en 1990, examina el uso de ingredientes bio-activos en formulaciones cosméticas, citando entre ellos a las arcillas. Otra revisión de las esmectitas y sus aplicaciones en cosmética en productos de cuidado personal fue presentada por Dell en 1993.

Este breve resumen de las aplicaciones de determinadas arcillas como excipiente de formas farmacéuticas y cosméticas sirve para destacar que no son, en absoluto, sustancias ajenas al campo de la tecnología farmacéutica, y en concreto al desarrollo de formas semisólidas de administración, en razón de sus propiedades (Viseras et al., 2007).

A la vista del empleo de las arcillas en la preparación de formas farmacéuticas semisólidas, no es de extrañar su uso en la elaboración de fangos terapéuticos.

## I.3.3. FORMULACIÓN DE FANGOS TERAPÉUTICOS

El agua mineromedicinal con frecuencia arrastra sólidos suspendidos hasta la superficie, que por decantación originan barros. Estos fangos, de composición obviamente muy variada, dependiendo de las rocas con las que el agua ha tenido contacto durante su ascenso hasta aparecer en superficie, han sido usados como “medicamentos naturales” de forma paralela a la del agua mineromedicinal. En la mayoría de los centros termales, los barros naturales son escasos y como sustitutivos se han preparado suspendiendo en el agua sólidos pulverulentos originarios de zonas próximas o adyacentes a la fuente termal. El origen, por tanto, de los fangos terapéuticos "artificiales" está ligado a la búsqueda de análogos de los sedimentos naturales, y hasta finales de siglo pasado, sino incluso en este mismo siglo, se ha hecho de forma empírica.

Las propiedades finales del producto dependen en gran medida de los materiales con los que se elabore, por lo que la selección de estos materiales es un proceso determinante. La elección de un material adecuado debe tener claramente en cuenta factores tales como composición mineral, quimismo, pH, granulometría, superficie específica, capacidad de intercambio catiónico, parámetros de consistencia, reología, comportamiento térmico y contenido en microorganismos y materia orgánica.

Una primera aproximación a la formulación de los fangos terapéuticos conduce a la necesidad de emplear materiales que presenten un elevado contenido en arcillas hinchables, con una granulometría fina y baja cantidad de minerales "abrasivos", para hacer agradable la aplicación del peloide y que no contengan elementos peligrosos (Viseras y López-Galindo, 1999; Viseras et al., 2006c; López-Galindo et al., 2007). En este sentido es importante el control del contenido de determinados elementos traza potencialmente tóxicos y de su movilidad durante el proceso de maduración, con objeto de evitar posibles intoxicaciones durante el tratamiento (Summa y Tateo, 1998, 1999; Mascolo et al., 1999).

La importancia de la identidad de los materiales que se van a utilizar para la elaboración de fangos terapéuticos ha hecho que sean numerosos los autores interesados (Cara et al., 2000a; Viseras et al., 2001a; Viseras et al., 2006c; Legido et al., 2007; Baschini et al., 2010; Abdel-Motelib et al., 2011; Rebelo et al., 2011a; Carretero et al., 2014; Khiari et al., 2014; Mefteh et al., 2014). En general, los autores coinciden en que de todas las arcillas, las bentonitas son las que resultan idóneas para la elaboración de fangos terapéuticos, y su porcentaje en la mezcla resulta un parámetro útil para discriminar la idoneidad de los mismos. Esto se debe al elevado índice de hinchamiento que presentan, a la alta plasticidad y calor específico, que hacen que mejoren las propiedades físicas y térmicas, y consecuentemente, la calidad (Cara et al., 2000b; Legido et al., 2007). Por ello, se considera que los fangos elaborados con bentonita son los mejores para la aplicación en fangoterapia (Morandi, 1999; Novelli, 2000). El uso de arcillas en fangoterapia es un campo emergente de aplicaciones biomédicas de estos materiales, en el que resulta imprescindible la adecuada caracterización de los materiales que vienen siendo empleados y su optimización, tanto en la preparación, como en su empleo adecuado, mejora de sus propiedades y personalización e individualización dependiendo de las características del paciente y su patología (Veniale et al., 2007; Karelina et al., 2009).

## I.3.4. ELABORACIÓN DE FANGOS TERAPÉUTICOS

La elaboración de un fango terapéutico consiste, obviamente, en la interposición del componente sólido en una fase líquida (el agua mineromedicinal) para obtener un sistema disperso sólido/líquido que denominamos fango. El fango resultado de esta interposición (mezclado) se considera "inmaduro" y para ser usado se somete a un procedimiento denominado "maduración", que se supone necesario para que el fango terapéutico sea adecuado para su empleo. Básicamente, la maduración consiste en almacenar el fango en un depósito durante un tiempo prolongado (meses) antes de ser usado. Durante ese tiempo puede estar sometido o no a distintas operaciones (cambio del agua sobrenadante, agitación periódica, etc…). La preparación y consiguiente maduración de un fango se produce de modo distinto en función del balneario. De manera habitual se produce al aire libre, bajo la acción de las condiciones ambientales del lugar. Durante la maduración tienen lugar reacciones entre el agua mineromedicinal y la arcilla, junto con procesos biológicos y bioquímicos relacionados con el crecimiento de microorganismos y algas (Curini et al., 1990). Diversos estudios han evaluado la influencia de los componentes usados, tanto minerales como orgánicos, en las propiedades del fango terapéutico (Ferrand e Yvon, 1991; Veniale, 1997; Summa y Tateo, 1998; Bettero et al., 1999; Cara et al., 2000a). El tiempo establecido de maduración es variable, normalmente entre dos meses (Galzigna et al., 1996 y 1998) y dos años (Veniale et al., 2004). Tolomio et al. (1999) consideran que la maduración comienza cuando el agua termal se mezcla con la arcilla, y que el tiempo de maduración depende de cuando se complete la colonización por parte de los microorganismos termófilos en el barro.

A la variabilidad composicional de las aguas mineromedicinales y del sólido se suma así otra variable: el procedimiento de elaboración con la maduración consiguiente, en el que hay que considerar la relación sólido/líquido, el tiempo, la temperatura y las condiciones de agitación (continua, discontinua, sin agitación). Se trata de un proceso en el que pueden influir distintos factores. Las condiciones de maduración han sido muy estudiadas en los últimos años. Veniale et al. (2004), estudiaron el efecto de la maduración de un fango formulado con la misma fase sólida y distintos tipos de agua; Carretero et al. (2007), estudiaron la influencia de las condiciones de agitación en mezclas de saponita y montmorillonita y agua de mar; Gámiz et al. (2009), estudiaron la influencia del tipo de agua y del tiempo de maduración en fangos en los que la fase sólida era una mezcla de kaolinita-saponita y Tateo et al. (2010), estudiaron fangos elaborados con agua mineral alcalino-sulfatada considerando la maduración a corto y largo plazo. Todos estos estudios conducen a resultados en los que se ponía de manifiesto la gran variabilidad de propiedades que derivan de la composición y método de elaboración. No obstante, el fin último de la maduración se supone que es mejorar las propiedades del producto, estabilizándolas y en lo posible exaltándolas u optimizándolas. Por tanto este proceso debería estar totalmente controlado.

El proceso de maduración de fangos a partir de materiales arcillosos puede provocar cambios en las propiedades técnicas de estos sistemas, variando sus propiedades reológicas, incluyendo la plasticidad, y térmicas, así como la granulometría de la fase dispersa. Además puede inducir cambios mineralógicos, del quimismo y de la textura de la fase sólida (De Bernardi y Pedrinazzi, 1996; Minguzzi et al., 1999; Veniale et al., 1999; Sánchez et al., 2002; Veniale et al. 2004; Viseras et al., 2006b; Gámiz et al., 2008). Diversos autores y estudios han evaluado los cambios que se producen durante la maduración (Curini et al., 1990; Galzigna et al., 1995, 1996, 1999a,b; Curri et al., 1997; Tolomio et al., 1999; Sánchez et al., 2002; Veniale et al., 2004; Viseras et al., 2006; Carretero et al., 2007; Gámiz et al., 2009; Karakaya et al., 2010; Quintela et al., 2010; Tateo et al., 2010). En palabras de Curini et al. (1990) estos cambios pueden ser de tres tipos; se pueden producir cambios en las propiedades físico-químicas de la arcilla, pueden aparecer nuevas especies químicas o que incrementen otras como resultado de la actividad biológica, o bien otras especies presentes pueden desaparecer o disminuir debido a la acción química, físico-química o física del agua mineral o a la actividad biológica.

Sánchez et al. (2002) vieron que durante la maduración no se producían cambios significativos mineralógicos, ni en la composición química, pero sí cambios en cuanto a la cristalinidad de las esmectitas, además observaron un cambio del área superficial específica, que aumentaba en el primer mes de maduración, debido a la degradación ilita-esmectita y posteriormente disminuía a los tres meses, puesto que el contenido en esmectitas se había reducido. Carretero et al. (2007) apreciaron un cambio en cuanto al tamaño de partícula, notándose una disminución de partículas menores de 2 μm, aumentando esta fracción progresivamente con la maduración. Ello parece conllevar una mejora de la textura del fango al eliminarse los agregados de mayor tamaño, al ir penetrando la fase líquida en la estructura.

Asimismo, algunos autores han puesto de manifiesto una mejora de la plasticidad, por el aumento de la capacidad de retención de agua, y esto posiblemente es lo que hace que pueda disminuir la velocidad de enfriamiento del sistema (Sánchez et al., 2002). Por tanto, también se podrían originar cambios relativos a las propiedades térmicas.

Por otro lado, la maduración implica cambios bioquímicos debido a la producción de componentes orgánicos. Puede aparecer un sulfoglicolípido (procedente de bacterias diatomeas), que podría estar relacionado con las propiedades antiinflamatorias que presentan los fangos terapéuticos (Galzigna et al., 1995, 1996, 1998; Tolomio et al., 1999, 2002, 2004; Quintela et al., 2010).

La maduración junto con el crecimiento de colonias de microorganismos termofílicos, puede dar lugar a la modificación las características físicas y químicas del fango.

Parece claro que el procedimiento de elaboración de estos sistemas tiene una enorme importancia en las propiedades resultantes.

## I.3.5. PROPIEDADES DE LOS FANGOS TERAPÉUTICOS

Los fangos deben presentar unas características adecuadas para su uso, y esas propiedades dependen en gran medida de las del material de partida y del proceso de elaboración/maduración. A continuación se describen las más significativas.

- Capacidad de absorción y adsorción

Lo ideal es que un fango presente una elevada capacidad de absorción (agua en la fase externa del sistema) y adsorción (agua retenida en la superficie y espacios interlaminares de las arcillas). Tanto la absorción como la adsorción de agua de un fango está determinada principalmente por los minerales que constituyen la fase interna del sistema sólido/líquido, especialmente por su contenido en esmectitas. La formación de sistemas estructurados (geles) por las partículas de estos minerales aumenta la cantidad de agua que el sistema sólido/líquido puede retener. Paralelamente, la capacidad de adsorción está directamente relacionada con el pequeño tamaño de partícula del sólido arcilloso y especialmente con la existencia de superficies estructurales internas y externas donde las moléculas de agua puedan quedar retenidas. Como resultado de todo lo anterior, la cantidad de agua que puede retener un fango puede variar entre el 30-90 %, en función del porcentaje de coloides hidrófilos y materia orgánica (Mourelle et al., 2014). Además, depende de la concentración iónica del medio de dispersión (Casás et al., 2011, 2013) dada su influencia en la estabilidad de los geles de esmectitas (Lagaly, 2006), con lo cual un factor determinante de la capacidad de absorción y adsorción será la presencia y concentración de electrolitos en la fase acuosa (Veniale et al., 2004; Lagaly, 2006; Gámiz et al., 2009; Knorst-Fouran et al., 2012).

- Capacidad de intercambio catiónico

Como hemos discutido anteriormente, los filosilicatos 2:1 se comportan como potentes intercambiadores de cationes. Los estratos tetraédricos y octaédricos se asocian para formar paquetes estructurales, que presentan una carga neta negativa, compensada por cationes localizados en el espacio interlaminar susceptibles de ser intercambiados. Los principales iones que se intercambian entre la arcilla y el agua pueden ser todos los que estén presenten en concentración suficiente, y en teoría el fango terapéutico resultante tendrá una composición que será diferente de la del agua mineromedicinal y que por la presencia de determinados oligoelementos resultará beneficiosa cuando se aplique sobre la piel (Baquero y García, 2014). De todo lo anterior, deriva que se consideren adecuados los fangos en los que se ha producido un intenso intercambio catiónico.

- Propiedades térmicas

Los efectos beneficiosos de los peloides se atribuyen principalmente al efecto térmico resultante de su aplicación tópica. Los fangos terapéuticos se suelen aplicar a elevada temperatura (40-45 °C), comportándose como agentes termoterápicos. Durante la aplicación deben mantener una temperatura superior a la de la piel del paciente para que se pueda producir la transferencia de calor desde el fango terapéutico. Por este motivo interesa que los fangos terapéuticos presenten un valor elevado de calor específico y una baja velocidad de enfriamiento.

Según la ley de enfriamiento de Newton, que describe la transferencia de calor entre dos cuerpos en contacto a diferentes temperaturas, el calor transferido por unidad de tiempo hacia un cuerpo por conducción, es aproximadamente proporcional a la diferencia de temperaturas entre el cuerpo y el medio externo, siempre y cuando este último mantenga su temperatura constante durante el proceso de enfriamiento. Esto queda recogido en la siguiente ecuación:

$$\frac{dQ}{dt} = \alpha S(T - T_A)$$

donde α es el coeficiente de intercambio de calor, S el área superficial del cuerpo expuesta y $T_A$, la temperatura ambiente. Si hay una diferencia de temperaturas, se experimentará una pérdida de calor, que será proporcional a la diferencia de temperaturas, expresándose esto en la siguiente ecuación:

$$dQ = -mC_p dT$$

donde m es la masa del cuerpo y $C_p$ su calor específico. El signo negativo indica la pérdida calorífica.

Combinando ambas ecuaciones, de forma simplificada resultaría la siguiente ecuación:

$$\frac{dT}{dt} = -k(T - T_A)$$

en la que k es una constante de proporcionalidad, definida como parámetro de enfriamiento.

Resolviendo esta ecuación diferencial para un material que enfría desde una temperatura $T_0$ hasta T, queda que:

$$(T - T_A) = (T_0 - T_A)e^{-kt}$$

A partir de la ley de enfriamiento de Newton, se pueden estudiar las propiedades térmicas de los fangos. Lo idóneo sería que durante al menos 20 minutos (considerado tiempo mínimo de aplicación), los fangos terapéuticos mantuvieran una temperatura superior a la de la piel ($\cong$ 32 °C) para que pueda producirse esa transferencia de calor (Ferrand e Ivon, 1991; Cara et al., 2000b). Por otro lado, se debe considerar el tiempo durante el cual el fango presenta una temperatura superior a 32 °C, puesto que transcurrido ese tiempo dejaría de producirse la transferencia de calor y el efecto térmico desaparecería.

El comportamiento térmico de los fangos ha sido estudiado por muchos autores. Lewis (1935) fue el primer autor que estudió las propiedades térmicas de los fangos terápeuticos. En su trabajo propuso un método para calcular la velocidad de enfriamiento, usando un calorímetro de cilindros concéntricos y sugirió que el aumento de la cantidad de agua provocaba un aumento de calor específico y a una disminución de la conductividad térmica. En 1965, Berbenni estudió la capacidad de retención de calor, indicando que la mayor capacidad de retención de calor se correspondía con la cantidad óptima de agua de cada fango. Rambud et al. (1986) realizaron un ajuste matemático, con una función exponencial, de curvas de enfriamiento. Posteriormente, Ferrand e Yvon (1991) estudiaron el comportamiento térmico de diferentes mezclas de arcilla y agua, proponiendo una función lineal mediante la cual se puede calcular el contenido de agua de un fango y su calor específico. Cara et al. (2000b) estudiaron las propiedades térmicas de suspensiones preparadas con diferentes bentonitas y distintas cantidades de agua, planteando una ecuación similar a la de Ferrand e Ivon para calcular el calor específico. Beer et al. (2003) realizaron un estudio comparativo del comportamiento térmico de un fango procedente de un pantano, de un fango terapéutico y del agua purificada, encontrando diferencias considerables. Legido et al. (2007) estudiaron la velocidad de enfriamiento de distintos sistemas arcilla/agua, concluyendo que los elaborados con arcillas puras mostraban un mejor comportamiento térmico y resaltando el uso de esmectitas para la elaboración de fangos terapéuticos. Casás et al. (2011, 2013) midieron el calor específico de mezclas de bentonita con agua purificada y agua de mar, resaltando que el calor específico depende en cierta medida de la concentración de la fase mineral y de la concentración de sales, relacionada ésta última con la cantidad de agua retenida.

Algunos autores han estudiado específicamente la conductividad térmica (propiedad física de los materiales que mide la capacidad de conducción de calor) de los fangos terapéuticos (Beziat et al., 1988; Ortiz de Zárate et al., 2010; Caridad et al., 2014).

A la vista de estos trabajos, se puede decir que esta propiedad depende principalmente del contenido en agua y de la densidad, y en menor medida de la temperatura, química y mineralogía. De manera que la conductividad térmica aumenta directamente con la densidad y el contenido en agua.

Es necesario estudiar el calor específico y la conductividad térmica para predecir y conocer la cinética de enfriamiento de un fango terapéutico (Ortiz de Zárate et al., 2010) y resaltar que el comportamiento térmico de los fangos está íntimamente relacionado con su contenido en agua (Cara et al., 2000b).

- Tamaño de partícula

La mayoría de los centros termales españoles aplican fangos en los que entre el 57-70 % de las partículas tienen un tamaño entre 2 y 20 μm (Armijo y Maraver, 2006). La granulometría determina la superficie reactiva del fangos, influyendo en la retención de agua y en la capacidad de intercambio iónico (Quintela et al., 2012). Que el tamaño de partícula sea el adecuado es importante, ya que porcentaje alto de partículas de gran tamaño puede provocar una disminución de la adhesividad del fango con la piel y dar lugar a sistemas con propiedades reológicas indeseables. Una propiedad derivada del tamaño de partícula es la abrasividad, de manera que un elevado contenido de partículas finas junto con una micromorfología favorable, contribuyen a la obtención de materiales más blandos y menos abrasivos (Klinkenberg et al., 2009). El valor del índice de abrasividad no debe exceder de 400-500 g/m$^2$ a 174.000 r.p.m. (Quintela et al., 2012).

- Propiedades reológicas

La reología es la parte de la mecánica que estudia la relación entre el esfuerzo y la deformación en los materiales que son capaces de fluir, como por ejemplo las suspensiones de arcilla. El comportamiento reológico de las suspensiones de arcillas que constituyen los fangos terapéuticos es complejo, puesto que depende de un gran número de factores que incluyen la concentración, naturaleza y estado de agregación de la fase sólida dispersa, la forma, tamaño, distribución y carga superficial de las partículas, la temperatura, etc. La propiedad reológica más importante en nuestro caso es la viscosidad aparente (relación entre esfuerzo de corte o cizalla y velocidad de corte o cizalla). La velocidad de cizalla se define como el cambio de la deformación de cizalla por unidad de tiempo.

Si la relación entre el esfuerzo deformante y la velocidad de cizalla es constante el cuerpo se denomina newtoniano. Las suspensiones de arcilla nunca se comportan siguiendo este patrón, sino que se desvían de la linealidad. Su comportamiento puede ser dilantate o con mucha mayor frecuencia pseudoplástico, presentado umbral de deformación ("yield point") y propiedades tixotrópicas, junto con una elevada viscosidad aparente (Güven, 1992; Mewis and Macosko, 1994) (Fig. I.4).

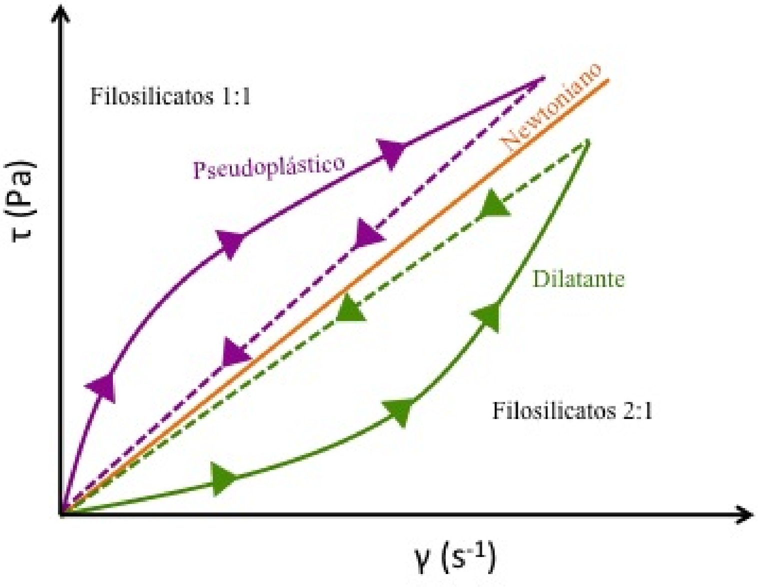


**Fig. I.4.** Perfiles de flujo ideales de las dispersiones de filosilicatos

Las suspensiones de caolinita de elevada concentración presentan un flujo dilantante (la viscosidad aparente aumenta al aumentar la velocidad de cizalla), con valores de viscosidad alrederor de 300 mPa s (para una suspensión acuosa al 70 % m/v). Este comportamiento se explica en razón del empaquetamiento que sufren las partículas de caolinita defloculadas, que al ser deformadas aumentan los contactos interparticulares y con ellos la resistencia al flujo. Lagaly (1989) señaló la importancia de la morfología de las partículas de caolinita y su carga superficial en el comportamiento reológico de sus suspensiones. Yuan y Murray (1997) compararon las propiedades reológicas de suspensiones de caolinita con distintas morfologías, encontrando una relación entre la forma de las partículas y las propiedades reológicas de los sistemas.

En cambio, cuando los minerales suspendidos dan lugar a sistemas floculados, como sucede con las esmectitas, el comportamiento es pseudoplástico (la viscosidad aparente disminuye al aumentar la velocidad de cizalla). Este comportamiento de flujo se explica como resultado de la progresiva destrucción de las estructuras tridimensionales de tipo gel que forman estas arcillas en medios polares. Al aumentar la velocidad de cizalla se destruyen las asociaciones interparticulares y el sistema fluye libremente. Es necesario aplicar un cierto valor inicial de esfuerzo deformante para que empiece a fluir (umbral de deformación o punto de flujo) y por otra parte, cuando cesa el esfuerzo deformante, el sistema es capaz de volver a estructurarse (tixotropía) (Lukham y Rossi, 1999; Mewis y Wagner, 2009). La velocidad de recuperación del sistema no es lineal, sino que en una primera etapa es muy elevada y posteriormente, las partículas individuales de arcilla que no están formando la red, se van uniendo a ésta aumentando la viscosidad de forma más progresiva. Cuando la red tridimensional cede ante un esfuerzo deformante, los acontecimientos se suceden a la inversa; la mayoría de la estructura se rompe rápidamente en un primer estadio, y posteriormente la ruptura es mucho más lenta. Las suspensiones de esmectitas son, por tanto, tixotrópicas; en reposo aumentan su viscosidad con el tiempo y bajo una deformación a velocidad constante, su viscosidad disminuye con el tiempo. Las suspensiones de esmectitas son también pseudoplásticas, ya que bajo una deformación de velocidad creciente, su viscosidad disminuye. La existencia de un umbral de deformación (punto de flujo) implica que la estructura tridimensional de gel formada por las partículas de esmectita permanezca inalterada hasta que se alcanza un cierto valor de esfuerzo. Obviamente, cuanto mayor es el punto de flujo, más estable es la suspensión.

Cuando se estudian las propiedades reológicas de las suspensiones de arcilla se emplean dispositivos que aplican un esfuerzo y miden una resistencia al flujo. Al representar los valores de resistencia al flujo frente a los esfuerzos se obtienen curvas de flujo. En otras palabras, la representación gráfica del esfuerzo de cizalla en función de la velocidad de cizalla se denomina curva de flujo. Dependiendo del tipo de curva cabe distinguir los diferentes tipos de flujo, y en cada caso se aplicará un modelo cuyas ecuaciones permitan ajustar los datos. Los modelos más sencillos para describir el flujo de un material son el de Newton (para fluidos newtonianos) y el de Bingham (utilizado para describir el flujo de un material plástico o pseudoplástico) y que viene definido por la siguiente ecuación:

$$\tau = \tau_0 + \eta_p \gamma$$

Donde $\tau_0$ es el punto de flujo, y $\eta_p$ es la viscosidad plástica, y ambos parámetros son constantes. Existen diferentes modelos para explicar flujo no lineal, como son el propuesto por Ostwald-de Waele, el de Herschel-Bulkley, el de Sisko (ley de la potencia) y el de Casson, entre otros. El modelo de Bingham permite explicar de forma satisfactoria el comportamiento de las suspensiones de arcillas en agua mineromedicinal y por tanto no existen razones para detallar los otros posibles tipos de ajuste.

Otra forma de representar el comportamiento al flujo es mediante las curvas de viscosidad, en donde se representa la viscosidad en función de la velocidad de cizalla. En el caso de materiales no newtonianos, la viscosidad es una función de la velocidad de cizalla, por ello se habla de viscosidad aparente ($\eta$ ($\gamma$)).

Por otro lado, la viscosidad depende de la temperatura del sistema y por lo general disminuye al aumentar la temperatura, por lo que es imprescindible informar de la temperatura en la que se ha determinado la viscosidad (o se ha obtenido la curva de flujo). La presión también influye en la viscosidad, pero de un modo mucho menos relevante, por lo que en general, el efecto de la presión no se considera, ya que los cambios son muy pequeños para diferencias de presión de ± 1 bar con respecto a la presión atmosférica (Moreno, 2005).

En cuanto a la relación de la estructura del sistema con los parámetros reológicos, lo anteriormente descrito para las suspensiones de arcilla fue recogido y detallado, en lo relativo a los tipos de estructuras tridimensionales, en una magnifica revisión (Lagaly, 2006). Las partículas de arcilla pueden agregarse de distintas maneras, dando lugar a diferentes tipos de estructuras. El modelo más conocido es el de “castillo de naipes”, en el que las partículas se mantienen unidas por contactos borde/cara. Esta estructura se forma cuando los bordes están cargados positivamente o en un medio ligeramente alcalino por encima de la concentración crítica de sales. En el caso de un pH por debajo de 6, se produce un fenómeno de heterocoagulación entre las cargas positivas de los bordes y las negativas de las caras de las partículas o de las capas de silicatos, dando lugar a contactos borde/cara. Este tipo de estructura se caracteriza por un flujo no-Newtoniano de las dispersiones y por la aparición de un umbral de deformación en medio ácido. Al aumentar el pH la estructura se desmorona y el esfuerzo de cizalla (dado a una determinada velocidad de cizalla) disminuye bruscamente, como ocurre en las dispersiones de montmorillonita sódica. A un pH por encima de 5, se produce un incremento del esfuerzo de cizalla como resultado del elevado grado de delaminación, y por tanto del aumento de partículas individuales. A un pH en torno a 7, se reduce el grado de delaminación y el efecto electroviscoso, provocando una disminución del esfuerzo de cizalla.

Otro tipo de estructura es la resultante de contactos cara/cara, conocida como "band-type networks". Esta estructura es más elástica que la de "castillo de naipes". En este caso la adicción de sales en dispersiones diluidas (< 2 % m/m) provoca determinados cambios en las propiedades reológicas. En ausencia de sales, los valores de viscosidad y del punto de flujo son bajos; tras la adición progresiva de cantidades ligeras de sales, estos parámetros disminuyen, para luego aumentar bruscamente. Por el contrario, una elevada concentración de sales puede reducir la viscosidad y el valor del punto de flujo. En el caso de ausencia o cantidades pequeñas de sales, las capas de silicatos o el conjunto son rodeadas por capas difusas de cationes, produciéndose una repulsión por las fuerzas electrostáticas (Van Olphen, 1977; Lagaly et al., 1997). Cuando la concentración de partículas es lo suficientemente alta (≥ 1 % m/m para dispersiones de montmorillonita sódica), se produce un aumento de la viscosidad y una disminución del punto de flujo, debido a que las capas iónicas difusas de las partículas o de las capas de los silicatos, les limitan el movimiento translacional y rotacional. La adición de sales reduce el espesor de la capa iónica difusa, incrementando la libertad de movimiento de las partículas y reduciendo los valores de viscosidad y umbral de deformación.

Desde un punto de vista práctico, el hecho de que las suspensiones concentradas de arcilla en agua presenten plasticidad (se deforman bajo un esfuerzo sin llegar a romperse y cesado el esfuerzo la deformación que ha producido permanece) viene determinado por las cantidad relativas de arcilla y de agua. Con estas premisas, el método de Atterberg establece el contenido de agua necesario para alcanzar una plasticidad que se entiende óptima, a través de dos parámetros: el límite plástico (cantidad relativa de agua por debajo de la cual no es posible moldear la pasta) y el límite líquido (cantidad relativa de agua por encima de la cual la masa de agua y arcilla fluye como un líquido). La diferencia entre ambos valores es lo que se denomina índice plástico. Muchos artículos se limitan a medir estos límites que tienen un carácter eminentemente práctico, pero que dan una información muy limitada del comportamiento reológico del sistema. Hay que tener en cuenta, por ejemplo, que la deformación de una pasta de arcilla no sólo depende del contenido en agua, también depende el tiempo necesario para que el sistema alcance su grado de estructuración, sin llegar a romperse.

Algunos autores han encontrado cambios relacionados con parámetros reológicos de los fangos, como resultado de la maduración (Galzigna et al., 1995; Sánchez et al., 2002; Veniale et al., 2004). Que el comportamiento reológico de un fango varíe con el tiempo es especialmente importante en este ámbito, puesto que la maduración de fangos terapéuticos implica períodos prolongados de tiempo desde la interposición del material arcilloso con el agua mineromedicinal hasta la aplicación, con lo cual es importante estudiar en profundidad las propiedades reológicas (Viseras et al., 1999, 2006b; Gómes et al., 2011). Los sistemas arcilla-agua mineromedicinal deben presentar una adecuada estabilidad física, asegurando que el producto cumple con su función inicial, a pesar de poder verse afectado por distintos parámetros, como son la temperatura, luz y humedad.
Por otro lado, durante la maduración, es posible que los fangos sean sometidos a procesos de agitación, y este procedimiento puede ser distinto, dependiendo de los protocolos de cada centro termal, pudiendo esto influir también en las propiedades reológicas finales del peloide (Viseras et al., 1999; Carretero et al., 2007).
Por tanto, las propiedades reólogicas son de gran interés e importancia; así se han estudiado de peloides elaborados con materiales procedentes de Portugal (Rebelo et al., 2011b), de peloides elaborados con distintos tipos de agua (Gómes et al., 2011), de peloides madurados en distintas condiciones y de la influencia de la maduración (Viseras et al., 2006b), y de arcillas usadas en la elaboración de mascarillas cosméticas (Ngomo et al., 2014), entre otros.

- pH

El pH de un peloide depende de su composición, tanto de la arcilla como del agua mineromedicinal con la que se haya elaborado. El valor de pH es importante en cuanto a que el fango se pondrá en contacto con la piel y por tanto un pH alcalino o ácido puede modificar el equilibrio fisiológico del manto cutáneo, lo que daría lugar a la modificación de su fisiología y propiedades. Generalmente, los fangos terapéuticos tienen un pH entre 6 y 10 (Baquero y García, 2014).

## I.3.6. ASPECTOS CLÍNICOS DE LA FANGOTERAPIA

La cura balnearia, en la que se incluye el uso de los fangos termales, sigue siendo un proceder terapéutico valioso, que de forma individual o bien como agente coadyuvante de otros tratamientos, puede facilitar la recuperación y rehabilitación de muchos enfermos (Armijo y San Martín, 1994). Debido al crecimiento en la actualidad del interés por los remedios naturales, se ha producido un aumento del uso de este tipo de terapia (Veniale et al., 2007; Hernández, 2014). No existen, en cambio, hasta la fecha estudios clínicos completos sobre los efectos asociados a la terapéutica con fangos termales. Por otra parte, a diferencia de otros tratamientos, la fangoterapia ha persistido a lo largo del tiempo, lo cual es un indicador de su utilidad como herramienta terapéutica (Giacomino y Michele, 2007).

La fangoterapia es usada con frecuencia en el tratamiento de enfermedades que afectan a las articulaciones. Los fangos se suelen aplicar en forma general o local en las partes seleccionadas del paciente, a una temperatura de 40-45 ºC, en capas de 1-2 cm de espesor y en sesiones de 20-30 minutos (Sánchez et al., 2002). La zona donde se aplica el fango se cubre con un material impermeable para que se conserve el calor y transcurridos los 20-30 minutos, el paciente se somete a un baño o ducha para retirar el producto. La duración del tratamiento puede ser de una a varias semanas (normalmente suele ser de 10-15 días), dependiendo del objetivo terapéutico (Veniale, 1997). La aplicación produce efectos relajantes, antiinflamatorios y analgésicos en el área tratada, debido a la vasodilatación, perspiración y estimulación del aparato circulatorio y respiratorio, y además se produce una respuesta inmunológica. Pero hay que tener en cuenta que los beneficios del tratamiento dependen de la composición del fango, de las condiciones de aplicación (temperatura, extensión y duración) y de la reactividad del organismo (Barbieri, 1996). Está comprobado que cuando la indicación está bien establecida y el tratamiento es el adecuado, produce resultados favorables, el problema es determinar con precisión la indicación y el encuadre en planes terapéuticos.

Por lo general el tratamiento es sintomático y procura el alivio y la mejoría funcional, haciendo la enfermedad más tolerable y llevadera. Principalmente se han empleado en afecciones crónicas del aparato locomotor, concretamente en procesos reumáticos. Se ha visto que los efectos beneficiosos en la artritis, derivados del uso de fangos, se deben a la reducción de la inflamación que provocan, a través de la disminución de la concentración del óxido nítrico y de la interleucina-1, mediadores de la inflamación, de la mieloperoxidasa, enzima que se relaciona con la intensidad de la inflamación y del factor de necrosis tumoral (TNF), que interviene en la inflamación y la destrucción articular (Bellometti et al., 2000; Cozzi et al., 2004) y además influye de manera positiva en la homeostasis y recuperación del cartílago (Bellometti et al., 2000; Britschka et al., 2007). Asimismo, se ha expuesto una mejora significativa de los pacientes con artritis reumatoide, una de las formas más comunes de la artritis, revelada por la mejoría en los índices clínicos y del alivio del dolor (Sukenik et al., 1992; Codish et al., 2005). La mejoría de los síntomas causados por la espondilitis anquilosante también han sido estudiado por diversos autores (Barnatskiĭ, 2007; Cozzi et al., 2007; Ciprian et al, 2013). También han resultado ser eficaces en fibromialgia (Bellometti y Galzigna, 1999; Fioravanti et al., 2007; Guidelli et al., 2012; Fraioli et al., 2013) y en el tratamiento del dolor crónico de espalda (Constant et al., 1995; 1998; Strauss-Blache et al., 2002).

El uso en el tratamiento de la artrosis (Grassi et al., 2003; Grigor´eva et al., 2001), y concretamente en el de la artrosis de rodilla (Wigler et al., 1995; Bellometti et al., 1997a,b; Flusser et al., 2002; Bellometti et al., 2005; Odabaşi et al., 2009; Bostan et al., 2010; Fioravanti et al., 2011; Fraioli et al., 2011; Espejo-Antúnez et al., 2013a, b; Tefner et al., 2013) se ha justificado a través de un amplio conjunto de estudios clínicos, que ponen de manifiesto que la fangoterapia produce una disminución de la prostaglandina E2, del leucotrieno B4, del TFN-α (Bellometti y Galzigna, 1998), de los niveles de interleucina-1 (Bellometti et al., 1997b; Bellometti y Galzigna, 1998; Untura et al., 2007), de la β-endorfina, de la hormona adrenocorticotropa (ACTH) y de cortisol (Pizzoferrato et al., 2000), un aumento de transferrina y ceruloplasmina, que implicaría una disminución de los radicales libres oxidantes y una disminución de malondialdehído, reflejado a su vez en una disminución de la concentración sérica de los productos resultantes de la peroxidación lipídica (Bellometti et al., 1996), un aumento de algunos aminoácidos, como son el triptófano, cisteína y citrulina (Bagnato et al., 2004) y la modificación de la actividad de los condrocitos, ya que parece ser que este tratamiento influye sobre las citoquinas relacionadas con la patogénesis y el mantenimiento de esta enfermedad (Bellometti et al., 1997b), aunque en las fases avanzadas es conveniente que se aplique junto con otras terapias (Bellometti et al., 2005).

Estos estudios, sin ser ensayos clínicos, permiten concluir que la fangoterapia tiene un efecto protector sobre el cartílago, reduciendo la reacción inflamatoria, lo que conlleva el alivio del dolor, junto con mejora de la capacidad funcional del paciente y de la función articular, mejorando la calidad de vida del paciente y reduciendo la necesidad de medicación.

La fangoterapia es, por tanto, una terapia alternativa y efectiva en el tratamiento de la artritis y artrosis. Pero no solo se emplea en estas afecciones, sino que también se ha estudiado el resultado en otras, como por ejemplo en afecciones respiratorias tales como la bronquitis crónica (Povazhnaia et al., 1990; Balaban y Ponomarenko, 2002; Ivanov et al., 2002) o la tuberculosis pulmonar (Strelis et al., 1989), en afecciones ginecológicas (Artymuk et al., 2010; Rondanelli et al., 2012), en la enfermedad isquémica del corazón (Davydova et al., 1994), en la úlcera duodenal (Petrakova, 2001), en el tratamiento de la microcirculación de la piel (Poensin et al., 2003) y como método alternativo para el tratamiento de pacientes con heridas (Rodríguez et al., 2004, 2005).

Por otra parte, cabe destacar que la balneoterapia en general se usa con éxito para tratar afecciones dermatológicas, principalmente la psoriasis y la dermatitis atópica, pero también se utiliza esta modalidad para el acné vulgar, dermatitis de contacto, dermatitis seborreica, dishidrosis, eczema, granuloma anular, ictiosis vulgar, liquen plano, liquen escleroso y atrófico, micosis fungoide, necrobiosis lipoídica, queratosis, prurito, rosácea, esclerodermia, úlcera crónica, urticaria pigmentosa, vitiligo, xerosis… (Torresani 1990; Nappi et al., 1996; Carabelli et al., 1998; Ubogui et al., 1998; Delfino et al., 2003; Matz et al., 2003; Argenziano et al., 2004; Mazzulla et al., 2004; Costantino y Lampa, 2005; Grether-Beck et al., 2008; Riyaz y Arakkal, 2011).

Hay también que resaltar el empleo de fangoterapia en el ámbito de la cosmética y medicina estética, para limpiezas cutáneas, prevención de la deshidratación de la piel y antienvejecimiento. Estos usos están asociados a sus propiedades antiinflamatorias, remineralizantes, antioxidantes y regenerativas (Tejero, 2014; Aguzzi al., 2014).

## I.3.7. MECANISMOS DE ACCIÓN DE LOS FANGOS TERAPÉUTICOS

Los estudios realizados hasta la fecha no han sido capaces de determinar los mecanismos de acción responsables de los efectos terapéuticos de los fangos, limitándose a indicar que las mejorías se deben a un conjunto de efectos térmicos, mecánicos, químicos e inmunológicos (Matz et al., 2003; Odabaşi et al., 2008).

Parece claro que los efectos terapéuticos se deben fundamentalmente a la temperatura de aplicación, que gracias a la presencia de arcilla puede ser mayor que la usada en balneoterapia con agua, por tanto pueden considerarse agentes terapéuticos termoterápicos. Ante una elevación de la temperatura, el organismo activa una serie de mecanismos termorreguladores, con lo cual se produce una vasodilatación periférica, un aumento de la frecuencia respiratoria y cardíaca, sudoración, un aumento de la circulación sanguínea y del metabolismo celular, perspiración cutánea e irradiación térmica, acompañada de una bajada de la tensión arterial, una sensación de calor agradable y una tendencia al sueño. Todo ello provoca un efecto antiespasmódico, analgésico, sedante y relajante muscular; además la estimulación térmica origina un incremento de los tejidos ricos en colágeno (Sukenik et al., 1992, 1999). El efecto analgésico puede ser atribuido, al menos parcialmente, al incremento de la concentración de la β-endorfina provocado por el calor (Jezora et al., 1985; Codish et al., 2005). El efecto antiinflamatorio puede deberse al aumento de secreción de cortisol y catecolaminas (Cozzi et al., 1995, 2004).

Este comportamiento térmico va a estar determinado en gran medida por la capacidad calorífica del peloide y por su cinética de enfriamiento, por ello lo ideal es que el sistema presente un elevado calor específico y que se libere lentamente, con el objeto de mantener este efecto durante el mayor tiempo posible. Por otra parte, esta acción está condicionada por la composición, relacionándose así con la cantidad de agua que es capaz de retener.

Mientras que los mecanismos térmicos han sido estudiados en profundidad y revisados previamente en esta memoria, son muy pocos los estudios relativos a los mecanismos químicos e inmunológicos. En cuanto a los mecanismos químicos, se deben al quimismo de las aguas y de los fangos, de modo que sus efectos dependerán de la posible penetración y/o permeación dérmica de los componentes presentes en el fango.

Sin embargo, hasta la fecha, los estudios centrados en la presencia de iones susceptibles de tener efectos biológicos (positivos o negativos) tras la aplicación de los fangos, son escasos y poco concluyentes (Summa y Tateo, 1998; Tateo y Summa, 2007; Tateo et al., 2009). No está claro qué elementos son esenciales y cuál es la concentración idónea de cada elemento para conseguir una respuesta óptima al tratamiento. La permeabilidad cutánea, esto es, la capacidad de la piel para permitir el paso de una determinada sustancia química, depende de distintos factores; lo que quiere decir que la presencia de un determinado elemento en el fango no implica necesariamente su biodisponibilidad en el organismo y puesto que son pocos los trabajos que existen, no se conoce la permeabilidad de iones desde fangos terapéuticos. De lo poco que se ha estudiado ha sido, la permeabilidad del calcio, favorecida por  iontoforesis, desde pastas de bentonita (Szántó y Papp, 1998) y la permeación desde suspensiones de metales pesados a través de la piel para evaluar los posibles efectos tóxicos (Van Lierde et al., 2006; Larese et al., 2006, 2007, 2009).

## I.3.8. CALIDAD DE FANGOS TERAPÉUTICOS

Si bien no existe un protocolo específico que permita la cualificación de un determinado fango terapéutico, en los últimos años se ha avanzado considerablemente en este sentido, dado al notable número de balnearios que utilizan este producto como agente terapéutico y notable interés. Para garantizar que el fango terapéutico desarrollado sea de la calidad requerida para el uso al que está destinado, se debería diseñar e implantar un sistema de calidad en el que se incluyan medidas relativas al diseño del fango, su producción (incluyendo interposición de los componentes y proceso de maduración) y control, el suministro y uso de las materias primas (agua mineromedicinal y excipientes minerales), el almacenamiento y empleo (Cerezo et al., 2014). Todo ello redundaría en asegurar la seguridad del producto y paralelamente permitiría abordar estudios de eficacia con los productos de calidad conocida.

Obviamente, la calidad se construye desde las materias primas, y en particular desde la calidad de las arcillas usadas. En este sentido, algunos minerales de la arcilla, como son la caolinita, el talco, la montmorillonita, la saponita, la sepiolita y la palygorskita, tienen su propia monografía en distintas farmacopeas (Viseras et al., 2007) y dichas monografías recogen las especificaciones requeridas para que dichos materiales puedan utilizarse en el ámbito farmacéutico y médico. Como materias primas para la elaboración de productos farmacéuticos deben cumplir una serie de requisitos, incluidos la precisa identificación de la sustancia, composición, contenido de elementos peligrosos e impurezas, manipulación y almacenamiento, propiedades físicas y químicas, estabilidad y reactividad y aspectos toxicológicos. Aunque no aparezca como un requisito específico en ninguna farmacopea, debe limitarse o excluirse la presencia de materiales abrasivos, como son el cuarzo y la calcita, y de los minerales fibrosos, o al menos su uso debería venir precedido de un control cuidadoso del tamaño de partícula (López-Galindo et al., 2007). En resumen, deben cumplir con una serie de requisitos en cuanto a identidad, pureza, riqueza, seguridad, estabilidad y eficacia.

Puesto que las arcillas empleadas con fines médicos/farmacéuticos deben cumplir una serie de requisitos, son varios los autores que han realizado estudios con esta finalidad (Gamiz et al., 1992; Summa y Tateo, 1999; Viseras y Lopez-Galindo, 1999; Tămăşan et al., 2010; Rebelo et al., 2011a). Varios trabajos han puesto de manifiesto que no todas las calidades de arcilla ni composiciones químicas son apropiadas para determinadas aplicaciones, de manera que muestras de idéntica mineralogía y composición pueden presentar comportamientos netamente distintos de flujo y compresibilidad, dependiendo de propiedades tales como su textura y/o microcomposición (Viseras et al., 2000a, b).

Las arcillas, una vez controladas y si es necesario modificadas, deben reunir los requisitos necesarios para alcanzar el nivel de sustancias medicinales adecuadas para la elaboración de los agentes terapéuticos o productos medicinales definitivos, que administrados convenientemente ejerzan el efecto deseado. En definitiva, es necesaria la investigación multidisciplinar para encontrar las posibles pautas de actuación para una optimización en la selección y empleo de estos materiales.

Bien es cierto que mientras que las arcillas para uso farmacéutico o cosmético deben cumplir una serie de especificaciones relativas a las propiedades tecnológicas y a la seguridad para ser usadas, los peloides se usan sin ningún control (Quintela et al., 2012). No obstante, parece un objetivo irrenunciable cambiar esta pauta de empleo y aproximarla en la medida de lo posible a los parámetros de calidad de uso en productos medicinales.

# CAPÍTULO II

## Objetivos y plan de trabajo

# II. 1. OBJETIVOS

Entendemos por fangos terapéuticos aquellos “productos medicinales de consistencia semisólida, constituidos por la interposición de sólidos arcillosos en agua mineromedicinal, que preparados convenientemente y administrados por vía tópica, en forma de aplicaciones locales o baños y en virtud de una serie de acciones biofísicas y/o bioquímicas se emplean en terapéutica para el tratamiento o prevención de ciertas patologías, o bien, para corregir sus efectos en el organismo” (Viseras et al., 2006). Los fangos terapéuticos sustituyen a los que de forma natural aparecen al sedimentar las partículas arrastradas por las aguas mineromedicinales. Hasta ahora estos “medicamentos” se formulan y elaboran siguiendo procedimientos que, a priori, intentan simular las condiciones en las que aparecen los peloides naturales (Veniale, 2004). No obstante, los fangos terapéuticos deberían ser considerados medicamentos en los que el agua mineromedicinal es la sustancia con actividad farmacológica (sustancia activa) y el resto de componentes, en particular las arcillas, son los excipientes que sirven de vehículo del agua, posibilitando su preparación, asegurando su estabilidad y permitiendo su aplicación (Cerezo et al., 2014). Esta tesis se enmarca en una línea de investigación que comprende el uso sostenible de determinadas arcillas, recursos minerales de marcado interés por sus aplicaciones industriales, y en particular farmacéuticas, derivadas de sus especiales propiedades. En este caso se usarán como excipientes destinados a la preparación de formas de administración del agua mineromedicinal.

De alguna forma, los fangos terapéuticos son un “fósil viviente” de la antigua tradición de preparación de remedios terapéuticos que ha alcanzado hasta nuestros días sin apenas cambios en su formulación, elaboración y control. Se trata, por tanto, de seguir el camino hace mucho tiempo iniciado por la Ciencia Farmacéutica por el que se dejó atrás el “hágase según arte” y se sustituyó por el “hágase según ciencia”, como brillantemente resumió el Prof. D. Rafael Cadórniga con motivo de su ingreso en la Real Academia Nacional de Farmacia (Cadórniga, 1983).

Con estas premisas se planteó el estudio de los aspectos formulativos, así como aquellos derivados de la preparación de los fangos terapéuticos desde un punto de vista farmacéutico con el objetivo de desarrollar fangos termales terapéuticos estables, seguros, eficaces y de calidad.

Los objetivos específicos de la tesis son:

1. Redefinir el concepto de Fango Terapéutico en el ámbito de la Medicina. Dado que actualmente se emplea para el tratamiento de enfermedades en centros sanitarios, bajo prescripción médica especializada, pero que su desarrollo y control escapan al ámbito farmacéutico, creemos imprescindible reflexionar sobre la importancia de este aspecto, a la par que recopilar la información que sirva para demostrar la necesidad de adecuar el lugar legislativo en que estos productos deberían estar encuadrados, esto es, en el de medicamentos de uso humano.
2. Establecer los atributos de identidad y seguridad que deben cumplir los materiales arcillosos empleados como excipientes en la elaboración de fangos terapéuticos.
3. Determinar las propiedades reológicas y mecanismos de formación de geles de arcilla en agua mineromedicinal que explican su comportamiento y garantizan su estabilidad, así como la correcta administración de los fangos terapéuticos sobre la piel.
4. Estudiar el procedimiento tradicional de elaboración de fangos terapéuticos denominado “maduración” en vistas a explicar las razones de su utilidad en la obtención de fangos terapéuticos.
5. Profundizar en el estudio farmacéutico de las propiedades de los fangos terapéuticos que explican sus efectos biofísicos y bioquímicos cuando son aplicados en los pacientes.

## II. 2. Plan de trabajo

De acuerdo con los objetivos citados con anterioridad, el plan de trabajo de la tesis doctoral se organizó en los siguientes apartados, correspondientes a capítulos en esta memoria de tesis:

**Capítulo III.** Las arcillas especiales son materias primas empleadas en farmacia y cuyas características y propiedades aparecen claramente especificadas en las correspondientes farmacopeas. No obstante, estos mismos materiales arcillosos son usados en la obtención de preparados empleados en la medicina complementaria y alternativa. Dadas las particularidades de los fangos terapéuticos y su relativa exclusión de la medicina convencional (son usados por la medicina convencional pero no son reconocidos como medicamentos), parece necesaria una revisión y actualización de los conocimientos en este ámbito del empleo de arcillas en medicinas no convencionales, que consideramos, por otra parte, el primer paso del método científico, en vistas al desarrollo galénico de fangos terapéuticos. En este apartado de la tesis se da cumplimento al primer objetivo planteado.

**Sánchez-Espejo, R., Aguzzi, C., Salcedo, I., Cerezo, P. y Viseras, C. (2014). Clays in complementary and alternative medicine. Mater. Technol. 29, B78-B81.**

**Capítulo IV.** En este capítulo se aborda el objetivo 2 de la tesis. En concreto se estudiaron distintos materiales arcillosos usados en la elaboración de fangos terapéuticos en centros termales españoles y de países limítrofes, en vistas a definir la identidad, pureza y riqueza de dichos materiales, así como su seguridad de empleo como excipientes.

**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. J. Ethnopharmacol. 155, 810-814.**

**Capítulo V.** En este capítulo se aborda el objetivos 3 de la tesis doctoral. En concreto, se estudió la influencia de las condiciones de maduración en los estados de agregación, la estructura de gel y el comportamiento reológico de suspensiones de arcillas comerciales de grado farmacéutico en agua mineromedicinal del balneario de Graena, comparándolas con las correspondientes en agua purificada. Se sentaron las bases para el estudio posterior y establecieron puentes de unión entre sistemas farmacéutico (los geles de arcillas de grado farmacéutico en agua purificada, del tipo de magma de bentonita) y los fangos terapéuticos obtenidos al interponer estas mismas arcillas en agua mineromedicinal.

**Aguzzi, C., Sánchez-Espejo, R., Cerezo, P., Machado, J., Bonferoni, C., Rossi, S., Salcedo, I., Viseras, C. (2013). Networking and rheology of concentrated clay suspensions "matured" in mineral medicinal water. Int. J. Pharm. 453, 473-479.**

**Capítulo VI.** Este capítulo se centra en los objetivos 4 y 5 de la tesis doctoral. En el se lleva a cabo la maduración de las arcillas seleccionadas como resultado del estudio llevado a cabo en el capítulo IV y el agua mineromedicinal empleada en el capítulo V. Se estudian los posibles cambios inducidos durante una maduración controlada de los fangos, que pudieran explicar modificaciones en la estabilidad o aplicabilidad (propiedades reológicas) o en la funcionalidad (propiedades térmicas y de liberación de iones).

**Sánchez-Espejo, R., Aguzzi, C., Salcedo, I., Cerezo, P., López-Galindo, A., Machado, J., Viseras, C. (2014). Folk pharmaceutical formulations in western Mediterranean: II. "Maturation" of clays in mineral medicinal water. (Enviado a J. Ethnopharmacol. 18/11/2014).**

# CAPÍTULO III

## Clays in complementary and alternative medicine

# ABSTRACT

In western countries complementary and alternative medicine is used to sustain the mainstream medical practice (biomedicine). Nowadays, it is used to treat diseases, but also in the prevention, health promotion and health maintenance. Complementary and alternative medicine includes treatments that do not involve use of material substances (mind body treatments), but also other therapies including the use of materials in their treatments. Clays are frequently used as biomaterials with clinical applications in complementary and alternative medicine, as actives and excipients. The uses of clay materials in these medicines are revised, including homeopathy, balneotherapy, natural health substances (i.e., food supplements), as well as other traditional therapeutic systems as Ayurveda and traditional Chinese medicine, with increasing presence in western countries.

# III.1. INTRODUCTION

In western countries, the mainstream medical practice is allopathic medicine or biomedicine, also denominated scientific medicine or modern medicine. Nevertheless, there are several other medical practices used as primary source of health care in the world. These practices are non-conventional in western countries, being used as complementary therapies to sustain biomedicine (particularly to treat chronic diseases) and they are usually denominated “complementary and alternative medicine” (CAM) or “traditional medicine” (TM). CAM is found in almost every country in the world[1] and has demonstrated efficacy in areas such as mental health, disease prevention, treatment of non-communicable diseases, and improvement of the quality of life for persons living with chronic diseases as well as for the ageing population. The demand for its services is increasing not only for disease treatments.[2]

Analysis of the status of CAM in western countries reveals that nearly 40 percent of adult USA citizens use some form of CAM, spending more than $30 billion/year.[3] The National Institutes of Health in USA invested in 2012 about $128 million into CAM-related research.[4] In Europe, even if most European countries provide universal health care systems, over 100 million of citizens use CAM.[2] CAM is also widely employed in Canada (70 % of population) [5] and Australia (2 of 3 persons).[6]

Boundaries between conventional medicine and CAM overlap and change with time. For example, in several western countries, acupuncture (previously a non-conventional treatment) is nowadays included in the conventional pain management. With these premises, WHO Traditional Medicine Strategy 2014–2023 is aimed to "support Member States in developing proactive policies and implementing action plans that will strengthen the role CAM plays in keeping populations healthy".[2]

## III.2. CONCEPTS

TM has been defined as "the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness".[2] TM is used as synonym of CAM in western countries. CAM has been defined as "a broad set of health care practices that are not part of that country's own tradition or conventional medicine and are not fully integrated into the dominant health care systems".[2] CAM is used in place of (alternative) or together with (complementary) conventional medicine (accepted by the health care system at a particular time and place). The National Center for Complementary and Alternative Medicine (NCCAM) of USA coined the term "integrative" to refer to the CAM used by evidence-based medicine to auxiliary their treatments.[4] Whether they are used as alternative, complement or integrated with biomedicine, these medicines include treatments that do not involve use of material substances (mind body medicines), but also other therapies involving the use of materials in their treatments (homeopathy, balneotherapy, food supplements, and other natural health care substances).

## III.3. CLAYS IN CAM

Man has used clays since prehistoric times for therapeutic purposes, due to their abundance and their particular properties. The first historical evidence of the use of clay for therapeutic purposes appears in a Sumerian-Mesopotamian tablet, dated around 2500 BC.[7] Many ancient Mediterranean/European medical texts also included clays as medical "simples".[8] Conventional western medicine continues to use clays as excipients and actives in medicinal products[9-12] as well as nanomaterials with promising abilities in drug delivery and tissue engineering.[13-15] Clays are included in medicinal products as pharmacologically inactive ingredients (abrasives, adsorbents, anticaking agents, glidants, coating agents, opacifying agents, viscosity-increasing agents, emulsion stabilizers, binders or suspending agents)[11, 16], or as pharmacologically active substances for the prevention, relief or cure of skin pathologies, inflammations, contusions, and gastrointestinal disorders.[10,12] Besides these conventional uses, clays are also present as ingredients of several CAM, as it will be discussed in this review.

### III.3.1. USE OF CLAYS IN HOMEOPATHY AND ANTHROPOSOPHIC MEDICINE

Homeopathy was developed in Germany arose in the late 19th century. It is one of the most used CAM in Europe and The United States. According to the 2007 National Health Interview Survey, about 4 million Americans adults used homeopathy in 2006.[4] Similar number of Europeans use homeopathy as CAM with biomedicine, even if no centralized data are available on homeopathic medicinal product registration and market authorization in EU Member States.[17, 18] Over 100 million European citizens use over-the-counter or prescribed homeopathic medicines.[19] Briefly, homeopathic treatments are based in three main principles, customization of the treatments, great dilution of the substances and use of materials that in normal doses produces symptoms similar to those of the treated disease. Anthroposophic medicine, founded in the 1920s by Rudolf Steiner, also used ultra-diluted remedies, similar to those also used in homeopathy. In both cases, most of the homeopathic/anthroposophic substances are of natural origin and many of them are plants or plant parts, but also inorganic materials (clays). Clays used in homeopathy/anthroposophy are listed in Table III.I. Most of the items came from the Natural Health Products ingredients database[20] of Canada that is based on three western homeopathic pharmacopoeias: Homeopathic Pharmacopoeia of the United States (HPUS), Encyclopedia of Homeopathic Pharmacopoeia (EHP) and German Homeopathic Pharmacopoeia (HAB). Beside these texts, the Anthroposophic Pharmaceutical Codex[21] includes "Nontronit" and *Terra medicinalis* (dried, finely-divided, naturally occurring clay and silt with a varied composition of aluminium oxide, silica, iron oxide and limestone) as well as *Terra rubra* (natural red clay) as ingredients of homeopathic/anthroposophic preparations. Nontronite and kaolin are also included in the regulation of homoeopathic and antroposophic medicines in Australia[22] and a modified bentonite is considered as homeopathic substance in FDA regulation.[23]

**Table III.1.** Clays used as homeopathic ingredients

| **Ingredient** | **Other denominations** | **Reference** |
|---|---|---|
| EHP Alumina silicata | Alumina silicata, Argilla, China clay, Kaolin, Porcelain clay, White bole | Natural Health Products Ingredients Database (NHPID) [20] |
| EHP Kaolinum | Aluminium silicate, China clay, Kaolinum | |
| EHP Talcum | French chalk, Talcum | |
| HPUS Alumina silicata | Alumina silicata, Argilla, Bolus alba, China clay, Kaolin, Kaolinum, Porcelain clay, White bole | |
| Nontronit | | Anthroposophic Pharmaceutical Codex[21] |
| *Terra medicinalis* | | |
| *Terra rubra* | Red clay | |
| Nontronite | | Regulation of Homoeopathic and Antroposophic Medicines in Australia[22] |
| Kaolin | | |
| Quartenium-18 Sodium bentonite | | Food and Drug Administration database[23] |

## III.3.2. USE OF CLAYS IN HYDROTHERAPY/BALNEOTHERAPY

Hydrotherapy can be defined as "the use of the water in different physical conditions and chemical compositions with many methodologies (traditional and scientific) for the preservation of health, prevention and cure".[24] Balneotherapy is the hydrotherapy with spring water (thermal water or mineral-medicinal water). Use of thermal water in Balneotherapy is part of the conventional medicine in several European countries, in which it is incorporated in medical curricula and the cares are dispensed by National Health Care systems. Nevertheless, this situation is far away to be general in western countries, where hydrotherapy is considered complementary and alternative medicine with different levels of integration to biomedicine.

Clays are used in hydrotherapy to prepare semisolid suspensions that are topically administered to the patients (thermal muds). Mud-pack therapy is effective in treating patients with knee osteoarthritis and other musculoskeletal disorders.[25-27] Thermal muds can be defined as "semisolid natural medicinal products prepared by interposition of organic and/or inorganic solids in mineral-medicinal water, that conveniently processes and administered topically locally or in baths, and as a result of a number of biophysical and/or biochemical actions, are used in therapeutics to treat or prevent some pathologies, or to correct their effects in the organism".[28] Ideally, these systems show thermal and rheological properties adequate to their therapeutic use.[29,30] Nevertheless, thermal muds are not legally recognized as medicinal products in any western country, and therefore they are not subject to any specific legislation. Most of thermal spas in Europe prepare muds with clays located in the neighbour of the thermal station. This situation clearly complicates their normalization and qualification, being desirable that in the next future these muds will comply with a number of requirements[31], such as adequate control of identity, purity, richness, security, efficacy and stability.[32-34]

## III.3.3. USE OF CLAYS IN NATURAL HEALTH PRODUCTS

This group includes a variety of products often sold as dietary supplements (USA and Canada) or food supplements (Europe). Dietary supplements are defined as products intended to supplement the diet, containing one or more dietary ingredients (vitamins, minerals, herbs or other botanicals, amino acids, and certain other substances) or their constituents, intended to be taken orally as tablets, capsules, powders, and other forms.[35] It is compulsory to study their effects and safety, as well as their interactions with medicines and other natural products.[4] In USA, Federal Government regulates dietary supplements through the FDA, but regulations for dietary supplements are different from those for prescription or over-the-counter medicines (OTC).[23]

In Europe, food supplements are defined as concentrated sources of nutrients or other substances with a nutritional or physiological effect whose purpose is to supplement the normal diet, marketed 'in dose' forms (pills, tablets, capsules, liquids and others).[36]

In natural products, clays are included because of their specific physiological effects (adsorbent of toxins, astringent, others) but also as additives or excipients with diverse functions (Table III.II). As a result of their multiple functionalities, clays are frequently employed in dietary supplements (Table III.III). Different clay denominations are included as dietary ingredients, appearing in some cases in the product names (TerraVita® - Clay Gray 450 mg; TerraVita® - Clay Green 450 mg; TerraVita® - Clay Green Powder; TERRAVITA® - Clay White 450 mg; doTERRA Holdings, LLC) and even in the brand name (TERRAMIN A California Living Clay).

**Table III.II.** Uses of clays in Natural health products[20]

| **Clay** | **Uses** |
|---|---|
| Aluminium magnesium silicate | Absorbent, anticaking, binder, disintegrant, emulsion stabilizer, opacifier, slip modifier, thickener, viscosity increasing agent. |
| Attapulgite | Surfactant-suspending agent, viscosity increasing agent. |
| Bentonite | Absorbent, bulking agent, emulsion stabilizer, stabilizing agent, viscosity increasing agent. |
| Kaolin | Abrasive, absorbent, anticaking, anticoagulant, bulking agent, coating agent, colour additive, diluent, filler, flow enhancer, glidant, lubricant, opacifier, slip modifier. |
| Magnesium trisilicate | Anticaking, clarifier, glidant, polishing agent. |
| Pyrophylite | Absorbent, colour additive, opacifier. |
| Talc | Abrasive, absorbent, anticaking agent, anticoagulant, bulking agent, coating agent, colour additive, diluent, filler, flow enhancer, glidant, lubricant, opacifier, slip modifier. |

**Table III.III.** Presence of clays in Dietary Supplements marketed in USA[37]

| Denomination | In the product name | As dietary ingredient | As additive |
|---|---|---|---|
| Clay | 5 | 6 | 40 |
| Gray clay | … | 1 | 1 |
| Green clay | … | 1 | 2 |
| White clay | … | 1 | 1 |
| Bentonite | … | 5 | 48 |
| Bentonite clay | … | 1 | 21 |
| Bentonite clay powder | … | 1 | 4 |
| Bentonite mineral powder | … | 1 | 2 |
| Hydrated bentonite | … | 1 | 1 |
| Pyrophyllite clay | … | 1 | 1 |
| Talc | … | 1 | 425 |
| Hydrated magnesium silicate | … | … | 2 |
| Magnesium silicate | … | … | 224 |
| Magnesium silicate hydroxide | … | … | 3 |
| Magnesium trisilicate | … | … | 23 |
| Terra | 1 | 3 | 8 |
| Terra alba | … | 1 | 1 |

## III.3.4. USE OF CLAYS IN OTHER CAMs

Clays are also used in other CAMs, such as traditional Chinese and Ayurvedic medicines. The Chinese Materia Medica describes thousands of medicinal substances, including some minerals. In particular, halloysite (Chi Shi Zhi, Halloysitum Rubrum) is used orally as astringent, to stop diarrhoea and bleeding; and topically to produce skin and cure ulcer. Talc (Hua Shi, Talcum) is used as diuretic and to clear heat and summer-heat, as well as to promote wound healing.[38] Ayurvedic pharmacopoeia of India comprises 21 minerals used to prepare medicinal products, including kaolinite (Kha°ikā) and biotite (Abhraka). Kaolinite is used orally to treat inflammations, diseases of eyes and diarrhoea as well as topically to treat disorders of skin, mouth and tooth. Biotite is always used after calcination (bhasma) with several therapeutic uses.[39]

# III.4. CONCLUSIONS

Clays are frequently used as biomaterials in CAM. These therapies and their products are gaining attention in occidental countries. Although research has explored many of CAM products, in most instances scientific evidence regarding efficacy or safety to support or refute their use is insufficient. Only some of these products have been studied in large, placebo-controlled trials. Research on others to determine whether they are effective and safe is required. It is imperative to determine the effects of these products in the human body and about their safety and potential interactions with medicines and with other natural products. Although further research, clinical trials, and evaluations are needed, complementary/alternative medicine has shown great potential to meet a broad spectrum of health care needs. Consequently, the resolution on traditional medicine adopted by the World Health Assembly in May 2009 (WHA62.13), urged Member States to “formulate national policies, regulations and standards, as part of comprehensive national health systems, to promote appropriate, safe and effective use of traditional medicine.” WHO also requests to "continue providing technical guidance to support countries in ensuring the safety, efficacy and quality” of these medicines and treatments. Obviously, safety and efficacy of these products would absolutely require a scientific orientation focused to assure high quality through following applicable requirements, being an interesting research area in biomaterials.

# CAPÍTULO IV

## Folk pharmaceutical formulations in western Mediterranean: Identification and safety of clays used in pelotherapy

# ABSTRACT

*Ethnopharmacological relevance:* Clays are naturally occurring ingredients of many natural health products, being included in most of ancient Mediterranean/European medical texts and currently used to prepare therapeutic hot-muds (peloids) in several thermal stations of the Mediterranean region. Clays are included in the formulation of peloids as vehicles of the mineral-medicinal water, to obtain inorganic gels with rheological and thermal properties suitable to be topically applied. Knowledge about formulations and preparation procedures of these traditional medicines has been orally transmitted since ancient times. Increasing recognition of the therapeutic utility of these traditional and natural health care substances make necessary a full ethnopharmaceutic research to ascertain those compositional characters that allow to establish quality attributes and corresponding requirements for these materials and products, including identity, purity, richness and safety.

*Materials and methods:* Five clay samples (A, B, C, D and E) currently used in various spa centers of southern European/Mediterranean countries were studied. X-Ray diffraction (XRD) and X-Ray fluorescence (XRF) data were used to asses sample identity and richness. Elemental impurities and microbiological contaminants were also determined and compared to normative limits. Particle size distribution was related to their safety as powder materials.

*Results:* Samples A, C, D and E were identified as “high purity clay”, while sample B was identified as a mix of clay minerals and carbonates. The presence of carbonates in this sample could compromise its suitability for pelotherapy. The studied clays meet the main normative limits for metals impurities, with the exception of arsenic in sample A and nickel in sample B. The samples comply with the microbiological limits proposed by European legislation for medicinal products. According to the particle size of the studied samples, prevention and control of dust exposure must be considered.

*Conclusions:* Despite their demonstrated longevity, the use of clays in traditional medicine formulations as peloids greatly requires comprehension of their identity and safety attributes. Continuity of these mineral substances as recognized health care ingredients oblige to conduct interdisciplinary research to know the features that sustain their traditional use in the preparation of medicines (ethnopharmaceutics).

# IV.1. INTRODUCTION

Traditional medicine involves the use of naturally occurring materials, mainly plants and plant parts, but also inorganic materials, and in particular, clays. On behalf of their healing properties, clays had been included in most of ancient Mediterranean/European medical texts (De Vos, 2010), being used to treat skin pathologies (antiseptic cataplasms), intestinal and stomach diseases (because of its astringent and adsorbent properties) as well as in balneotherapy (Carretero et al., 2006).

Nowadays, modern medical care continues to use clays as highly significant health care ingredients in industrial products (López Galindo and Viseras, 2004) as well as in natural remedies and dietary supplements (http://www.dsld.nlm.nih.gov/dsld/). In western medicine, clays are used in patent medicinal products both as pharmacologically inactive ingredients (abrasives, adsorbents, anticaking agents, glidants, coating agents, opacifying agents, viscosity-increasing agents, emulsion stabilizers, binders or suspending agents) (Viseras et al., 2007; Rowe et al., 2009), and as pharmacologically active substances for the prevention, relief or cure of skin pathologies, inflammations, contusions, and gastrointestinal disorders (López-Galindo et al., 2011; Carretero et al., 2013).

In the last century, the use of clays in traditional European medicine had been practically limited to medical hidrology (pelotherapy), with only some exceptions in folk European (Pieroni et al., 2004) or migrant communities pharmacopoeias (Ellena et al., 2012). Despites the longevity of clays as therapeutic ingredients ("Terra sigillata" was probably the first patented medicine (Finkelman, 2006)), the dominant European health care system, based on allopathic medicine, considers the use of clays in traditional medicine as "complementary", "alternative" or "non-conventional" remedies. However, traditional medicine in developed countries is gaining popularity because it offers less adverse effects and gentler means of managing chronic diseases (heart disorders, cancer, diabetes...) in comparison with allopathic medicine (WHO, 2005). In particular, several studies have analyzed the effectiveness of pelotherapy in patients with rheumatic diseases versus allopathic therapies, noticing improvements in quality of life or drug consumption (Bostan et al., 2010; Fioravanti et al., 2012; Espejo Antúnez et al., 2013). Pelotherapy involves the topical application of heated muds, constituted by semisolid dispersions of clayey solid phases into mineral-medicinal waters (Veniale et al., 2004). The selection of mud components, as well as their preparation and application procedures follows traditional knowledge; the systems are frequently prepared and used without proper understanding of their composition and quality controls. However, confirmation (or refutation) of the possible effectiveness of these natural health care products would absolutely require a scientific orientation focused to assure applicable requirements (López-Galindo et al., 2007). It is desirable to design and implement quality systems involving adequate control of raw materials, procedures and final products.

Clays are included in the formulation of peloids as vehicles of the mineral-medicinal water, to obtain inorganic gels with rheological and thermal properties suitable to be topically applied. Knowledge about formulations and preparation procedures of these traditional medicines has been orally transmitted since ancient times. The study of these folk medicinal products involving identification of the ingredients and preparation procedures fall into the concept of ethnopharmaceutics, as a part of ethnopharmacy (Heinrich and Pieroni, 2001).

With these premises, this study is a first step in a much larger project aimed to address the traditional therapeutic use of clays in European/Mediterranean countries in view of establishing the minimum compositional requirements that should be comply to make possible the evaluation of clinical efficacy of the treatments based on these natural inorganic substances. For these purposes, and following pharmacopoeias and international guidelines (www.healthcanada.gc.ca/nhp; ICH, 2013), this study aims to determine compositional attributes of clay samples used in European/Mediterranean thermal stations, including identity, purity, richness and safety of these natural ingredients.

## IV.2. MATERIALS AND METHODS

Five clay samples (A, B, C, D and E) used to prepare peloids in 17 spa centers of three southern European/Mediterranean countries (Italy, Spain and Tunisia) were studied. Sample A was Volcangel© gifted by BENESA (Spain). Sample B was gifted under confidential agreement. Sample C was Peloide Minerale® from SO.MI.ES. (Italy). Sample D, came from Jebel Aidoudi deposits (Tunisia) and sample E was Innogel LBB® clay purchased from Aplicaciones especiales del Vallés S.L. (Spain). Selected samples were representative of the raw materials used by a large number of thermal spas. All samples were milled, sieved (< 125 μm) and dried in oven (40 °C) before carrying out any test.

## IV.2.1. IDENTITY AND RICHNESS

X-Ray diffraction (XRD) data and chemical analysis (major elements) were used to asses sample identity and richness. The mineral quantification was based on the measurements of peak areas in the diffractograms according to Biscaye (1965) and Moore and Reynolds (1989), and corrected with chemical data (López-Galindo et al., 1996) using the XPOWERs program (http://www. xpowder.com/) (Martín-Ramos, 2004). XRD analysis was done by using a Philips® X-Pert (Philips, Holanda) diffractometer equipped with automatic slit (CuKα, 4–70° 2θ, 6°/min, 40 kV) both on bulk random and oriented aggregated samples (glycolated and heated to 550 °C). 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).

## IV.2.2. PURITY

### IV.2.2.1. TRACE ELEMENTS

The following elements were analyzed by an ICP/MS Perkin-Elmer® SCIEX Elan-5000 equipment prior sample digestion with “aqua regia” (HNO3 + 3HCl): Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Se and Tl. Accuracy was in 1–3% range, depending on the element amount. The analyses were carried out by Activation Laboratories Ltd. (Canada).

### IV.2.2.2. MICROBIOLOGICAL CONTAMINANTS

Microbiological test were carried out according to European Pharmacopoeia methods (EP 7.0., 2011). The analysis included total viable aerobic, contaminating fungi (yeast and mold), *Salmonella spp*., *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

### IV.2.3. PARTICLE SIZE DISTRIBUTION

Particle size analysis was performed with a Malvern® Master-sizer 2000 LF granulometer. Prior to the analysis, a few milligrams of powder sample were dispersed in purified water and sonified for several minutes.

## IV.3. RESULTS AND DISCUSSION

### IV.3.1. IDENTITY AND RICHNESS

Mineralogical compositions of the samples (Table IV.I) were determined on the basis of XRPD patterns (Fig. IV.1) and chemical compositions (Table IV.II). Samples A, C, D and E were identified as “high purity clays” as the sum of minerals assigned to this group (smectites, kaolinite, illite and chlorite) was > 70 % w/w. Sample B was identified as a mix of clay minerals (illite, chlorite and kaolinite) and carbonates (calcite and dolomite). Significant presence of carbonates (13 % w/w) was also detected in sample C. Several authors have pointed out the importance of the presence of high content of clay minerals (in particular smectites) in samples to be used in pelotherapy, because rheological, thermal and chemical features associated to these minerals improve some performances (Veniale et al., 2004; Carretero et al., 2007; Rebelo et al., 2011). Carbonates can be considered as “inert” impurities, even if their high relative amount in sample B could compromise its suitability for pelotherapy. Crystalline silica (quartz) was also measured in all the studied samples. This mineral is usually present in all clay deposits, but its occurrence in health care products must be reduced as much as possible as it is classified by the International Agency for Research on Cancer (IARC) as a product with sufficient evidence of carcinogenicity in laboratory animals and limited evidence in humans (Group 1) (IARC, 1997). In a most recent monograph, it has been corroborated that crystalline silica in the form of quartz or cristobalite dust is carcinogenic to humans (Group 1) (IARC, 2012). It must be pointed out that crystalline silica did not show the same carcinogenic potency in all circumstances (IARC, 1997). In particular, the association of quartz with clay minerals inhibits most adverse effects (Duffin et al., 2001; Schins et al., 2002, Creutzenberg et al., 2008; Miles et al., 2008).

**Table IV.I.** Mineral composition (w/w %) of the studied samples

| | A | B | C | D | E |
|---|---|---|---|---|---|
| Smectites | 65 | traces | 42 | 68 | 80 |
| Quartz | 5 | 13 | 10 | 5 | 7 |
| Calcite | 5 | 31 | 11 | 4 | - |
| Albite | 10 | 4 | 4 | - | - |
| K-Feldspars | 3 | 2 | - | - | - |
| Kaolinite | traces | 7 | 9 | 5 | 12 |
| Illite | 6 | 23 | 22 | 7 | - |
| Clinoptilolite | 6 | - | - | - | - |
| Dolomite | - | 6 | 2 | - | - |
| Chlorite | - | 12 | - | - | - |
| Gypsum | - | traces | - | 11 | 2 |

**Table IV.II.** Major-element content (w/w %) of the studied samples

| | $SiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | MnO | MgO | CaO | $Na_2O$ | $K_2O$ | $TiO_2$ | $P_2O_5$ | LOI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 56.71 | 11.17 | 3.36 | 0.05 | 12.62 | 3.81 | 2.89 | 1.47 | 0.37 | 0.10 | 7.11 |
| B | 33.82 | 11.37 | 4.39 | 0.06 | 4.17 | 19.66 | 0.62 | 2.43 | 0.44 | 0.12 | 22.00 |
| C | 48.64 | 17.23 | 6.52 | 0.18 | 2.35 | 7.97 | 1.26 | 2.46 | 0.76 | 0.15 | 10.90 |
| D | 45.83 | 17.23 | 6.67 | 0.03 | 2.03 | 6.32 | 3.14 | 1.72 | 0.96 | 0.29 | 8.56 |
| E | 50.98 | 18.63 | 9.31 | 0.03 | 2.42 | 1.00 | 2.33 | 1.20 | 1.35 | 0.14 | 10.80 |

LOI (loss on ignition)

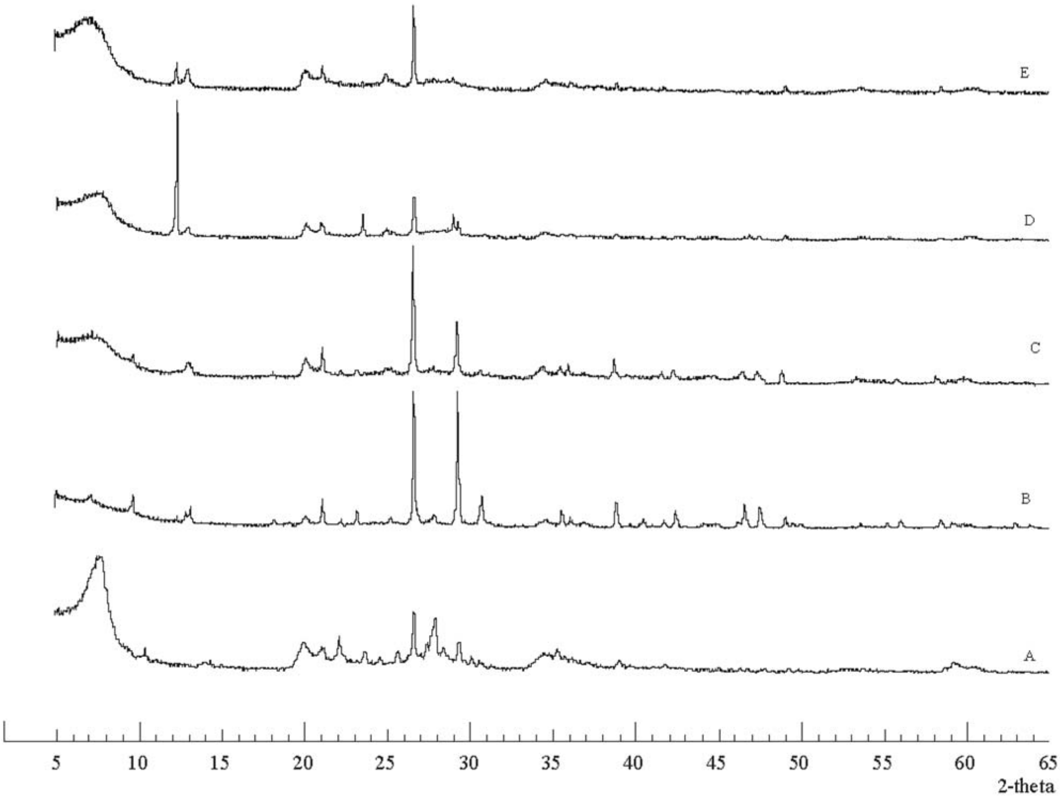


**Fig. IV.1.** XRD patterns of the studied samples

## IV.3.2. PURITY

### IV.3.2.1. TRACE ELEMENTS

All the trace elements considered are naturally associated to mined materials. The speciation and physicochemical state of these elements are important factors in considering their bioavailability and toxicity. Most of the elements that can exert adverse effects even at low concentration should be maintained within acceptable limits. In absence of a specific legislation, the measured values of elemental impurities in the studied samples were compared to the limits proposed for clays to be used as pharmaceutical ingredients by European and USA pharmacopoeias (EP 7.0., 2011; USP 36–NF 31, 2013), those recommended for topical products by the Natural Health Product Directorate (NHPD) of Canada (www.healthcanada.gc.ca/nhp) and those included in the

“Guideline for elemental impurities” in drug products, recently issued by the International Conference of Harmonization (ICH, 2013).

Pharmacopoeial impurities only refer to arsenic and lead content. As shown in Table 3, all samples met the pharmacopoeial limits, with the exception of arsenic for sample A. However, it must be pointed out that systemic absorption of arsenic via the skin is very low (NRC, 1999; ATSDR, 2007). The use of sample A in pelotherapy is therefore unlikely to be harmful, even if treatment to reduce arsenic would be desirable.

As regards to other significant elements with health concern, contents of Cd, Hg and Sb were compared to limits proposed in NPHD guide for topical products (Table IV.III). All samples meet the required limits for these elements. The presence of other elements (Mo, Se, Co, Ag, Au, Tl, Ba, Cr, Cu, and Ni) with relative low toxicity or toxicity based on route of administration were also evaluated and compared with limits included in guide Q3D of ICH (Table IV.III). The Q3D guide defines relevant thresholds for elemental impurities in raw materials (both actives and excipients) and medicinal products administered by oral, parenteral and inhalatory routes. The studied samples meet the required limits, with the exception of Co content. Topical exposure to Co induces dermatitis (ATSDR, 2004). Nevertheless, it must be pointed that allergic topical effects of Co appear mainly from exposure to the metal itself, rather than to aqueous Co salts (Nielsen et al., 2000). Ni content in sample B was also greater than the required limit. Allergic contact dermatitis is also frequently induced by Ni chlorate and sulfate (ATSDR, 2005). Ni is a natural constituent of soil in concentrations ranging from 4 to 80 ppm (ATSDR, 2005). The presence on > 80 ppm of Ni in sample B should be take into consideration, since human and animal studies showed that Ni can penetrate the skin (Norgaard, 1955; Norgaard, 1957; Lloyd, 1980; Fullerton et al., 1986).

**Table IV.III.** Trace elements (ppm, aqua regia digestion) in the samples with safety concerns accordingly to European and USA pharmacopoeias (EP, USP); Canadian Natural Health Products Guide (NHPG) and Q3D guideline (Q3DG)

| Element (detection limit, ppm) | A | B | C | D | E | Acceptable limit (ppm) | Normative | Class |
|---|---|---|---|---|---|---|---|---|
| As (0.5) | 15.6 | 3.8 | 4.4 | 5.0 | 1.8 | ≤ 8 | EP, USP | |
| Pb* (0.1) | 11.4 | 12.9 | 13.4 | 9.6 | 8.1 | ≤ 50 | | |
| Cd (0.1) | < 0.1 | 0.2 | 0.1 | < 0.1 | < 0.1 | 3 | NHPG | |
| Hg (0.01) | < 0.01 | 0.04 | 0.04 | < 0.01 | < 0.01 | 1 | | |
| Sb (0.1) | 0.2 | 0.5 | 0.2 | < 0.1 | < 0.1 | 5 | | |
| Mo (0.1) | 0.2 | 1.8 | 1.7 | 0.7 | 0.6 | 18 | Q3DG** | 2A |
| Se (0.5) | 0.7 | 1.7 | 0.9 | 1.3 | 1.4 | 17 | | |
| Co (0.1) | 5.9 | 13.8 | 19.2 | 12.7 | 12.9 | 5 | | |
| Ag (0.1) | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | 17 | | 2B |
| Au (0.01) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | 13 | | |
| Tl (0.1) | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.80 | | |
| Ba (1) | 359 | 102 | 158 | 25 | 183 | 1300 | | 3 |
| Cr (1) | 16 | 76 | 58 | 101 | 86 | 1100 | | |
| Cu (0.1) | 21.7 | 26.1 | 62.9 | 15.9 | 39.2 | 130 | | |
| Ni (0.1) | 6.3 | 88.4 | 54.5 | 33.1 | 41.9 | 60 | | |

*"Heavy metals" assay in European Pharmacopoeia match to Pb determination (EP, 2011).

** Oral limits in drug products, drug substances and excipients, accordingly to table A.2.2 of Q3D guide.

### IV.3.2.2. MICROBIOLOGICAL CONTAMINANTS

The presence of certain microorganisms in non-sterile preparations may have the potential to adversely affect the health of the patients and must be consequently carefully determined. Results of microbial examination of the studied samples were compared to normative limits proposed by European legislation (EP 7.0., 2011) (Table IV.IV). The limit for total aerobic microbial count is $10^3$ CFU/g in “non-sterile substances for pharmaceutical use", whereas for "non-sterile oral dosage forms containing raw materials of natural (animal, vegetal o mineral) origin" it increases to $10^4$ CFU/g, reaching $10^5$ CFU/g in some "herbal medicinal products". Similarly, the limit for combined yeasts/molds is in the range $10^2 – 10^4$ CFU/g, depending on the nature of the starting material and the manufacturing process. None of the samples exceeded the acceptance criteria for "herbal medicinal products". With exception of sample A, the samples also complied with limits for "non-sterile oral dosage forms containing raw materials of natural (animal, vegetal o mineral) origin". As regards of pathogen microorganisms, all the samples complied with the required absence of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella spp*. and *Staphylococcus aureus*.

**Table IV.IV.** Results of microbial examination of the samples and acceptance criteria for "Non-sterile substances" (NSS), "Oral dosage forms containing materials of natural origin" (MNO) and "Herbal medicinal products" (HMP) accordingly to European Pharmacopoeia

| Microorganism (CFU/g) | A | B | C | D | E | NSS | MNO | HMP |
|---|---|---|---|---|---|---|---|---|
| Total aerobic microbial | 14000 | 4400 | 3300 | < 1000 | 10000 | $10^3$ | $10^4$ | $10^5$ |
| Yeasts and Moulds | < 10 | < 10 | < 10 | < 10 | 86 | $10^2$ | $10^2$ | $10^4$ |
| *Escherichia coli* | A | A | A | A | A | A | A | A |
| *Pseudomonas aeruginosa* | A | A | A | A | A | - | - | - |
| *Salmonella* spp. | A | A | A | A | A | A | A | A |
| *Staphylococcus aureus* | A | A | A | A | A | A | - | - |

**A (absence)**

## IV.3.3. PARTICLE SIZE

The granulometric distribution of the samples was almost unimodal with mean particle diameters in the range 4.4 (sample D) – 16.7 µm (sample A) (Fig. IV.2). The pathogenic inhalatory potential of particulate matters (dust) are related to their composition, as for example the presence of crystalline silica or chemical impurities, as discussed previously, but also to their physical properties, and in particular to the particle size distribution (WHO, 1999). Depending on their size, particles of raw clay materials used to prepare peloids could become hazardous to worker health, particularly when suspended in air. The respirable dust range harmful to workers' health is defined by those particles at, or below, 10 µm size range (Cecala et al., 2012). Smaller airborne particles of dust, which can remain suspended in air for hours, pose a greater risk to the respiratory system when inhaled. Particles having a diameter of greater than 10 µm are expected to be deposited in the nasopharyngeal region, whereas particles with diameter under 5 µm may achieve the bronchial and alveolar regions. Cumulative particle size distribution of the studied samples (Fig. IV.3) was used to determine the fraction of particles likely to be deposited in the alveolar region of the lungs when inhaled (respirable dust). More than 99 % of the particles had a diameter under 100 µm and consequently prevention and control of dust exposure must be considered. Cumulative % of particles with diameters under 5 µm were noticeable, ranging from 27.4 (sample A) to 69.8 (sample D). Taking into account that studied samples also present quartz contents > 5 % w/w, strategies of controlling occupational exposure, as well as implementation of control measures should be desirable.

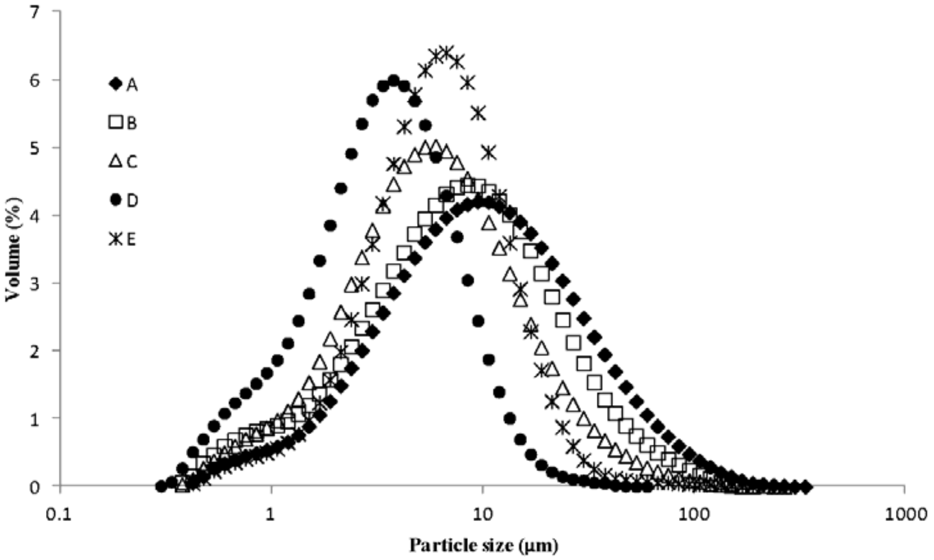


**Fig. IV.2.** Granulometric distribution of the samples

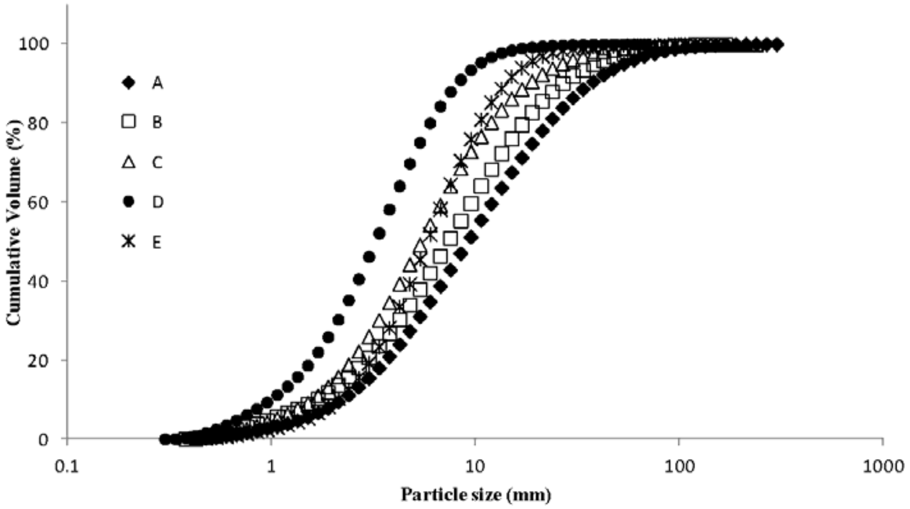


**Fig. IV.3.** Cumulative particle size distribution of the studied samples

# IV.4. CONCLUSIONS

Despite their demonstrated longevity and therapeutic effectiveness, the use of clays in traditional medicine greatly requires comprehension of their action mechanism and composition related effects. Continuity of these mineral substances as recognized health care ingredients obliges to conduct interdisciplinary research to understand the features that sustain their therapeutic uses. Accordingly to their mineralogical composition, the studied samples were identified as "high purity clays" with important differences in richness. In particular, the presence of carbonates in sample B characterizes this raw material. As regards of the presence of mineral impurities with safety concerns, quartz amounts in the samples was not relevant for patient's health, even if the presence of this mineral would require adequate controls during the preparation stages of the thermal muds to prevent any hazard in workers. Elemental impurities in the studied samples were compared to the limits proposed in three related legislations, finding that As in sample A and Ni in sample B should be reduced. The samples comply with the microbiological limits proposed by European legislation. Finally, as regards of the particle sizes of the studied samples, control of occupational exposure is advised.

# CAPÍTULO V

# Networking and rheology of concentrated clay suspensions “matured” in mineral medicinal water

# ABSTRACT

This work studied the influence of “maturation” conditions (time and agitation) on aggregation states, gel structure and rheological behaviour of a special kind of pharmaceutical semisolid products made of concentrated clay suspensions in mineral medicinal water. Maturation of the samples was carried out in distilled and sulphated mineral medicinal water, both in static conditions (without agitation) and with manual stirring once a week, during a maximum period of three months. At the measured pH interval (7.5-8.0), three-dimensional band-type networks resulting from face/face contacts were predominant in the laminar (disk-like) clay suspensions, whereas the fibrous (rod-like) particles formed micro-aggregates by van der Waals attractions. The high concentration of solids in the studied systems greatly determined their behaviour. Rod-like sepiolite particles tend to align the major axis in aggregates promoted by low shearing maturation, whereas aggregates of disk-like smectite particles did not have a preferential orientation and their complete swelling required long maturation time, being independent of stirring. Maturation of both kinds of suspensions resulted in improved rheological properties. Laminar clay suspensions became more structured with time, independently from static or dynamic maturation conditions, whereas for fibrous clay periodic agitation was also required. Rheological properties of the studied systems have been related to aggregation states and networking mechanisms, depending on the type of clay minerals constituents. Physical stability of the suspensions was not impaired by the specific composition of the Graena medicinal water.

# V.1. INTRODUCTION

Rheological properties of semisolid pharmaceutical products greatly determine their physical stability and applications (Lee et al., 2009). Clay minerals are prominent excipients used to achieve adequate semisolid formulations, having been the subject of numerous studies (Viseras et al., 2007). Peloids are special kind of health care semisolid systems consisting of a solid phase rich in clay minerals dispersed in mineral medicinal water. Pelotherapy, intended as the therapeutically use of naturally occurring sediments, is one of the most popular techniques in thermal treatments (Viseras and Cerezo, 2006).

It consists in the local application of peloids for the treatment of musculoskeletal and skin diseases (Torresani, 1990; Fabiani et al., 1996; Sukenik et al., 1999; Argenziano et al., 2004; Cozzi et al., 2007; Fioravanti et al., 2007). In the last decade, beauty and anti-stress treatments were also added to these traditional uses (Morganti et al., 2001; Varvaresou et al., 2011). A very important aspect to consider in pelotherapy concerns on the need of peloids for specialised treatments. In fact, treatments with different purposes, such as traditional treatments (musculo-skeletal, rheumatic, skin) and cosmetic ones (beauty and anti-stress) should require the use of peloids with specific features suitable as appropriate. Therefore, formulation of exclusive peloids has been defined as the "new frontier" of pelotherapy (Veniale et al., 2007). For this purpose a systematic approach is needed, able to determine optimum characteristics of these special clay/water suspensions depending on the required treatment. This objective may be achieved studying not only the peloid itself, but also their components (mineromedicinal water and clay minerals) and the process of maturation (contact between the solid and water medium during a prolonged time period). For example, several studies have been published focusing on characteristics of peloids and their constituents, including mineralogical and chemical compositions (Summa and Tateo, 1998, 1999; Cara et al., 2000a; Sanchez et al., 2002; Veniale et al., 2004; Tateo et al., 2009; Baschini et al., 2010; Carretero et al., 2010; Karakaya et al., 2010; Rebelo et al., 2011a), thermal behaviour (Ferrand and Yvon, 1991; Cara et al., 2000b; Ortiz de Zárate et al., 2010; Casas et al., 2011; Knorst-Fouran et al., 2012) and biochemical and microbiological aspects (Andreoli and Rascio, 1975; Ferrara et al., 1999; Galzigna et al., 1996). Moreover, peloids are semisolid products that require a detailed characterisation of their rheological properties (Bettero et al., 1999). In such systems, flow behaviour affect the entire life of the product, including filling, mixing, packaging, removal from the container and define the *in vivo* behaviour (Lee et al., 2009). In general, formulations with high viscosity and thixotropic properties are desirable, i.e. showing high consistency at rest, continuous decrease of viscosity with time when stress is applied and subsequent recovery of viscosity in time when the stress is discontinued (Mewis and Wagner, 2009). Peloids shows these rheological features, as they are concentrated clay suspensions resulting in structured gels (Viseras et al., 2006b, 2007; Rebelo et al., 2011b). Clay gelling structures came from the aggregation of clay minerals particles by different mechanisms mainly depending on the type of clay minerals dispersed, pH and ionic strength (Lagaly, 2006). Clay mineral powders are constituted by single mineral particles (assembly of clay mineral layers), aggregates (assembly of particles) and assembly of aggregates (Bergaya and Lagaly, 2006). Traditionally, peloids were prepared with common clays, mostly consisting of mixtures of smectites, illite and kaolinite, but also including carbonates, quartz and others minerals in various proportions. Frequently, peloids need to be heated before being applied, because their therapeutic effects mainly depend on their capacity to transfer heat slowly (low cooling rates). It has been demonstrated that the use of almost pure phyllosilicates (main mineral > 90 %) gave rise to systems with better thermal performance in comparison with common mixtures (Legido et al., 2007). Therapeutic activity of peloids is also related to the ionic composition of the liquid phase. It was suggested that peloids based on phyllosilicates with high cation exchange capacity (saponite, montmorillonite) may be enriched with ions provided by dispersion medium, showing potential creno-therapeutic activity (Carretero et al., 2007).

It must be taken into account that rheological behaviour of a semisolid product can change over time, leading to inadequate formulations (Martin, 1993). These changes could be largely important in pelotherapy, since maturation of peloids implies prolonged periods of time from the interposition of the solid particles in the mineral medicinal water to the moment of their application. Moreover, during maturation peloids are mixed or remain undisturbed, depending on the specific protocols of each thermal station (Veniale et al., 2004). Peloids are therefore complex systems, whose rheological stability should be evaluated over maturation time. As therapeutic products, these suspensions must comply with a number of requirements, and in particular, they must realize quality constancy attributes when applied, including identity, purity and stability features.

Given these premises, aim of this work was to study the influence of maturation conditions (time and agitation) on physical stability of peloids prepared with laminar and fibrous clay samples suspended in a mineral medicinal water. The effects of particle-particle interactions (leading to new aggregates and changing the system properties) were followed by particle size measures and rheological characteristics. Maturation of the samples was carried out in static conditions (without agitation) and with manual stirring once a week, during a maximum period of three months.

# V. 2. MATERIALS AND METHODS

Two clay samples were used; Veegum© HV (VHV), a pharmaceutical grade magnesium aluminium silicate (Vanderbilt Ltd., USA), and sepiolite from Vicalvaro (SV) (Tolsa SA, Spain). Both samples were fully characterised in previous works in terms of mineralogical, chemical, textural and technological properties (Viseras and López-Galindo, 1999, 2000; Viseras et al., 2000, 2001a,b; Cerezo et al., 2001; Aguzzi et al., 2005). Accordingly, VHV was composed (> 85 % w/w) of at least four types of laminar shape minerals, each one corresponding to an intermediate mineral phase between di-(montmorillonite) and tri-(saponite) octahedral smectites, while SV is composed of fibrous particles of sepiolite as a percentage above 95 % (w/w). 50.07 meq/100 g $Na^+$ and 18.28 meq/100 g $Ca^{2+}$ were the main exchangeable cations in VHV (Aguzzi et al., 2005).

Medicinal mineral water, hereafter referred as Graena water (Table V.I), came from the thermal spring of Graena (Cortes y Graena, Granada, Spain), being used for several purposes, including pelotherapy with local clays. A Crison® pHmeter mod. Basic 20+ with 5010T and 5071 sensors was used to determine pH and conductivity, respectively. Dissolved ions were determined by specific titration procedures (Porretta, 1990). Distilled water was also used to compare with Graena water. The measured pH of distilled water was 5.5 (± 0.12).

**Table V.I.** Physicochemical characteristics of Graena water (means values ± s.d.; n = 3)

| | |
|---|---|
| **pH** | 7.98 ± 0.380 |
| **Conductivity (20ºC) (μS/cm)** | 1729.75 ± 235.968 |
| **$Cl^-$ (ppm)** | 17.75 ± 1.775 |
| **$SO_4^{2-}$ (ppm)** | 1440.58 ± 13.757 |
| **$NO3^-$ (ppm)** | 1.00 ± 0.000 |
| **$NO^{2-}$ (ppm)** | 0.01 ± 0.007 |
| **$CO_3^{2-}$ (ppm)** | 10.50 ± 3.000 |
| **$HCO_3^-$ (ppm)** | 116.66 ± 11.780 |
| **$Ca^{2+}$ (ppm)** | 438.88 ± 34.092 |
| **$Mg^{2+}$ (ppm)** | 116.73 ± 20.829 |
| **$NH^+_4$ (ppm)** | 0.14 ± 0.121 |

## V.2.1. PREPARATION OF PELOIDS

Clay/water suspensions were prepared by dispersing 25 g of VHV and SV powders in 75 g of distilled or Graena water, using a turbine stirrer (Ultraturrax T25, Janke and Kunkel GMBH & Co., G) at 10000 rpm for 5 min. The resultant suspensions were stored at room temperature (25 ºC) in airtight polyethylene containers, to avoid contamination, for three months without stirring (static maturation) or manually stirred by means of a glass rod performing planetary movements for 30 min every seven days (dynamic maturation). At time 0, 1, 2 and 3 months, aliquots of the suspensions were taken to be characterized. Name, composition and maturation conditions of each sample are shown in Table V.II.

**Table V.II.** Components and maturation conditions of the samples

| Clay mineral | Dispersion medium | Maturation | Peloid |
|---|---|---|---|
| VHV | Distilled water | Dynamic | $VHV/Dist^{+}$ |
| | | Static | $VHV/Dist^{-}$ |
| | Graena water | Dynamic | $VHV/Graena^{+}$ |
| | | Static | $VHV/Graena^{-}$ |
| SV | Distilled water | Dynamic | $SV/Dist^{+}$ |
| | | Static | $SV/Dist^{-}$ |
| | Graena water | Dynamic | $SV/Graena^{+}$ |
| | | Static | $SV/Graena^{-}$ |

## V.2.2. WATER CONTENT, pH AND PARTICLE SIZE

The water content of suspensions was determined by weight loss on drying of 1 g of sample. pH was measured by using a pHmeter Crison (mod. 2001) equipped with a semisolid sensor (5053T). Particle size analysis was performed by a Coulter Counter method (Coulter Multisizer II, Coulter electronics Ltd., UK) with 50 µm (VHV) and 140 µm (SV) orifice tube. Prior to the analysis, a few milligrams of each sample were dispersed in isotonic NaCl (0.9% w/v) as conductive medium. Data were on line collected by means of AccuComp Windows Software for Z2 (Beckman Coulter, I) and particle volume equivalent diameters ($d_{10}$, $d_{50}$, $d_{90}$) were calculated. At least three replicates were performed for each test.

### V.2.3. RHEOLOGICAL ANALYSIS

Rheological analysis was carried out with a Bohlin CS® rheometer (Bohlin Instrument Division, Metrics Group Ltd., UK) connected to a "personal computer" to set analysis parameters, process and record data. A cone/plate combination (CP 4/20) was used as measuring system. Measurements were carried out at room temperature (25 ºC) after a rest time of 90 s. Rheological properties of the samples were measured in the range 70-800 $s^{-1}$ following a “constant rate test” procedure. This interval was selected as representative of the stress produced by technological operations and manipulation, like skin spreading (10-200 $s^{-1}$), manual mixing (100-200 $s^{-1}$) and container removal (400-2000 $s^{-1}$) (Schott, 1995).

Rheological characterization included the area of hysteresis between the flow curves obtained with increasing and decreasing shear rate, the yield point and the apparent viscosity of the samples. Six replicates were performed on each sample.

### V.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). The comparison of two groups (t Student test) was performed with Statgraphics® 5.0 statistical package (Statistical Graphics Corporation, Rockville, MD, USA). Differences between groups were considered to be significant at a level of P less than 0.05.

## V.3. RESULTS AND DISCUSSION

### V.3.1. WATER CONTENT, pH AND PARTICLE SIZE

The influence of a possible decrease of water content should be controlled and taken into account for rheological studies. As expected, at time zero water content ranged between 73-76 % (w/w) (Table V.III). Implicit manipulation of the suspensions during maturation did not induced significant changes on water content values. Samples manually agitated showed slight reduction on water content, and therefore increase of solid fraction, related to manipulation, but these changes were in all cases < 2 % (w/w).

pH of the samples was in the interval 7.42–8.30 (Table V.III). These values are included in the region of the isoelectric point of smectite edges (Avena and de Pauli, 1998). It is well known that house-of-cards aggregation of smectite particles requires edge/face contacts between positive charged edges and negative charged faces (van Olphen, 1977). At the measured pH interval face/face contacts should be predominant resulting in formation of three-dimensional band-type networks (Weiss and Frank, 1961; Weiss, 1962). Regarding the sepiolite peloids, at the measured pH and in a similar way to that described for palygorskite suspensions (Neaman and Singer, 2011), the magnitude of the negative surface charge is expected to be low. Consequently, van der Waals attraction predominates over electrostatic repulsion resulting in micro-aggregates of fibrous particles.

**Table V.III.** Water content and pH of the samples (mean values ± s.e.; n = 3)

| PELOID | Water content (w/w %) | | | | pH (20ºC) | | | |
|---|---|---|---|---|---|---|---|---|
| | **Time zero** | **One month** | **Two months** | **Three months** | **Time zero** | **One month** | **Two months** | **Three months** |
| **VHV/Dist+** | 74,30±0,071 | 72,97±0,145 | 72,84±0,855 | 72,67±1,103 | 8,14±0,303 | 7,89± 0,032 | 7,83 ± 0,081 | 7,85 ± 0,234 |
| **VHV/Dist-** | 75,74±0,227 | 75,76±0,155 | 75,73±0,029 | 75,07±0,032 | 8,08±0,303 | 8,06 ± 0,026 | 8,02 ± 0,130 | 7,92 ± 0,169 |
| **VHV/Graena+** | 73,85±0,816 | 72,74±0,213 | 72,48±0,626 | 72,37±0,947 | 7,55±0,303 | 7,44 ± 0,144 | 7,47 ± 0,090 | 7,42 ± 0,148 |
| **VHV/Graena-** | 74,20±0,727 | 73,87±0,243 | 73,36±0,100 | 73,44±0,804 | 7,79±0,303 | 7,84 ± 0,070 | 8,08 ± 0,044 | 7,98 ± 0,090 |
| **SV/Dist+** | 74,40±0,238 | 72,48±1,900 | 72,57±1,292 | 72,59±1,717 | 8,30 ± 0,244 | 8,09 ± 0,049 | 7,88 ± 0,032 | 8,08 ± 0,092 |
| **SV/Dist-** | 74,55±0,501 | 73,71±0,920 | 73,38±2,135 | 72,95±1,700 | 8,20 ± 0,026 | 8,16 ± 0,118 | 8,15 ± 0,136 | 8,10 ± 0,474 |
| **SV/Graena+** | 74,24±0,803 | 73,28±0,903 | 72,87±1,279 | 72,56±0,989 | 7,85 ± 0,061 | 7,89 ± 0,030 | 7,87 ± 0,167 | 7,82 ± 0,029 |
| **SV/Graena-** | 74,63±0,062 | 73,81±0,347 | 73,49±0,806 | 73,01±0,610 | 7,87 ± 0,353 | 7,86 ± 0,076 | 7,81 ± 0,093 | 7,79 ± 0,031 |

Particle size analysis of the suspensions showed trending in aggregation states of the particles as a result of maturation. Initially, VHV suspensions showed $d_{90} \leq 12$ µm, $d_{50} \leq 2$ µm and $d_{10} \leq 1$ µm (Fig. V.1). During maturation $d_{90}$ did not show significant changes compared to the initial value. On the other hand, $d_{50}$ progressively increased until 8 µm and $d_{10}$ increased only at the end of the maturation process. This trend was observed independently of water medium (distilled or Graena water) or maturation conditions (dynamic or static). It must be remarked that the studied systems were very concentrated suspensions and complete swelling of smectite particles in concentrated suspensions requires long period of time (Lapides and Yariv, 2004). Therefore, long maturation time probably induced dimension increase of the aggregates, but also their interaction with individual swelled particles and consequently the number of individual particles decreased, to be less of 10 % after three months of maturation. It is well known that ionic strength induces electrostatic changes on the aggregation states of very dilute smectite dispersions (≤ 1-2 % w/v) (Lagaly, 2006). Accordingly, the aggregation behaviour of distilled and Graena suspensions should be clearly different, but it was not the case. It may be assumed that the high particle concentration (25 % w/w of solids) reduced their mobility, even when the thickness of the double diffuse layer around the particles decreased as a result of higher ionic concentration (Graena water). Subsequently, the ratio of clay to water in the studied systems overwhelms the ionic content of the Graena water (Table V.I). The relative importance of solid fraction in electrokinetic behaviour and aggregation states of these concentrated clay minerals suspensions (≥ 25% w/w) has produced some controversy in the last years. An increase of clay fraction in saponite and montmorillonite maturated in seawater was explained as a result of exchange of $Ca^{2+}$ for $Na^{+}$ (Carretero et al., 2007). On the other hand, in other works it was found that maturation of saponite and kaolinite in bidistilled and sulphate mineral water increased the size of particle aggregates and the connexions between them, resulting in enlarged pores (Gamiz et al., 2009). Probably, as suggested by Tateo et al. (2011), after measuring grain size distributions trends on different clays maturated in sulphate mineral water, "case by case" studies are needed to describe the behaviour of these systems. In our opinion, the high concentration of solids in these systems (peloids) greatly determines their behaviour. Even in much more diluted systems, it has been observed the influence of solid concentration on the aggregation state and properties of clay suspensions (Lapides and Yariv, 2004).

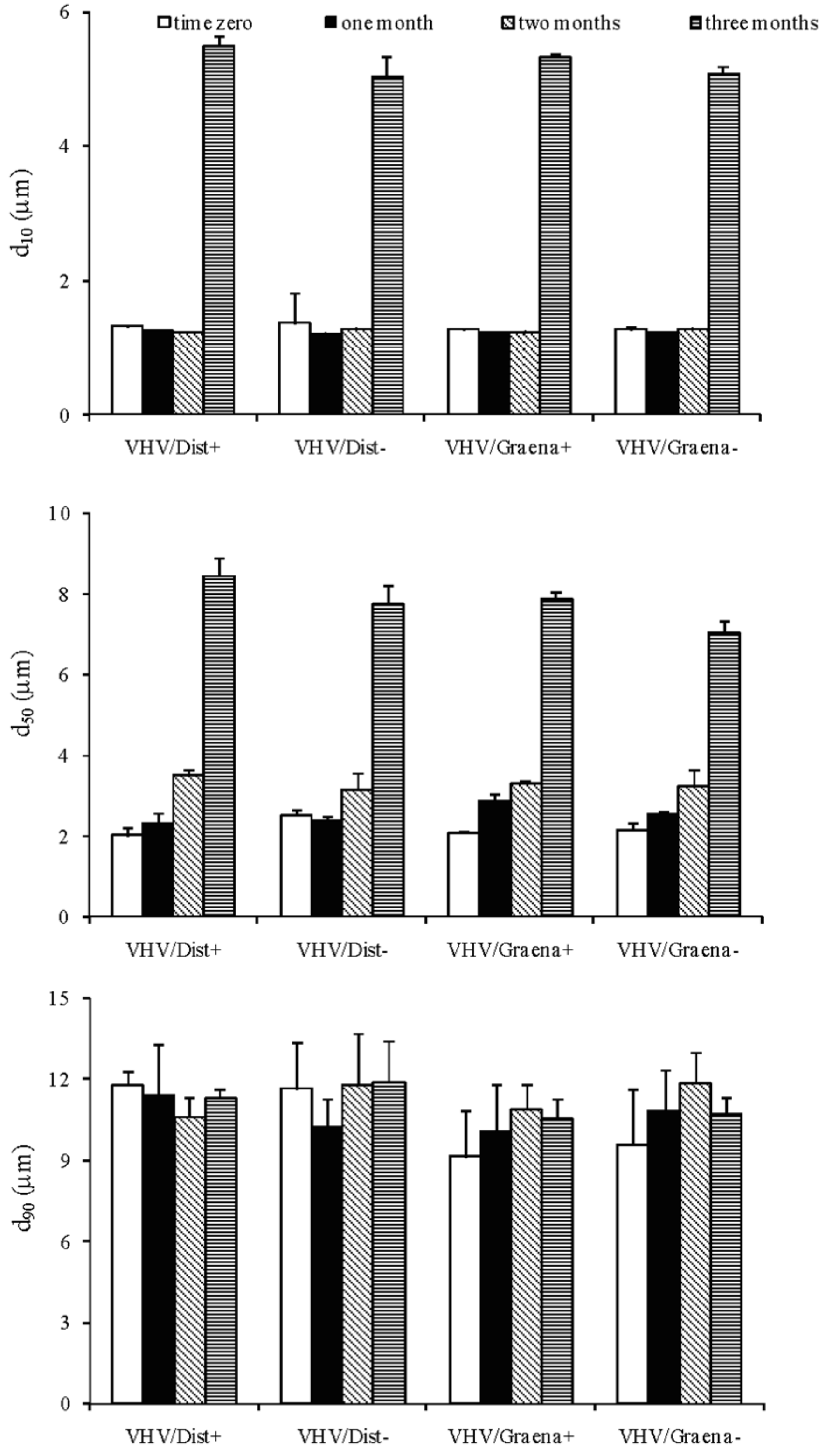


**Fig. V.1.** Statical diameters of VHV suspensions (mean values ± s.e.; n=3-6)

Particle aggregates in SV suspensions (Fig. V.2) showed average diameter at time zero ranging from 4 µm ($d_{10}$) to 40 µm ($d_{90}$), approximately. As a result of maturation, significant differences in statistical diameters (compared to time zero) were only observed in periodically shaken suspensions. In particular, $d_{10}$ values increased from 4 µm to 6 µm, and $d_{50}$ from 12 µm to approximately 18 µm at the end of maturation. It can be hypothesised that sample agitation led to collisions between particles and small aggregates, promoting their aggregation.

Differences between laminar and fibrous dispersions may be explained on the basis of their aggregation mechanisms (Viseras et al., 1999, 2001b). Rod-like (fibrous) sepiolite particles tend to align the major axis in aggregates, and low shearing promotes sticking of new particles (or aggregates), whereas aggregates of disk-like (laminar) smectite particles do not have a preferential orientation being structurally independent from hydrodynamic effects due to low agitation.

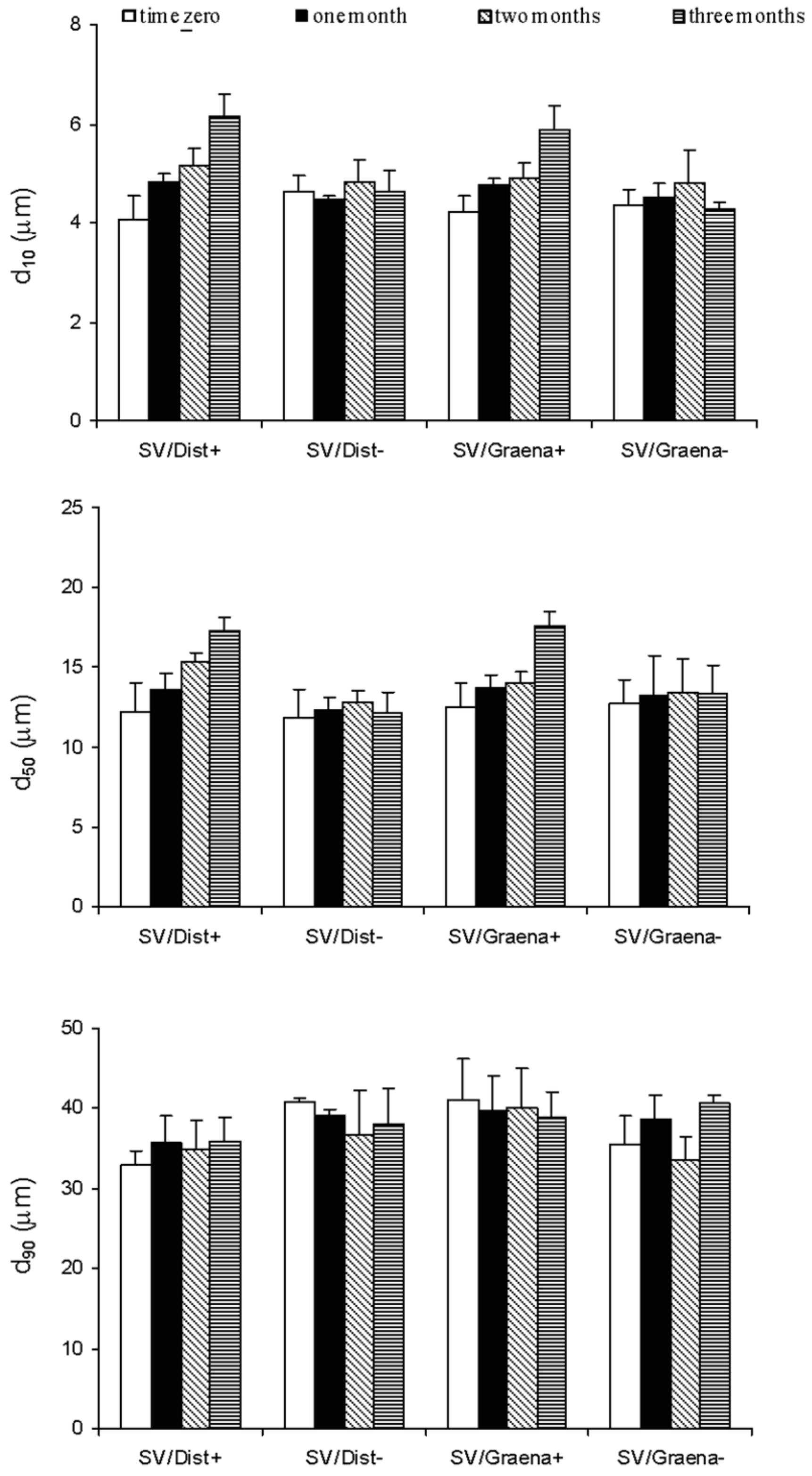


**Fig. V.2.** Statical diameters of SV suspensions (mean values ± s.e.; n=3-6)

## V.3.2. RHEOLOGICAL PROPERTIES

The studied peloids showed, whatever the maturation time and dispersion medium, typical non-Newtonian viscoplastic (i.e. pseudoplastic with yield points) flow curves, as expected with concentrated suspensions of flocculated clay particles (Van Olphen, 1977). As representative example, flow curves (shear stress vs. shear flow) at time zero are shown in Fig. V.3, in which the progressive decline in shear stress slope as shear rate increased is characteristic of pseudoplastic systems, that can be accurately described by the Bingham model (Güven, 1992). Laminar particle gels (VHV) showed spur peaks (typical of very concentrated suspensions (Barry, 1974)) and thixotropic behaviour whereas rod-like particle gels (SV) did not show any initial spur and were rheopectic. In thixotropic VHV peloids, application of shear rate determined a reversible rupture of the internal network, the recovery of which gradually went on once the applied stress was removed or reduced. Negative thixotropy observed in SV peloids represents a reversible increase, rather than a decrease, of the consistency of the sample in the curve of return. It could be said that the structure of negative thixotropic materials is formed during the shear and vice versa weakens when the stress stops. Orientation of the fibres during the flow align them respect to their major axis, increasing their contact area and resulting in an augment of required energy to flow in the return curve of the rheogram.

From the flow curves, it was possible to obtain the apparent viscosities (at 350 $s^{-1}$), yield values (characteristic value of shear stress necessary to break the attractions between adjacent particles, representative of the flow resistance of the formed gel) and hysteresis area (correlated to thixotropy). Yield values of SV were calculated according to the Bingham model as the intercept of the linear portion of the flow curve with the stress

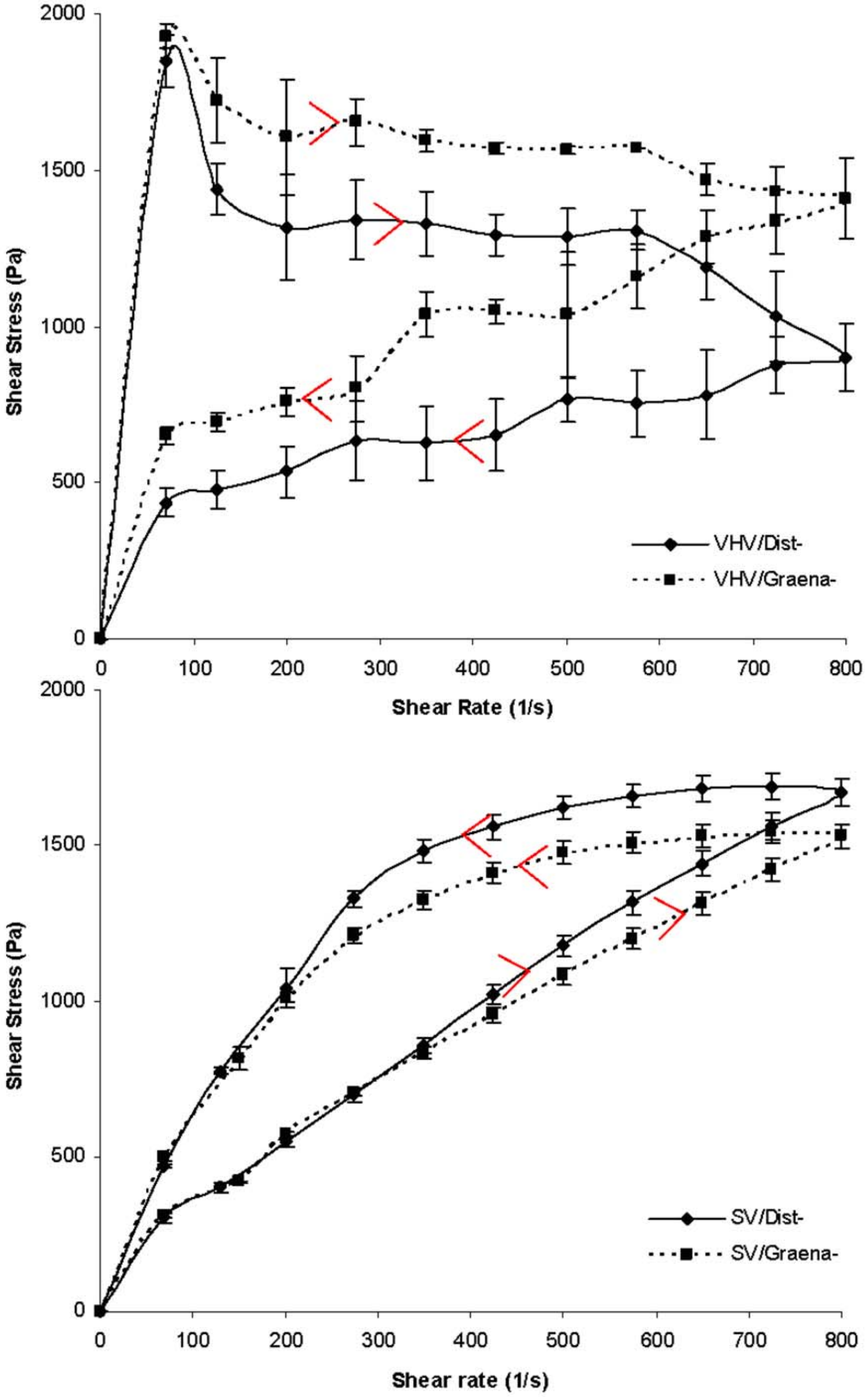


**Fig. V.3.** Flow curves of VHV and SV suspensions obtained at time zero and static maturation. Up: VHV; down: SV (mean values ± s.e.; n=6)

In the case of VHV, yield stresses were directly obtained from the shear stress value corresponding to the experimental spur point (Barry, 1974).

The influence of maturation process in apparent viscosities and yield points of the studies gels are shown in Table V.IV. Maturation in laminar gels resulted in significant increase of apparent viscosity values, independently from agitation conditions. In particular, after three months of maturation the level of significance (with respect to time zero) was 99.9 % in all cases ($P < 0.001$, 1-way ANOVA, post hoc Scheffé test, "Simple Contrast between Means"). Fibrous gels only showed minor increases of apparent viscosities in samples that had been maturated with agitation.

Changes of yield values followed the same pattern that apparent viscosities. These results indicated that maturation increased the degree of structure of the laminar systems (as a result of delamination and swelling). Yield values in systems constituted by flocculated particles in concentrated suspensions are associated to attractions between adjacent particles established by virtue of van der Waals forces, which must be broken before the material can start to flow. In the case of VHV, these links were particularly intense, and flow curves showed spurs typical of structured systems with high flow resistance (Barry, 1974; Martin, 1993, Mewis and Wagner, 2009).

Regarding time-dependent characteristics of the gels, area of hysteresis of VHV peloids increased with progressing maturation time (Fig. V.4). These results suggested that samples became more structured, in line with the observed increase in apparent viscosities and yield values.

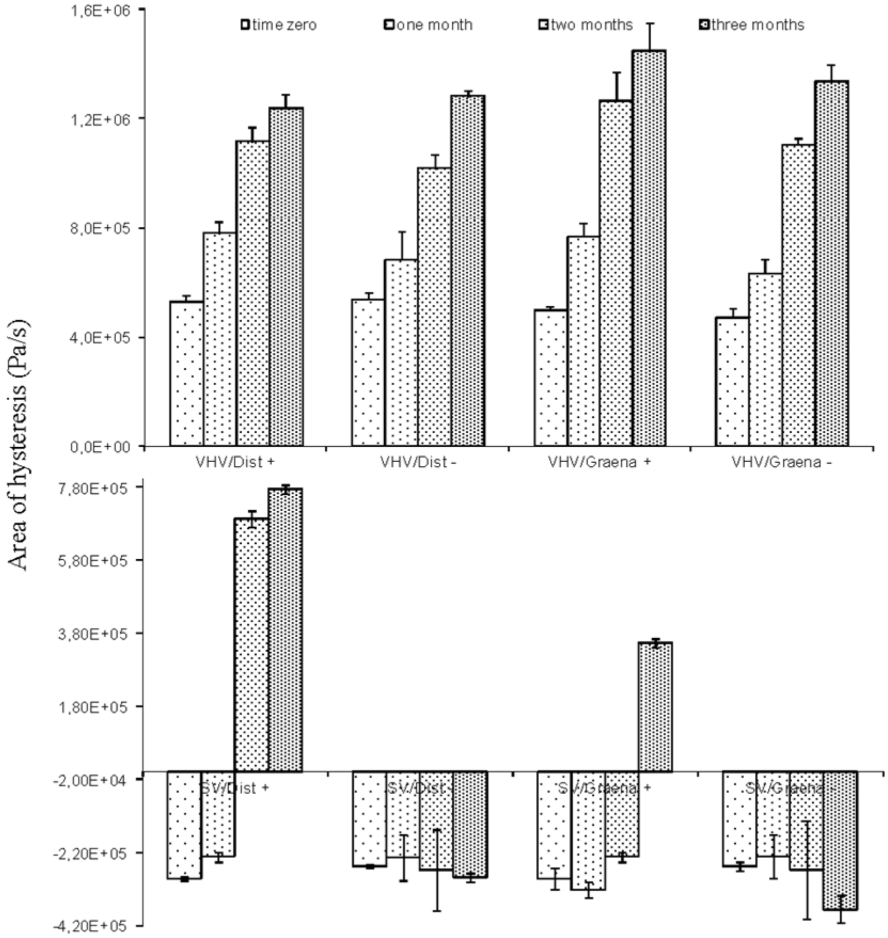


**Fig. V.4.** Area of hysteresis of the samples. Up: VHV; down: SV (mean values ± s.e.; n=6)

Fibrous peloids of SV showed negative thixotropy, as it has been also described for aqueous suspensions of palygorskite (Neaman and Singer, 2000). It has been hypothesized that, similarly to what is observed in a system subjected to mechanical stirring, shearing may increase the frequency of collision between suspended particles, promoting their aggregation and the formation of a three-dimensional system structured (Martin, 1993; Barnes, 1997, Mewis and Wagner, 2009). When the flow stops, the structure weakens and the system gradually recovers to its initial state. This hypothesis agrees with the theory that attributes the formation of gels of sepiolite mainly to hydrodynamic factors and not to electrostatic interactions (Viseras et al., 1999, 2001b; López-Galindo et al., 2011). In the case of dynamic maturation, the time dependent behaviour of SV gels, initially anti-thixotropic, changed significantly, to become thixotropic after 2-3 months of maturation (Fig. V.4). Probably, the periodic agitation of the samples, by promoting the assembly of fibres, produced a stable structured system over time, capable of manifesting a thixotropic behaviour in flow conditions. To support this idea, the observed time-depended behaviour changes concurred with high increases in yield point values (Table V.IV) observed at 2-3 months of maturation on periodic agitated samples.

**Table V.IV.** Apparent viscosities (Pa·s; 350 $s^{-1}$; 25ºC) and yield values (Pa) of the samples (mean values ± s.e.; n = 6)

| Maturation time (months) | Viscosity | Yield value | Viscosity | Yield value | Viscosity | Yield value | Viscosity | Yield value |
|---|---|---|---|---|---|---|---|---|
| | **VHV/Dist+** | | **VHV/Dist-** | | **VHV/Graena+** | | **VHV/Graena-** | |
| 0 | 4.04 ± 0.060 | 2037 ± 125.0 | 3.78 ± 0.260 | 2009 ± 223.4 | 4.18 ± 0.180 | 2177 ± 170.0 | 4.01 ± 0.145 | 2065 ± 95.0 |
| 1 | 6.67 ± 0.493 | 2860 ± 80.0 | 6.46 ± 0.288 | 2558 ± 173.2 | 6.29 ± 0.250 | 2658 ± 67.4 | 5.11 ± 0.137 | 2464 ± 178.1 |
| 2 | 7.45 ± 0.257 | 3005 ± 52.0 | 7.28 ± 0.352 | 2970 ± 74.0 | 6.70 ± 0.609 | 3120 ± 147.0 | 6.80 ± 0.045 | 2004 ± 151.0 |
| 3 | 8.00 ± 0.200 | 3108 ± 256.4 | 7.60 ± 0.479 | 3050 ± 87.2 | 8.04 ± 0.465 | 3450 ± 99.2 | 7.77 ± 0.140 | 2560 ± 88.2 |
| | **SV/Dist+** | | **SV/Dist-** | | **SV/Graena+** | | **SV/Graena-** | |
| 0 | 2.46 ± 0.074 | 160 ± 18.4 | 2.48 ± 0.058 | 218 ± 19.4 | 2.60 ± 0.104 | 151 ± 24.6 | 2.38 ± 0.051 | 209 ± 15.2 |
| 1 | 2.78 ± 0.131 | 190 ± 12.7 | 2.54 ± 0.212 | 189 ± 28.7 | 3.25 ± 0.081 | 211 ± 26.3 | 2.77 ± 0.199 | 222 ± 19.1 |
| 2 | 3.32 ± 0.037 | 1165 ± 24.2 | 3.11 ± 0.492 | 225 ± 21.5 | 3.16 ± 0.001 | 336 ± 11.4 | 2.69 ± 0.067 | 232 ± 32.5 |
| 3 | 3.41 ± 0.008 | 1208 ± 43.5 | 2.83 ± 0.094 | 215 ± 18.2 | 3.40 ± 0.238 | 957 ± 25.9 | 3.23 ± 0.084 | 231 ± 25.6 |

# V.4. CONCLUSIONS

Particle and aggregate sizes, as well as rheological properties of concentrated suspension of laminar and fibrous clay mineral particles depended on the networking mechanisms; laminar clay gels, at the studied pH, were made by "face-face" interactions between individual particles resulting in band-type networks, whereas fibrous clay gels were formed by hydrodynamic interparticle contacts. Maturation time increased dimensions of laminar aggregates, whereas fibrous aggregates only enlarged under agitation. Rheological properties of these systems resulted from their aggregation states; apparent viscosities and yield points increased with particle aggregation. Consequently, maturation of clay suspensions resulted in improved rheological properties, as peloids should easily flow when applied and posses sufficient consistency at rest. In the case of laminar clay suspensions the systems became more structured with time of maturation, whereas for fibrous clay systems it was also necessary to agitate the suspensions periodically during maturation process to obtain thixotropic gels. The observed changes in the properties of the systems as a result of maturation were independent from the water type (distilled or Graena). Consequently, as concern the studied properties, the physical stability of the suspensions was not impaired by the specific composition of the Graena medicinal water.

# CAPÍTULO VI

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## Folk pharmaceutical formulations in western mediterranean: II. “maturation” of clays in mineral medicinal water

# ABSTRACT

*Ethnopharmacological relevance:* 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 pelotherapic centres. 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. Ethnopharmaceutical 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 to physical and/or chemical therapeutic mechanisms that sustain the traditional use of maturation in the preparation of therapeutic muds.

*Materials and Methods:* Two clay samples were used to prepare thermal muds with mineral medicinal water from 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 possible mechanisms of action (composition, pH, particle size distribution, cation exchange capacity, thermal properties and amount of cations released).

*Results:* Maturation of the studied therapeutic muds induced alteration of smectites to intermediate mixed-layer clays (illite-smectite) and consequently a decrease in amplitude of particle size distribution, changes in pH and disappearance of thixotropic behaviour. Maturation increased the release of cations from therapeutic muds but did not improve their thermal properties.

*Conclusions:* In the studied case, thermophysical activity did not require of mud maturation. Conversely, maturation increased the amount of cations released from the muds, appearing as a beneficial process for possible chemical therapeutic effects associated to 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.

# VI.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.

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.

# VI.2. MATERIALS AND METHODS

## VI.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).

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).

## VI.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 $II_3$).

## VI.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).

### VI.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, 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).

### VI.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 were 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. Data were on line 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.

### VI.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.

### VI.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 25 ºC 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.

### VI.2.3.5. THERMAL STUDIES

Cooling kinetics were studied following Cara et al. (2000b). Briefly, known amounts of muds were conditioned at 60 °C in a cylindrical polyethylene terephthalate cell and then immersed in a thermostatic bath at 25 ºC, measuring the cooling of the samples up to 32 ºC, 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 (25 ºC), $T_{max}$ was the initial temperature (50 ºC), 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.

### VI.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), receptor phase was withdrawn, and amount of cations released was dosed by ICP-OES (Optima 8300 ICP-OES Spectrometer, Perkin Elmer, USA).

### VI.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.

## VI.3. RESULTS AND DISCUSSION

### VI. 3.1. MINERALOGY

Mineralogical compositions of the therapeutic muds at different maturation times were calculated and compared to those of the initial raw clay materials (Table VI.I). These results were obtained on the basis of XRPD patterns (Fig. VI.1) and chemical compositions (Table VI.II).

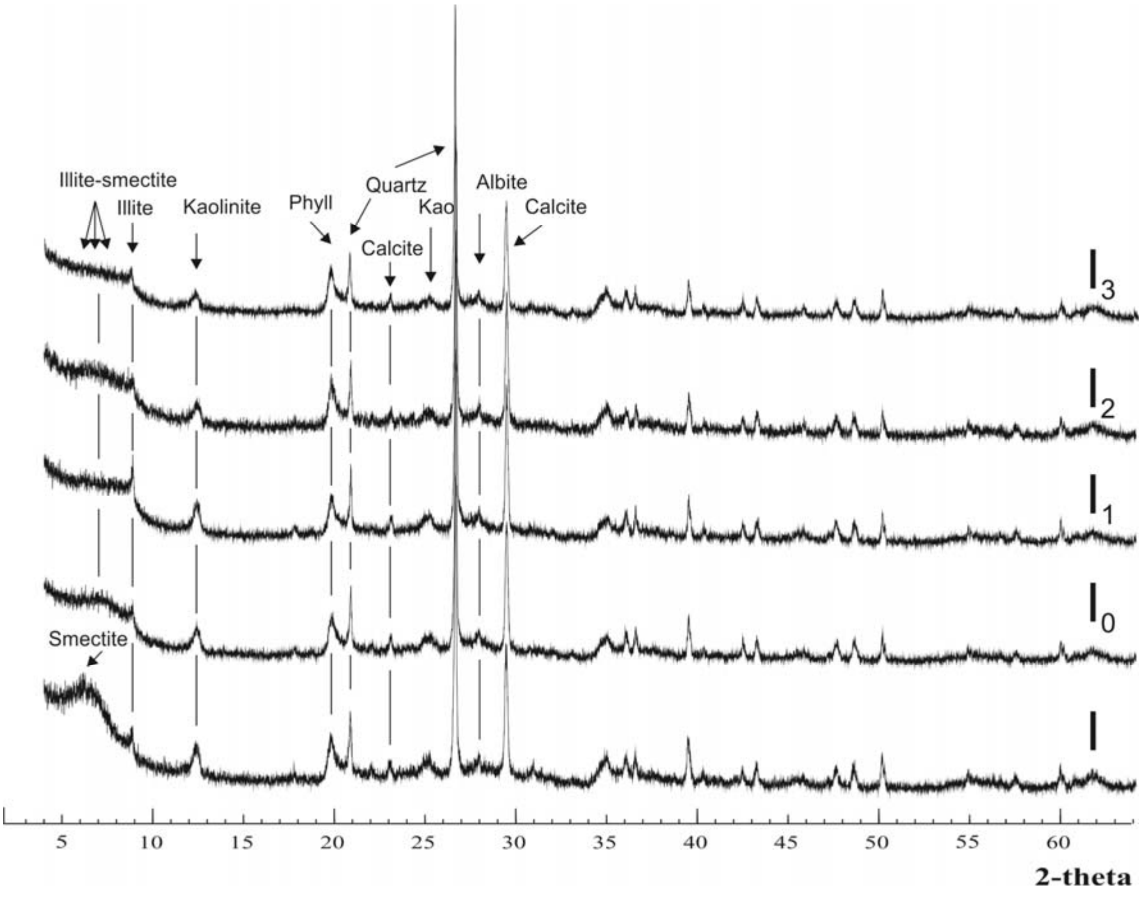

**Fig. VI.1.** XRD patterns of clay I and its corresponding muds

As a result of maturation process it was observed in both samples a clear evolution of smectites to intermediate mixed-layer clays (illite-smectite) (Fig. VI.1 and VI.2). In sample I, diffraction peak corresponding to the basal spacing of the $Na^+$ smectite was altered to broader diffraction reflections in the matured muds, as a result of the degradation of smectite to mixed-layer structures of illite and smectite (Fig. VI.1). Progressive disaggregation and reduction in smectite crystal dimensions continued during the maturation process, achieving the almost complete smectite disappearance at three month of maturation (Table VI.I). Similar degradation of smectite structure by mineral water was also observed by Sánchez et al. (2002). It must be pointed out that secondary peaks of clay minerals remained unaltered during all the process, corroborating the transformation of smectite in illite-smectite mixed layer clay structures (Fig. VI.1). In sample II, during the first 48 hours 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) ($II_0$) (Fig. VI.2).

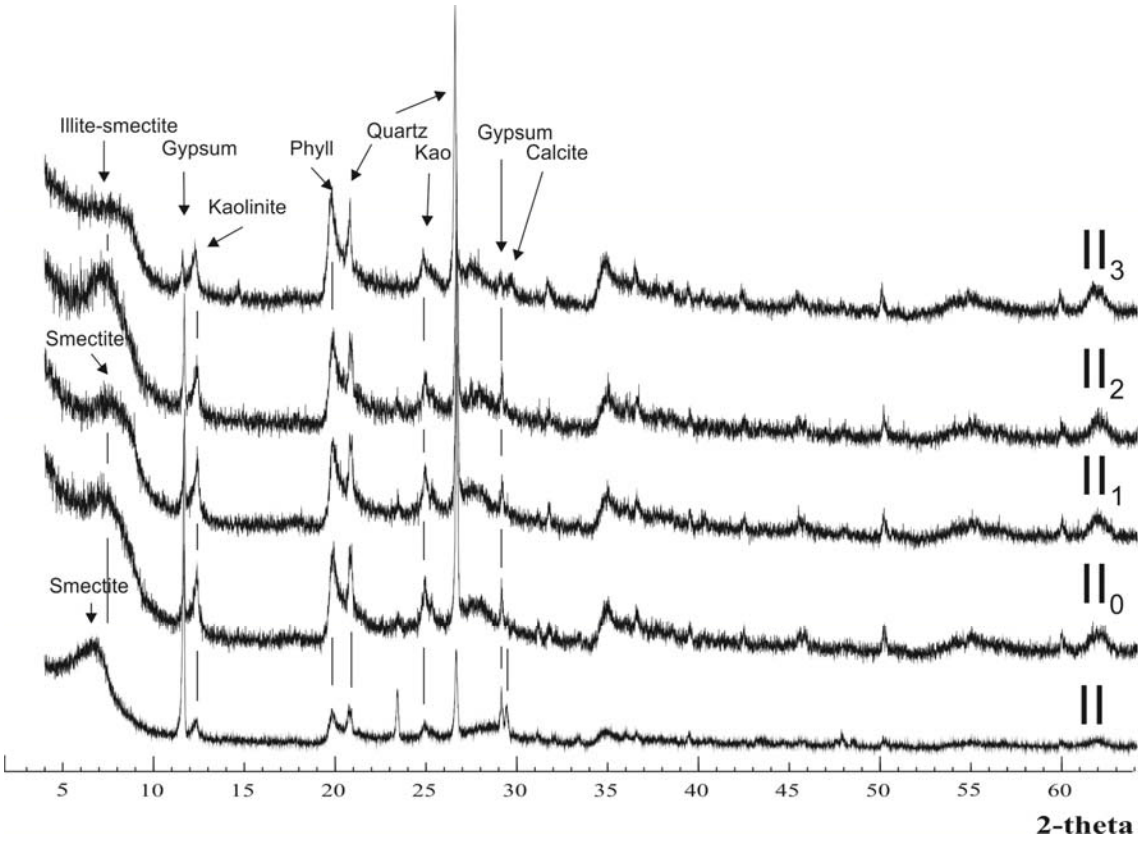

**Fig. VI.2.** XRD patterns of clay I and its corresponding muds

During the rest of the maturation process, a similar progressive alteration to that previously described for sample I was observed. As regards to the mineral impurities, dolomite in sample I dissolved completely after 48 hours of maturation. Similarly, the amount of gypsum in sample II greatly decrease during the first hours of maturation and halite completely dissolved in the first maturation month (Table VI.I).

**Table VI.I.** Mineral composition (w/w %) of the studied samples

| | I* | $I_0$ | $I_1$ | $I_2$ | $I_3$ | II* | $II_0$ | $II_1$ | $II_2$ | $II_3$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Smectites | 42 | 25 | 20 | 15 | 10 | 66 | 50 | 45 | 35 | 15 |
| Illite-Smectite | - | 17 | 22 | 27 | 32 | - | 22 | 28 | 38 | 58 |
| Quartz | 10 | 10 | 10 | 10 | 10 | 5 | 6 | 6 | 6 | 6 |
| Calcite | 11 | 11 | 11 | 11 | 11 | 4 | 4 | 4 | 4 | 4 |
| Albite | 4 | 4 | 4 | 4 | 4 | - | - | - | - | - |
| Hallite | - | - | - | - | - | 2 | 1 | - | - | - |
| Kaolinite | 9 | 9 | 9 | 9 | 9 | 5 | 6 | 6 | 6 | 6 |
| Illite | 22 | 22 | 22 | 22 | 22 | 7 | 8 | 8 | 8 | 8 |
| Dolomite | 2 | - | - | - | - | - | - | - | - | - |
| Gypsum | - | - | - | - | - | 11 | 3 | 3 | 3 | 3 |

* Taken from Sánchez-Espejo et al., 2014.

**Table VI.II.** Major-element content (w/w %) of the studied samples

| | I* | $I_0$ | $I_1$ | $I_2$ | $I_3$ | II* | $II_0$ | $II_1$ | $II_2$ | $II_3$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $SiO_2$ | 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 |

* Taken from Sánchez-Espejo et al., 2014.
LOI (loss on ignition)

## VI.3.2. WATER CONTENT, PH AND PARTICLE SIZE DISTRIBUTION

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

**Table VI.III.** 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 |

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 VI.III). 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 achieved two (sample I, $P < 0.01$) or three months (sample II, $P < 0.05$) of maturation (Table VI.IV). 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). The reduction in the percentage of small particles is in agreement with the mineralogical alteration of smectite to mixed-layer illite-smectite clay minerals. Evolution of fundamental-particle size during illitization of smectite was studied by Srodon et al. (2000). Illite nucleation is initially restricted to the size of the original smectite layers. After nucleation finish, illite crystal may continue to grow generating three dimensional growths of the mixed-layer particles. As a result of these mineralogical changes, the amplitude in particle size distribution decreased during maturation, as confirmed by the reduction in SPAN factor values (Table VI.IV).

**Table IV.IV.** 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 |

## VI.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. VI.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 to illite-smectite mixed-layers.

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 VI.V). 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). However, alteration of smectite to interstratified illite-smectite prevented the typical thixotropic behavior of the pure smectite gels.

**Table IV.V.** 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 |

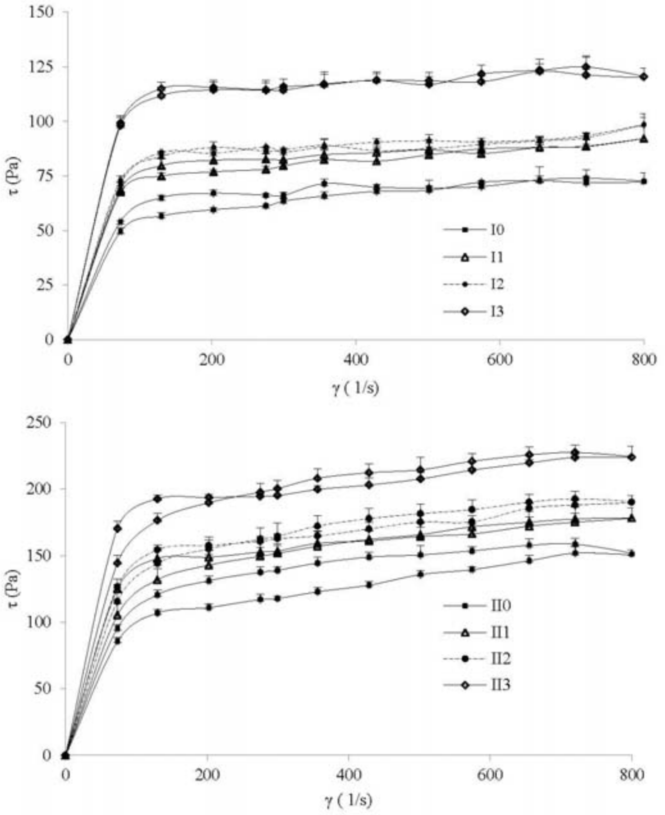

**Fig. VI.3.** Flow curves of the studied samples. Up (muds prepared with clay I) and down (muds prepared with clay II)

## VI.3.4. THERMAL STUDIES

Experimental specific heats of the therapeutic muds are shown in Table VI.VI. In the table are also included the time required to achieve 32 ºC ($t_{32}$) 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). 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 35 ºC 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. This is congruent with the hypothesis that thermal behaviour of clay muds is mainly dependent of their water content (Cara et al., 2000b).

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

| | $C_p$ (J/g K) | $t_{32ºC}$ (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 |

## VI.3.5. CATION EXCHANGE CAPACITY AND IN VITRO CATION RELEASE

The total CEC of the therapeutic muds was independent of maturation time (Table VI.VII). However, some significant changes occurred in the exchanged amounts of certain cations (Table VI.VII). Exchangeable $Ca^{2+}$ and $Mg^{2+}$ decreased in sample I ($P < 0.05$) as a result of dissolution of dolomite. In sample II, 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).

**Table VI.VII.** 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 |

The amount of cations released in the Franz cells experiments are reported in Table VI.VIII. 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 from the second month of maturation. Apparently, the alteration of the smectite to mixed-layer clay structures causes the release of $Na^{+}$ cations presented in the smectite interlayer.

**Table VI.VIII.** 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 |

# VI.4. CONCLUSIONS

Maturation of the studied clay samples in mineral medicinal water of Graena induced alteration of smectites to intermediate mixed-layer clays (illite-smectite). This mineralogical alteration resulted in a decrease in amplitude of particle size distribution as well as changes in pH of the muds and disappearance of thixotropic behaviour of initial smectite gels. Nevertheless, the viscosity and yield point values of the muds increased and the thermal properties remained unaltered. Maturation 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 ethnopharmaceutical 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 to that maturation period.

# CAPÍTULO VII

## Conclusiones/Conclusions

En esta Tesis doctoral se han desarrollado fangos terapéuticos con arcillas y agua mineromedicinal del balneario de Graena (España), evaluando sus propiedades físicas, químicas y tecnológicas en vistas a su empleo terapéutico en el tratamiento de distintas patologías humanas. Fruto del trabajo desarrollado se han publicado (uno aún pendiente de publicación) cuatro artículos JCR situados entre los primeros de su categoría, en los que se abordan los objetivos planteados en la Tesis. En cada uno de ellos se dan conclusiones parciales que han quedado plasmadas en los capítulos anteriores. En el presente capítulo de conclusiones de esta memoria de Tesis doctoral se señalan aquellas que consideramos conclusiones generales a las que cabe llegar, que son las siguientes:

1. Las arcillas se emplean con frecuencia en medicina complementaria y alternativa, y en particular en fangoterapia, como materias primas de productos terapéuticos que requieren de estudios destinados a establecer los atributos de calidad mínimos para su uso seguro y eficaz.
2. Resulta imperativo el cumplimiento de la resolución de la Organización Mundial de la Salud en la que se hace un llamamiento a la promoción del uso y del estudio científico de estas medicinas alternativas, entre las que cabe destacar la fangoterapia y con ella de los fangos terapéuticos.
3. Las arcillas usadas en numerosos centros termales que han sido estudiadas en esta tesis son por lo general ricas en minerales de la arcilla.
4. Las principales impurezas que pueden limitar el empleo de las arcillas estudiadas en esta tesis y que vienen siendo usadas en la elaboración de fangos termales en centros españoles y de países limítrofes son el cuarzo y en algunos casos impurezas metálicas.
5. En todos los casos las arcillas que han sido estudiadas cumplen con los requisitos microbiológicos de empleo en formas sólidas que recoge la normativa europea.
6. Las suspensiones de arcillas comerciales de grado farmacéutico en agua purificada y agua mineromedicinal del balneario de Graena se comportan de forma similar. La composición del agua no afecta de forma significativa a la estabilidad física o propiedades del sistema formado.

7. Las suspensiones concentradas de arcillas laminares del tipo de las esmectitas originan geles resultado de interacciones electrostáticas cara-cara entre las partículas de arcilla.
8. Las suspensiones concentradas de arcillas fibrosas forman geles resultado de contactos interparticulares hidrodinámicos carentes de carácter electrostático.
9. La maduración de suspensiones concentradas de arcillas laminares aumenta la estructuración del gel resultante y con ella las propiedades reológicas. La agitación manual durante la maduración no afecta ni positiva ni negativamente al aumento del grado de estructuración.
10. La maduración de suspensiones concentradas de arcillas fibrosas requiere de agitación manual periódica para que se produzca un aumento del grado de estructuración y con ello de las propiedades reológicas.
11. La maduración de arcillas usadas en balnearios constituidas por una mezcla de esmectitas y otros minerales produce cambios en las esmectitas que comienzan con el intercambio de los cationes interlaminares por otros presentes en el medio de suspensión y prosiguen con la alteración de la esmectita y aparición de interestratificados illita-esmectita.
12. La alteración de las esmectitas es responsable directa de cambios en la distribución granulométrica, el pH o la tixotropía de los sistemas.
13. La maduración no mejora las propiedades térmicas del fango terapéutico relacionadas con su efecto terapéutico. La capacidad calorífica del fango recién preparado y madurado a distintos tiempos es similar.
14. La maduración del fango mejora la liberación de cationes, lo que podría explicar su necesidad en vistas a optimizar sus efectos químicos.

Como corolario de la Tesis Doctoral puede inferirse que:

Las arcillas deben ser consideradas materias primas susceptibles de empleo en el desarrollo de formas farmacéuticas de administración del agua mineromedicinal, en las que el agua es la sustancia activa y la arcilla el excipiente que permite su administración. Para la concepción, desarrollo y control de estos “medicamentos” resulta imprescindible la participación del experto en medicamentos, esto es del Farmacéutico.

Therapeutic muds with mineral medicinal water of Graena (Spain) were developed in this Thesis, determining their physical, chemical and technological properties in view of their therapeutic use in the treatment of various human diseases. The objectives and results of the Thesis have been published in four articles (one of them submitted for publication) located at the top of their JCR subject. Partial conclusions were included in each one of the articles. In this chapter of the Thesis, partial and general conclusions are listed. In particular:

1. Clays are frequently used in complementary and alternative medicine, especially in fangotherapy, as raw materials to prepare therapeutic products, in which it would be compulsory to study the minimal quality attributes that sustain their safety and efficacy.
2. It is enforced to comply with the World Health Organization directive asking member states to promote appropriate, safe and effective use of traditional medicine, providing technical guidance to support countries in ensuring the safety, efficacy and quality of these medicines and treatments, among which stands out the Fangotherapy.
3. The clays studied in this Thesis are used in several spa centers, being, in general, rich in clay minerals.
4. In some cases, the presence of quartz or metal impurities could limit the use of the studied clay samples, even if they are used in several spa centers in Spain and adjacent countries.
5. The studied clays comply with European microbiological limits for use as raw materials in the preparation of solid dosage forms.
6. Muds prepared with pharmaceutical grade clays in mineral medicinal water of Graena and purified water showed similar properties. The characteristics of the water did not affect the stability neither the properties of the systems.
7. Concentrated suspensions of smectitic laminar clays gellified as a result of face to face electrostatic interactions between the clay particles.
8. Concentrated suspensions of fibrous clays gellified as a result of hydrodynamic interparticle contacts, without electrostatic character.

9. Maturation of concentrated laminar clay suspensions increased gellification and therefore rheological properties. Manual stirring during maturation did not affect the degree of structuration.
10. Maturation of concentrated fibrous clay suspensions required periodic manual stirring during maturation to increase the degree of structuration of the gel and therefore the rheological properties.
11. As a result of maturation, selected clays used in the spa centers, constituted by smectites and other minerals underwent to mineralogical changes, including exchange of the interlayer cations with those presented in the medium and alteration of the smectites to mixed layer illite-smectite structures.
12. Smectite alteration was responsible of the observed changes in granulometric distribution, pH and thixotropic behavior of muds during maturation.
13. Maturation did not improve thermophysical properties related with therapeutic use of the muds. Specific heats of initial and matured muds were similar.
14. Maturation improved cation release from the muds, justifying the necessity of this operation to optimize the therapeutic effect associated to chemical properties of the mud.

As a corollary of the doctoral thesis it can be inferred that:

Clays must be considered raw materials to be used in the development of pharmaceutical dosage forms of mineral medicinal waters, in which the water is the active substance and the clays are the excipients that allow water administration. For the design, development and control of these “medicinal products” the participation of the pharmacist is compulsory.

# CAPÍTULO VIII

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# ANEXOS

# Clays in complementary and alternative medicine

R. Sánchez-Espejo[1], C. Aguzzi[2], I. Salcedo[2], P. Cerezo[2] and C. Viseras*[1,2]

In western countries complementary and alternative medicine is used to sustain the mainstream medical practice (biomedicine). Nowadays, it is used to treat diseases, but also in the prevention, health promotion and health maintenance. Complementary and alternative medicine includes treatments that do not involve use of material substances (mind body treatments), but also other therapies including the use of materials in their treatments. Clays are frequently used as biomaterials with clinical applications in complementary and alternative medicine, as actives and excipients. The uses of clay materials in these medicines are revised, including homeopathy, balneotherapy, natural health substances (i.e. food supplements), as well as other traditional therapeutic systems as Ayurveda and traditional Chinese medicine, with increasing presence in western countries.

**Keywords:** Clays, Complementary and alternative medicine, Homeopathy, Balneotherapy, Natural health products, Dietary or food supplements

***This paper is part of a special issue on Layered clay biomaterials and human health***

## Introduction

In western countries, the mainstream medical practice is allopathic medicine or biomedicine, also denominated scientific medicine or modern medicine. Nevertheless, there are several other medical practices used as primary source of health care in the world. These practices are non-conventional in western countries, being used as complementary therapies to sustain biomedicine (particularly to treat chronic diseases) and they are usually denominated 'complementary and alternative medicine' (CAM) or 'traditional medicine' (TM). CAM is found in almost every country in the world[1] and has demonstrated efficacy in areas such as mental health, disease prevention, treatment of non-communicable diseases, and improvement of the quality of life for persons living with chronic diseases as well as for the ageing population. The demand for its services is increasing not only for disease treatments.[2]

Analysis of the status of CAM in western countries reveals that nearly 40% of adult USA citizens use some form of CAM, spending more than $30 billion/year.[3] The National Institutes of Health in USA invested in 2012 about $128 million into CAM related research.[4] In Europe, even if most European countries provide universal health care systems, over 100 million of citizens use CAM.[2] CAM is also widely employed in Canada (70% of population)[5] and Australia (two of three persons).[6]

Boundaries between conventional medicine and CAM overlap and change with time. For example, in several western countries, acupuncture (previously a non-conventional treatment) is nowadays included in the conventional pain management. With these premises, WHO Traditional Medicine Strategy 2014–2023 is aimed to 'support Member States in developing proactive policies and implementing action plans that will strengthen the role CAM plays in keeping populations healthy'.[2]

## Concepts

TM has been defined as 'the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness'.[2] TM is used as synonym of CAM in western countries. CAM has been defined as 'a broad set of health care practices that are not part of that country's own tradition or conventional medicine and are not fully integrated into the dominant health care systems'.[2] CAM is used in place of (alternative) or together with (complementary) conventional medicine (accepted by the health care system at a particular time and place). The National Center for Complementary and Alternative Medicine (NCCAM) of USA coined the term 'integrative' to refer to the CAM used by evidence-based medicine to auxiliary their treatments.[4] Whether they are used as alternative, complement or integrated with biomedicine, these medicines include treatments that do not involve use of material substances (mind body medicines), but also other therapies involving the use of materials in their treatments (homeopathy, balneotherapy, food supplements, and other natural health care substances).

## Clays in CAM

Man has used clays since prehistoric times for therapeutic purposes, due to their abundance and their particular properties. The first historical evidence of the

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use of clay for therapeutic purposes appears in a Sumerian-Mesopotamian tablet, dated around 2500 BC.[7] Many ancient Mediterranean/European medical texts also included clays as medical 'simples'.[8] Conventional western medicine continues to use clays as excipients and actives in medicinal products[9–12] as well as nanomaterials with promising abilities in drug delivery and tissue engineering.[13–15] Clays are included in medicinal products as pharmacologically inactive ingredients (abrasives, adsorbents, anticaking agents, glidants, coating agents, opacifying agents, viscosity increasing agents, emulsion stabilizers, binders or suspending agents),[11,16] or as pharmacologically active substances for the prevention, relief or cure of skin pathologies, inflammations, contusions, and gastrointestinal disorders.[10,12] Besides these conventional uses, clays are also present as ingredients of several CAM, as it will be discussed in this review.

## Use of clays in homeopathy and anthroposophic medicine

Homeopathy was developed in Germany arose in the late 19th century. It is one of the most used CAM in Europe and The United States. According to the 2007 National Health Interview Survey, about 4 million Americans adults used homeopathy in 2006.[4] Similar number of Europeans use homeopathy as CAM with biomedicine, even if no centralised data are available on homeopathic medicinal product registration and market authorization in EU Member States.[17,18] Over 100 million European citizens use over-the-counter or prescribed homeopathic medicines.[19] Briefly, homeopathic treatments are based in three main principles, customization of the treatments, great dilution of the substances and use of materials that in normal doses produces symptoms similar to those of the treated disease. Anthroposophic medicine, founded in the 1920s by Rudolf Steiner, also used ultra-diluted remedies, similar to those also used in homeopathy. In both cases, most of the homeopathic/anthroposophic substances are of natural origin and many of them are plants or plant parts, but also inorganic materials (clays). Clays used in homeopathy/anthroposophy are listed in Table 1. Most of the items came from the Natural Health Products ingredients database[20] of Canada that is based on three western homeopathic pharmacopoeias: Homeopathic Pharmacopoeia of the United States (HPUS), Encyclopedia of Homeopathic Pharmacopoeia (EHP) and German Homeopathic Pharmacopoeia (HAB). Beside these texts, the Anthroposophic Pharmaceutical Codex[21] includes 'Nontronit' and *Terra medicinalis* (dried, finely-divided, naturally occurring clay and silt with a varied composition of aluminium oxide, silica, iron oxide and limestone) as well as *Terra rubra* (natural red clay) as ingredients of homeopathic/anthroposophic preparations. Nontronite and kaolin are also included in the regulation of homoeopathic and antroposophic medicines in Australia[22] and a modified bentonite is considered as homeopathic substance in FDA regulation.[23]

## Use of clays in hydrotherapy/balneotherapy

Hydrotherapy can be defined as 'the use of the water in different physical conditions and chemical compositions with many methodologies (traditional and scientific) for the preservation of health, prevention and cure'.[24] Balneotherapy is the hydrotherapy with spring water (thermal water or mineral-medicinal water). Use of thermal water in Balneotherapy is part of the conventional medicine in several European countries, in which it is incorporated in medical curricula and the cares are dispensed by National Health Care systems. Nevertheless, this situation is far away to be general in western countries, where hydrotherapy is considered complementary and alternative medicine with different levels of integration to biomedicine.

Clays are used in hydrotherapy to prepare semisolid suspensions that are topically administered to the patients (thermal muds). Mud-pack therapy is effective in treating patients with knee osteoarthritis and other musculoskeletal disorders.[25–27] Thermal muds can be defined as 'semisolid natural medicinal products prepared by interposition of organic and/or inorganic solids in mineral–medicinal water, that conveniently processes and administered topically locally or in baths, and as a result of a number of biophysical and/or biochemical actions, are used in therapeutics to treat or prevent some pathologies, or to correct their effects in the organism'.[28] Ideally, these systems show thermal and rheological properties adequate to their therapeutic use.[29,30] Nevertheless, thermal muds are not legally recognised as medicinal products in any western country, and therefore they are not subject to any specific legislation. Most of thermal spas in Europe prepare muds with clays located in the neighbour of the thermal station. This situation clearly complicates their normalisation and qualification, being desirable that in the next future these

**Table 1 Clays used as homeopathic ingredients**

| Ingredient | Other denominations | Reference |
|---|---|---|
| EHP Alumina silicata | Alumina silicata, Argilla, China clay, Kaolin, Porcelain clay, White bole | Natural Health Products Ingredients Database (NHPID)[20] |
| EHP Kaolinum | Aluminium silicate, China clay, Kaolinum | |
| EHP Talcum | French chalk, Talcum | |
| HPUS Alumina silicata | Alumina silicata, Argilla, Bolus alba, China clay, Kaolin, Kaolinum, Porcelain clay, White bole | |
| Nontronit | | Anthroposophic Pharmaceutical Codex[21] |
| *Terra medicinalis* | | |
| *Terra rubra* | Red clay | |
| Nontronite | | Regulation of Homoeopathic and Antroposophic Medicines in Australia[22] |
| Kaolin | | |
| Quartenium-18 | | Food and Drug Administration database[23] |
| Sodium bentonite | | |

muds will comply with a number of requirements,[31] such as adequate control of identity, purity, richness, security, efficacy and stability.[32–34]

## Use of clays in natural health products

This group includes a variety of products often sold as dietary supplements (USA and Canada) or food supplements (Europe). Dietary supplements are defined as products intended to supplement the diet, containing one or more dietary ingredients (vitamins, minerals, herbs or other botanicals, amino acids, and certain other substances) or their constituents, intended to be taken orally as tablets, capsules, powders, and other forms.[35] It is compulsory to study their effects and safety, as well as their interactions with medicines and other natural products.[4] In USA, Federal Government regulates dietary supplements through the FDA, but regulations for dietary supplements are different from those for prescription or over-the-counter medicines (OTC).[23]

In Europe, food supplements are defined as concentrated sources of nutrients or other substances with a nutritional or physiological effect whose purpose is to supplement the normal diet, marketed 'in dose' forms (pills, tablets, capsules, liquids and others).[36]

In natural products, clays are included because of their specific physiological effects (adsorbent of toxins, astringent, others) but also as additives or excipients with diverse functions (Table 2). As a result of their multiple functionalities, clays are frequently employed in dietary supplements (Table 3). Different clay denominations are included as dietary ingredients, appearing in some cases in the product names (TerraVita-Clay Gray 450 mg; TerraVita-Clay Green 450 mg; TerraVita-Clay Green Powder; TERRAVITA-Clay White 450 mg; doTERRA Holdings, LLC) and even in the brand name (TERRAMIN A California Living Clay).

## Use of clays in other CAMs

Clays are also used in other CAMs, such as traditional Chinese and Ayurvedic medicines. The Chinese Materia Medica describes thousands of medicinal substances, including some minerals. In particular, halloysite (Chi Shi Zhi, Halloysitum Rubrum) is used orally as astringent, to stop diarrhoea and bleeding; and topically to produce skin and cure ulcer. Talc (Hua Shi, Talcum) is used as diuretic and to clear heat and summer-heat, as well as to promote wound healing.[38] Ayurvedic pharmacopoeia of India comprises 21 minerals used to prepare medicinal products, including kaolinite (Kha°ikā) and biotite (Abhraka). Kaolinite is used orally to treat inflammations, diseases of eyes and diarrhoea as well as topically to treat disorders of skin, mouth and tooth. Biotite is always used after calcination (bhasma) with several therapeutic uses.[39]

**Table 2 Uses of clays in Natural health products[20]**

| Clay | Uses |
|---|---|
| Aluminium magnesium silicate | Absorbent, anticaking, binder, disintegrant, emulsion stabiliser, opacifier, slip modifier, thickener, viscosity increasing agent. |
| Attapulgite | Surfactant-suspending agent, viscosity increasing agent. |
| Bentonite | Absorbent, bulking agent, emulsion stabiliser, stabilizing agent, viscosity increasing agent. |
| Kaolin | Abrasive, absorbent, anticaking, anticoagulant, bulking agent, coating agent, colour additive, diluent, filler, flow enhancer, glidant, lubricant, opacifier, slip modifier. |
| Magnesium trisilicate | Anticaking, clarifier, glidant, polishing agent. |
| Pyrophylite | Absorbent, colour additive, opacifier. |
| Talc | Abrasive, absorbent, anticaking agent, anticoagulant, bulking agent, coating agent, colour additive, diluent, filler, flow enhancer, glidant, lubricant, opacifier, slip modifier. |

**Table 3 Presence of clays in Dietary Supplements marketed in USA[37]**

| Denomination | In the product name | As dietary ingredient | As additive |
|---|---|---|---|
| Clay | 5 | 6 | 40 |
| Gray clay | … | 1 | 1 |
| Green clay | … | 1 | 2 |
| White clay | … | 1 | 1 |
| Bentonite | … | 5 | 48 |
| Bentonite clay | … | 1 | 21 |
| Bentonite clay powder | … | 1 | 4 |
| Bentonite mineral powder | … | 1 | 2 |
| Hydrated bentonite | … | 1 | 1 |
| Pyrophyllite clay | … | 1 | 1 |
| Talc | … | 1 | 425 |
| Hydrated magnesium silicate | … | … | 2 |
| Magnesium silicate | … | … | 224 |
| Magnesium silicate hydroxide | … | … | 3 |
| Magnesium trisilicate | … | … | 23 |
| Terra | 1 | 3 | 8 |
| Terra alba | … | 1 | 1 |

## Conclusions

Clays are frequently used as biomaterials in CAM. These therapies and their products are gaining attention in occidental countries. Although research has explored many of CAM products, in most instances scientific evidence regarding efficacy or safety to support or refute their use is insufficient. Only some of these products have been studied in large, placebo-controlled trials. Research on others to determine whether they are effective and safe is required. It is imperative to determine the effects of these products in the human body and about their safety and potential interactions with medicines and with other natural products. Although further research, clinical trials, and evaluations are needed, complementary/alternative medicine has shown great potential to meet a broad spectrum of health care needs. Consequently, the resolution on traditional medicine adopted by the World Health Assembly in May 2009 (WHA62·13), urged Member States to 'formulate national policies, regulations and standards, as part of comprehensive national health systems, to promote appropriate, safe and effective use of traditional medicine.' WHO also requests to 'continue providing technical guidance to support countries in ensuring the safety, efficacy and quality' of these medicines and treatments. Obviously, safety and efficacy of these products would absolutely require a scientific orientation focused to assure high quality through following applicable requirements, being an interesting research area in biomaterials.

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Research Paper

# Folk pharmaceutical formulations in western Mediterranean: Identification and safety of clays used in pelotherapy

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ABSTRACT

*Ethnopharmacological relevance:* Clays are naturally occurring ingredients of many natural health products, being included in most of ancient Mediterranean/European medical texts and currently used to prepare therapeutic hot-muds (peloids) in several thermal stations of the Mediterranean region. Clays are included in the formulation of peloids as vehicles of the mineral-medicinal water, to obtain inorganic gels with rheological and thermal properties suitable to be topically applied. Knowledge about formulations and preparation procedures of these traditional medicines has been orally transmitted since ancient times. Increasing recognition of the therapeutic utility of these traditional and natural health care substances make necessary a full ethnopharmaceutic research to ascertain those compositional characters that allow to establish quality attributes and corresponding requirements for these materials and products, including identity, purity, richness and safety.

*Materials and methods:* Five clay samples (A, B, C, D and E) currently used in various spa centers of southern European/Mediterranean countries were studied. X-Ray diffraction (XRD) and X-ray fluorescence (XRF) data were used to asses sample identity and richness. Elemental impurities and microbiological contaminants were also determined and compared to normative limits. Particle size distribution was related to their safety as powder materials.

*Results:* Samples A, C, D and E were identified as “high purity clay”, while sample B was identified as a mix of clay minerals and carbonates. The presence of carbonates in this sample could compromise its suitability for pelotherapy. The studied clays meet the main normative limits for metals impurities, with the exception of arsenic in sample A and nickel in sample B. The samples comply with the microbiological limits proposed by European legislation for medicinal products. According to the particle size of the studied samples, prevention and control of dust exposure must be considered.

*Conclusions:* Despite their demonstrated longevity, the use of clays in traditional medicine formulations as peloids greatly requires comprehension of their identity and safety attributes. Continuity of these mineral substances as recognized health care ingredients oblige to conduct interdisciplinary research to know the features that sustain their traditional use in the preparation of medicines (ethnopharmaceutics).

## 1. Introduction

Traditional medicine involves the use of naturally occurring materials, mainly plants and plant parts, but also inorganic materials, and in particular, clays. On behalf of their healing properties, clays had been included in most of ancient Mediterranean/European medical texts (De Vos, 2010), being used to treat skin pathologies (antiseptic cataplasms), intestinal and stomach diseases (because of its astringent and adsorbent properties) as well as in balneotherapy (Carretero et al., 2006).

Nowadays, modern medical care continues to use clays as highly significant health care ingredients in industrial products (López Galindo and Viseras, 2004) as well as in natural remedies and dietary supplements (http://www.dsld.nlm.nih.gov/dsld/). In western medicine, clays are used in patent medicinal products both as pharmacologically inactive ingredients (abrasives, adsorbents, anticaking agents, glidants, coating agents, opacifying agents, viscosity-increasing agents, emulsion stabilizers, binders or suspending agents) (Viseras et al., 2007; Rowe et al., 2009), and as pharmacologically active substances for the prevention, relief or cure of skin pathologies, inflammations, contusions, and

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gastrointestinal disorders (López-Galindo et al., 2011; Carretero et al., 2013).

In the last century, the use of clays in traditional European medicine had been practically limited to medical hidrology (pelotherapy), with only some exceptions in folk European (Pieroni et al., 2004) or migrant communities pharmacopoeias (Ellena et al., 2012). Despites the longevity of clays as therapeutic ingredients ("Terra sigillata" was probably the first patented medicine (Finkelman, 2006)), the dominant European health care system, based on allopathic medicine, considers the use of clays in traditional medicine as "complementary", "alternative" or "non-conventional" remedies. However, traditional medicine in developed countries is gaining popularity because it offers less adverse effects and gentler means of managing chronic diseases (heart disorders, cancer, diabetes…) in comparison with allopathic medicine (WHO, 2005). In particular, several studies have analyzed the effectiveness of pelotherapy in patients with rheumatic diseases versus allopathic therapies, noticing improvements in quality of life or drug consumption (Bostan et al., 2010; Fioravanti et al., 2012; Espejo Antúnez et al., 2013). Pelotherapy involves the topical application of heated muds, constituted by semisolid dispersions of clayey solid phases into mineral-medicinal waters (Veniale et al., 2004). The selection of mud components, as well as their preparation and application procedures follows traditional knowledge; the systems are frequently prepared and used without proper understanding of their composition and quality controls. However, confirmation (or refutation) of the possible effectiveness of these natural health care products would absolutely require a scientific orientation focused to assure applicable requirements (López-Galindo et al., 2007). It is desirable to design and implement quality systems involving adequate control of raw materials, procedures and final products.

Clays are included in the formulation of peloids as vehicles of the mineral-medicinal water, to obtain inorganic gels with rheological and thermal properties suitable to be topically applied. Knowledge about formulations and preparation procedures of these traditional medicines has been orally transmitted since ancient times. The study of these folk medicinal products involving identification of the ingredients and preparation procedures fall into the concept of ethnopharmaceutics, as a part of ethnopharmacy (Heinrich and Pieroni, 2001).

With these premises, this study is a first step in a much larger project aimed to address the traditional therapeutic use of clays in European/Mediterranean countries in view of establishing the minimum compositional requirements that should be comply to make possible the evaluation of clinical efficacy of the treatments based on these natural inorganic substances. For these purposes, and following pharmacopoeias and international guidelines (www.healthcanada.gc.ca/nhp; ICH, 2013), this study aims to determine compositional attributes of clay samples used in European/Mediterranean thermal stations, including identity, purity, richness and safety of these natural ingredients.

## 2. Materials and methods

Five clay samples (A, B, C, D and E) used to prepare peloids in 17 spa centers of three southern European/Mediterranean countries (Italy, Spain and Tunisia) were studied. Sample A was Volcangel© gifted by BENESA (Spain). Sample B was gifted under confidential agreement. Sample C was Peloide Minerale® from SO.MI.ES. (Italy). Sample D, came from Jebel Aidoudi deposits (Tunisia) and sample E was Innogel LBB® clay purchased from Aplicaciones especiales del Vallés S.L. (Spain). Selected samples were representative of the raw materials used by a large number of thermal spas. All samples were milled, sieved ($<125$ μm) and dried in oven (40 °C) before carrying out any test.

### 2.1. Identity and richness

X-Ray diffraction (XRD) data and chemical analysis (major elements) were used to asses sample identity and richness. The mineral quantification was based on the measurements of peak areas in the diffractograms according to Biscaye (1965) and Moore and Reynolds (1989), and corrected with chemical data (López-Galindo et al., 1996) using the XPOWER® program (http://www.xpowder.com/) (Martín-Ramos, 2004). XRD analysis was done by using a Philips® X-Pert (Philips, Holanda) diffractometer equipped with automatic slit (CuKα, 4–70° 2θ, 6°/min, 40 kV) both on bulk random and oriented aggregated samples (glycolated and heated to 550 °C). 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).

### 2.2. Purity

#### 2.2.1. Trace elements

The following elements were analyzed by an ICP/MS Perkin-Elmer® SCIEX Elan-5000 equipment prior sample digestion with "aqua regia" ($HNO_3+3HCl$): Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Se and Tl. Accuracy was in 1–3% range, depending on the element amount. The analyses were carried out by Activation Laboratories Ltd. (Canada).

#### 2.2.2. Microbiological contaminants

Microbiological test were carried out according to European Pharmacopoeia methods (EP 7.0., 2011). The analysis included total viable aerobic, contaminating fungi (yeast and mold), *Salmonella spp., Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.*

### 2.3. Particle size distribution

Particle size analysis was performed with a Malvern® Mastersizer 2000 LF granulometer. Prior to the analysis, a few milligrams of powder sample were dispersed in purified water and sonified for several minutes.

## 3. Results and discussion

### 3.1. Identity and richness

Mineralogical compositions of the samples (Table 1) were determined on the basis of XRPD patterns (Fig. 1) and chemical compositions (Table 2). Samples A, C, D and E were identified as "high purity clays" as the sum of minerals assigned to this group (smectites, kaolinite, illite and chlorite) was $>70\%$ w/w. Sample B was identified as a mix of clay minerals (illite, chlorite and kaolinite) and carbonates (calcite and dolomite). Significant presence of carbonates (13% w/w) was also detected in sample C. Several authors have pointed out the importance of the presence of high content of clay minerals (in particular smectites) in samples to be used in pelotherapy, because rheological, thermal and chemical features associated to these minerals improve some performances (Veniale et al., 2004; Carretero et al., 2007; Rebelo et al., 2011). Carbonates can be considered as "inert" impurities, even if their high relative amount in sample B could compromise its suitability for pelotherapy. Crystalline silica (quartz) was also measured in all the studied samples. This mineral is usually present in all clay deposits, but its occurrence in health care

**Table 1**
Mineral composition (w/w%) of the studied samples.

| | A | B | C | D | E |
|---|---|---|---|---|---|
| Smectites | 65 | Traces | 42 | 68 | 80 |
| Quartz | 5 | 13 | 10 | 5 | 7 |
| Calcite | 5 | 31 | 11 | 4 | – |
| Albite | 10 | 4 | 4 | – | – |
| K-Feldspars | 3 | 2 | – | – | – |
| Kaolinite | Traces | 7 | 9 | 5 | 12 |
| Illite | 6 | 23 | 22 | 7 | – |
| Clinoptilolite | 6 | – | – | – | – |
| Dolomite | – | 6 | 2 | – | – |
| Chlorite | – | 12 | – | – | – |
| Gypsum | – | Traces | – | 11 | 2 |

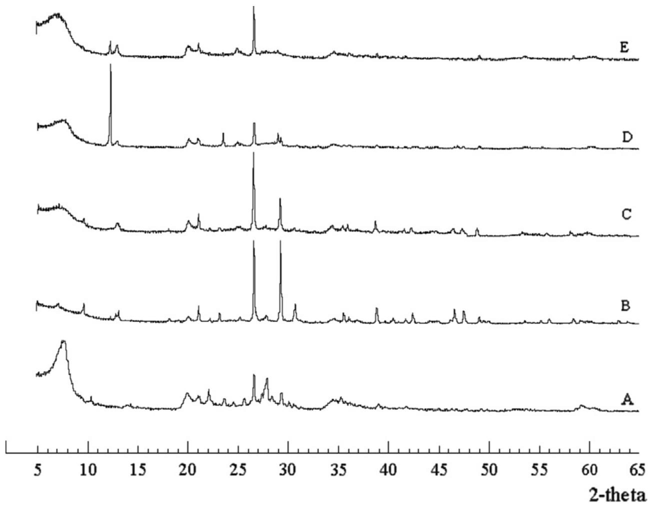

**Fig. 1.** XRD patterns of the studied samples.

**Table 2**
Major-element content (w/w%) of the studied samples.

| | $SiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | MnO | MgO | CaO | $Na_2O$ | $K_2O$ | $TiO_2$ | $P_2O_5$ | LOI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 56.71 | 11.17 | 3.36 | 0.05 | 12.62 | 3.81 | 2.89 | 1.47 | 0.37 | 0.10 | 7.11 |
| B | 33.82 | 11.37 | 4.39 | 0.06 | 4.17 | 19.66 | 0.62 | 2.43 | 0.44 | 0.12 | 22.00 |
| C | 48.64 | 17.23 | 6.52 | 0.18 | 2.35 | 7.97 | 1.26 | 2.46 | 0.76 | 0.15 | 10.90 |
| D | 45.83 | 17.23 | 6.67 | 0.03 | 2.03 | 6.32 | 3.14 | 1.72 | 0.96 | 0.29 | 8.56 |
| E | 50.98 | 18.63 | 9.31 | 0.03 | 2.42 | 1.00 | 2.33 | 1.20 | 1.35 | 0.14 | 10.80 |

LOI (loss on ignition)

products must be reduced as much as possible as it is classified by the International Agency for Research on Cancer (IARC) as a product with sufficient evidence of carcinogenicity in laboratory animals and limited evidence in humans (Group 1) (IARC, 1997). In a most recent monograph, it has been corroborated that crystalline silica in the form of quartz or cristobalite dust is carcinogenic to humans (Group 1) (IARC, 2012). It must be pointed out that crystalline silica did not show the same carcinogenic potency in all circumstances (IARC, 1997). In particular, the association of quartz with clay minerals inhibits most adverse effects (Duffin et al., 2001; Schins et al., 2002, Creutzenberg et al., 2008; Miles et al., 2008).

## 3.2. *Purity*

### 3.2.1. *Trace elements*

All the trace elements considered are naturally associated to mined materials. The speciation and physicochemical state of these elements are important factors in considering their bioavailability and toxicity. Most of the elements that can exert adverse effects even at low concentration should be maintained within acceptable limits. In absence of a specific legislation, the measured values of elemental impurities in the studied samples were compared to the limits proposed for clays to be used as pharmaceutical ingredients by European and USA pharmacopoeias (EP 7.0., 2011; USP 36–NF 31, 2013), those recommended for topical products by the Natural Health Product Directorate (NHPD) of Canada (www.healthcanada.gc.ca/nhp) and those included in the “Guideline for elemental impurities” in drug products, recently issued by the International Conference of Harmonization (ICH, 2013).

Pharmacopoeial impurities only refer to arsenic and lead content. As shown in Table 3, all samples met the pharmacopoeial limits, with the exception of arsenic for sample A. However, it must be pointed out that systemic absorption of arsenic via the skin is very low (NRC, 1999; ATSDR, 2007). The use of sample A in pelotherapy is therefore unlikely to be harmful, even if treatment to reduce arsenic would be desirable.

As regards to other significant elements with health concern, contents of Cd, Hg and Sb were compared to limits proposed in NPHD guide for topical products (Table 3). All samples meet the required limits for these elements. The presence of other elements (Mo, Se, Co, Ag, Au, Tl, Ba, Cr, Cu, and Ni) with relative low toxicity or toxicity based on route of administration were also evaluated and compared with limits included in guide Q3D of ICH (Table 3). The Q3D guide defines relevant thresholds for elemental impurities in raw materials (both actives and excipients) and medicinal products administered by oral, parenteral and inhalatory routes. The studied samples meet the required limits, with the exception of Co content. Topical exposure to Co induces dermatitis (ATSDR, 2004). Nevertheless, it must be pointed that allergic topical effects of Co appear mainly from exposure to the metal itself, rather than to aqueous Co salts (Nielsen et al., 2000). Ni content in sample B was also greater than the required limit. Allergic contact dermatitis is also frequently induced by Ni chlorate and sulfate (ATSDR, 2005). Ni is a natural constituent of soil in concentrations ranging from 4 to 80 ppm (ATSDR, 2005). The presence on $>$ 80 ppm of Ni in sample B should be take into consideration, since human and animal studies showed that Ni can penetrate the skin (Norgaard, 1955; Norgaard, 1957; Lloyd, 1980; Fullerton et al., 1986).

### 3.2.2. *Microbiological contaminants*

The presence of certain microorganisms in non-sterile preparations may have the potential to adversely affect the health of the patients and must be consequently carefully determined. Results of microbial examination of the studied samples were compared to normative limits proposed by European legislation (EP 7.0., 2011) (Table 4). The limit for total aerobic microbial count is $10^3$ CFU/g in “non-sterile substances for pharmaceutical use”, whereas for “non-sterile oral dosage forms containing raw materials of natural (animal, vegetal o mineral) origin” it increases to $10^4$ CFU/g, reaching $10^5$ CFU/g in some “herbal medicinal products”. Similarly, the limit for combined yeasts/molds is in the range $10^2$–$10^4$ CFU/g, depending on the nature of the starting material and the manufacturing process. None of the samples exceeded the acceptance criteria for “herbal medicinal products”. With exception of sample A, the samples also complied with limits for “non-sterile oral dosage forms containing raw materials of natural (animal, vegetal o mineral) origin”. As regards of pathogen microorganisms, all the samples complied with the required absence of *Escherichia coli, Pseudomonas aeruginosa,Salmonella spp. and Staphylococcus aureus*.

**Table 3**
Trace elements (ppm, aqua regia digestion) in the samples with safety concerns accordingly to European and USA pharmacopoeias (EP, USP); Canadian Natural Health Products Guide (NHPG) and Q3D guideline (Q3DG).

| Element (detection limit, ppm) | A | B | C | D | E | Acceptable limit (ppm) | Normative | Class |
|---|---|---|---|---|---|---|---|---|
| As (0.5) | 15.6 | 3.8 | 4.4 | 5.0 | 1.8 | ≤ 8 | EP, USP | |
| Pb[a] (0.1) | 11.4 | 12.9 | 13.4 | 9.6 | 8.1 | ≤ 50 | | |
| Cd (0.1) | < 0.1 | 0.2 | 0.1 | < 0.1 | < 0.1 | 3 | NHPG | |
| Hg (0.01) | < 0.01 | 0.04 | 0.04 | < 0.01 | < 0.01 | 1 | | |
| Sb (0.1) | 0.2 | 0.5 | 0.2 | < 0.1 | < 0.1 | 5 | | |
| Mo (0.1) | 0.2 | 1.8 | 1.7 | 0.7 | 0.6 | 18 | Q3DG[b] | 2A |
| Se (0.5) | 0.7 | 1.7 | 0.9 | 1.3 | 1.4 | 17 | | |
| Co (0.1) | 5.9 | 13.8 | 19.2 | 12.7 | 12.9 | 5 | | |
| Ag (0.1) | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | 17 | | 2B |
| Au (0.01) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | 13 | | |
| Tl (0.1) | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.80 | | |
| Ba (1) | 359 | 102 | 158 | 25 | 183 | 1300 | | 3 |
| Cr (1) | 16 | 76 | 58 | 101 | 86 | 1100 | | |
| Cu (0.1) | 21.7 | 26.1 | 62.9 | 15.9 | 39.2 | 130 | | |
| Ni (0.1) | 6.3 | 88.4 | 54.5 | 33.1 | 41.9 | 60 | | |

[a] "Heavy metals" assay in European Pharmacopoeia match to Pb determination (EP 7.0., 2011).
[b] Oral limits in drug products, drug substances and excipients, accordingly to table A.2.2 of Q3D guide.

**Table 4**
Results of microbial examination of the samples and acceptance criteria for "Non-sterile substances" (NSS), "Oral dosage forms containing materials of natural origin" (MNO) and "Herbal medicinal products" (HMP) accordingly to European Pharmacopoeia.

| Microorganism (CFU/g) | A | B | C | D | E | NSS | MNO | HMP |
|---|---|---|---|---|---|---|---|---|
| Total aerobic microbial | 14,000 | 4400 | 3300 | < 1000 | 10,000 | $10^3$ | $10^4$ | $10^5$ |
| Yeasts and molds | < 10 | < 10 | < 10 | < 10 | 86 | $10^2$ | $10^2$ | $10^4$ |
| *Escherichia coli* | A | A | A | A | A | A | A | A |
| *Pseudomonas aeruginosa* | A | A | A | A | A | – | – | – |
| *Salmonella* spp. | A | A | A | A | A | A | A | A |
| *Staphylococcus aureus* | A | A | A | A | A | A | – | – |

A (absence)

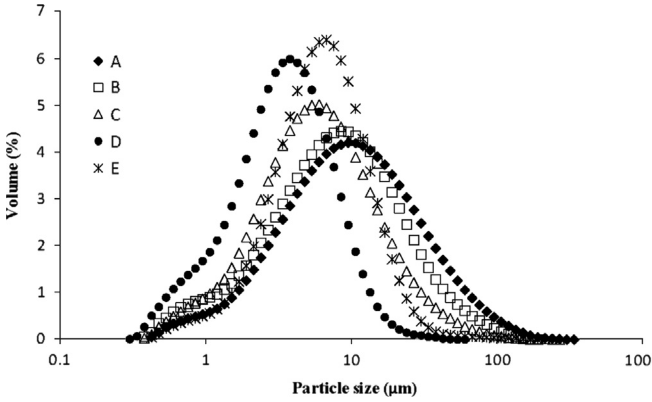

**Fig. 2.** Granulometric distribution of the samples.

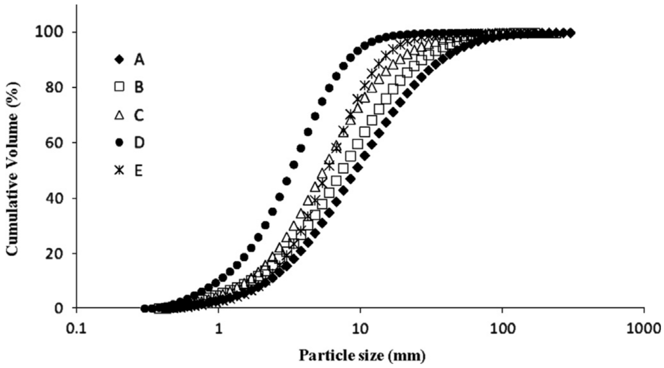

**Fig. 3.** Cumulative particle size distribution of the studied samples.

### 3.3. Particle size

The granulometric distribution of the samples was almost unimodal with mean particle diameters in the range 4.4(sample D)–16.7 μm (sample A) (Fig. 2). The pathogenic inhalatory potential of particulate matters (dust) are related to their composition, as for example the presence of crystalline silica or chemical impurities, as discussed previously, but also to their physical properties, and in particular to the particle size distribution (WHO, 1999). Depending on their size, particles of raw clay materials used to prepare peloids could become hazardous to worker health, particularly when suspended in air. The respirable dust range harmful to workers' health is defined by those particles at, or below, 10 μm size range (Cecala et al., 2012). Smaller airborne particles of dust, which can remain suspended in air for hours, pose a greater risk to the respiratory system when inhaled. Particles having a diameter of greater than 10 μm are expected to be deposited in the nasopharyngeal region, whereas particles with diameter under 5 μm may achieve the bronchial and alveolar regions. Cumulative particle size distribution of the studied samples (Fig. 3) was used to determine the fraction of particles likely to be deposited in the alveolar region of the lungs when inhaled (respirable dust). More than 99% of the particles had a diameter under 100 μm and consequently prevention and control of dust exposure must be considered. Cumulative % of particles with diameters under 5 μm were noticeable, ranging from 27.4 (sample A) to 69.8 (sample D). Taking into account that studied samples also present quartz contents > 5% w/w, strategies of controlling occupational exposure, as well as implementation of control measures should be desirable.

## 4. Conclusions

Despite their demonstrated longevity and therapeutic effectiveness, the use of clays in traditional medicine greatly requires comprehension of their action mechanism and composition related effects. Continuity of these mineral substances as recognized health care ingredients obliges to conduct interdisciplinary research to understand the features that sustain their therapeutic uses. Accordingly to their mineralogical composition, the studied samples were identified as “high purity clays” with important differences in richness. In particular, the presence of carbonates in sample B characterizes this raw material. As regards of the presence of mineral impurities with safety concerns, quartz amounts in the samples was not relevant for patient's health, even if the presence of this mineral would require adequate controls during the preparation stages of the thermal muds to prevent any hazard in workers. Elemental impurities in the studied samples were compared to the limits proposed in three related legislations, finding that As in sample A and Ni in sample B should be reduced. The samples comply with the microbiological limits proposed by European legislation. Finally, as regards of the particle sizes of the studied samples, control of occupational exposure is advised.

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# Networking and rheology of concentrated clay suspensions “matured” in mineral medicinal water

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## ABSTRACT

This work studied the influence of “maturation” conditions (time and agitation) on aggregation states, gel structure and rheological behaviour of a special kind of pharmaceutical semisolid products made of concentrated clay suspensions in mineral medicinal water. Maturation of the samples was carried out in distilled and sulphated mineral medicinal water, both in static conditions (without agitation) and with manual stirring once a week, during a maximum period of three months. At the measured pH interval (7.5–8.0), three-dimensional band-type networks resulting from face/face contacts were predominant in the laminar (disc-like) clay suspensions, whereas the fibrous (rod-like) particles formed micro-aggregates by van der Waals attractions. The high concentration of solids in the studied systems greatly determined their behaviour. Rod-like sepiolite particles tend to align the major axis in aggregates promoted by low shearing maturation, whereas aggregates of disc-like smectite particles did not have a preferential orientation and their complete swelling required long maturation time, being independent of stirring. Maturation of both kinds of suspensions resulted in improved rheological properties. Laminar clay suspensions became more structured with time, independently from static or dynamic maturation conditions, whereas for fibrous clay periodic agitation was also required. Rheological properties of the studied systems have been related to aggregation states and networking mechanisms, depending on the type of clay minerals constituents. Physical stability of the suspensions was not impaired by the specific composition of the Graena medicinal water.

## 1. Introduction

Rheological properties of semisolid pharmaceutical products greatly determine their physical stability and applications (Lee et al., 2009). Clay minerals are prominent excipients used to achieve adequate semisolid formulations, having been the subject of numerous studies (Viseras et al., 2007). Peloids are special kind of health care semisolid systems consisting of a solid phase rich in clay minerals dispersed in mineral medicinal water. Pelotherapy, intended as the therapeutic use of naturally occurring sediments, is one of the most popular techniques in thermal treatments (Viseras and Cerezo, 2006). It consists in the local application of peloids for the treatment of musculoskeletal and skin diseases (Torresani, 1990; Fabiani et al., 1996; Sukenik et al., 1999; Argenziano et al., 2004; Cozzi et al., 2007; Fioravanti et al., 2007). In the last decade, beauty and anti-stress treatments were also added to these traditional uses (Morganti et al., 2001; Varvaresou et al., 2011). A very important aspect to consider in pelotherapy concerns on the need of peloids for specialized treatments. In fact, treatments with different purposes, such as traditional treatments (musculo-skeletal, rheumatic, skin) and cosmetic ones (beauty and anti-stress) should require the use of peloids with specific features suitable as appropriate. Therefore, formulation of exclusive peloids has been defined as the “new frontier” of pelotherapy (Veniale et al., 2007). For this purpose a systematic approach is needed, able to determine optimum characteristics of these special clay/water suspensions depending on the required treatment. This objective may be achieved studying not only the peloid itself, but also its components (mineromedicinal water and clay minerals) and the process of maturation (contact between the solid and water medium during a prolonged time period). For example, several studies have been published focusing on characteristics of

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peloids and their constituents, including mineralogical and chemical compositions (Summa and Tateo, 1998, 1999; Cara et al., 2000a; Sanchez et al., 2002; Veniale et al., 2004; Tateo et al., 2009; Baschini et al., 2010; Carretero et al., 2010; Karakaya et al., 2010; Rebelo et al., 2011a), thermal behaviour (Ferrand and Yvon, 1991; Cara et al., 2000b; Ortiz de Zárate et al., 2010; Casás et al., 2011; Knorst-Fouran et al., 2012) and biochemical and microbiological aspects (Andreoli and Rascio, 1975; Ferrara et al., 1999; Galzigna et al., 1996). Moreover, peloids are semisolid products that require a detailed characterization of their rheological properties (Bettero et al., 1999). In such systems, flow behaviour affects the entire life of the product, including filling, mixing, packaging, removal from the container and defines the in vivo behaviour (Lee et al., 2009). In general, formulations with high viscosity and thixotropic properties are desirable, i.e. showing high consistency at rest, continuous decrease of viscosity with time when stress is applied and subsequent recovery of viscosity in time when the stress is discontinued (Mewis and Wagner, 2009). Peloids shows these rheological features, as they are concentrated clay suspensions resulting in structured gels (Viseras et al., 2006, 2007; Rebelo et al., 2011b). Clay gelling structures come from the aggregation of clay minerals particles by different mechanisms mainly depending on the type of clay minerals dispersed, pH and ionic strength (Lagaly, 2006). Clay mineral powders are constituted by single mineral particles (assembly of clay mineral layers), aggregates (assembly of particles) and assembly of aggregates (Bergaya and Lagaly, 2006). Traditionally, peloids were prepared with common clays, mostly consisting of mixtures of smectites, illite and kaolinite, but also including carbonates, quartz and others minerals in various proportions. Frequently, peloids need to be heated before being applied, because their therapeutic effects mainly depend on their capacity to transfer heat slowly (low cooling rates). It has been demonstrated that the use of almost pure phyllosilicates (main mineral > 90%) gave rise to systems with better thermal performance in comparison with common mixtures (Legido et al., 2007). Therapeutic activity of peloids is also related to the ionic composition of the liquid phase. It was suggested that peloids based on phyllosilicates with high cation exchange capacity (saponite, montmorillonite) may be enriched with ions provided by dispersion medium, showing potential creno-therapeutic activity (Carretero et al., 2007).

It must be taken into account that rheological behaviour of a semisolid product can change over time, leading to inadequate formulations (Martin, 1993). These changes could be largely important in pelotherapy, since maturation of peloids implies prolonged periods of time from the interposition of the solid particles in the mineral medicinal water to the moment of their application. Moreover, during maturation peloids are mixed or remain undisturbed, depending on the specific protocols of each thermal station (Veniale et al., 2004). Peloids are therefore complex systems, whose rheological stability should be evaluated over maturation time. As therapeutic products, these suspensions must comply with a number of requirements, and in particular, they must realize quality constancy attributes when applied, including identity, purity and stability features.

Given these premises, aim of this work was to study the influence of maturation conditions (time and agitation) on physical stability of peloids prepared with laminar and fibrous clay samples suspended in a mineral medicinal water. The effects of particle-particle interactions (leading to new aggregates and changing the system properties) were followed by particle size measures and rheological characteristics. Maturation of the samples was carried out in static conditions (without agitation) and with manual stirring once a week, during a maximum period of three months.

**Table 1**
Physicochemical characteristics of Graena water (means values ± s.d.; $n = 3$).

| | |
|---|---|
| pH | 7.98 ± 0.380 |
| Conductivity (20 °C) (μS/cm) | 1729.75 ± 235.968 |
| $Cl^-$ (ppm) | 17.75 ± 1.775 |
| $SO_4^{2-}$ (ppm) | 1440.58 ± 13.757 |
| $NO_3^-$ (ppm) | 1.00 ± 0.000 |
| $NO_2^-$ (ppm) | 0.01 ± 0.007 |
| $CO_3^{2-}$ (ppm) | 10.50 ± 3.000 |
| $HCO_3^-$ (ppm) | 116.66 ± 11.780 |
| $Ca^{2+}$ (ppm) | 438.88 ± 34.092 |
| $Mg^{2+}$ (ppm) | 116.73 ± 20.829 |
| $NH^+_4$ (ppm) | 0.14 ± 0.121 |

## 2. Materials and methods

Two clay samples were used; Veegum© HV (VHV), a pharmaceutical grade magnesium aluminium silicate (Vanderbilt Ltd., USA), and sepiolite from Vicalvaro (SV) (Tolsa, SA, Spain). Both samples were fully characterized in previous works in terms of mineralogical, chemical, textural and technological properties (Viseras and Lopez-Galindo, 1999, 2000; Viseras et al., 2000, 2001a,b; Cerezo et al., 2001; Aguzzi et al., 2005). Accordingly, VHV was composed (>85% w/w) of at least four types of laminar shape minerals, each one corresponding to an intermediate mineral phase between di-(montmorillonite) and tri-(saponite) octahedral smectites, while SV is composed of fibrous particles of sepiolite as a percentage above 95% (w/w). 50.07 meq/100 g $Na^+$ and 18.28 meq/100 g $Ca^{2+}$ were the main exchangeable cations in VHV (Aguzzi et al., 2005).

Medicinal mineral water, hereafter referred as Graena water (Table 1), came from the thermal spring of Graena (Cortes y Graena, Granada, Spain), being used for several purposes, including pelotherapy with local clays. A Crison® pHmeter mod. Basic 20+ with 5010T and 5071 sensors was used to determine pH and conductivity, respectively. Dissolved ions were determined by specific titration procedures (Porretta, 1990). Distilled water was also used to compare with Graena water. The measured pH of distilled water was 5.5 (±0.12).

### 2.1. Preparation of peloids

Clay/water suspensions were prepared by dispersing 25 g of VHV and SV powders in 75 g of distilled or Graena water, using a turbine stirrer (Ultraturrax T25, Janke and Kunkel GMBH & Co., G) at 10,000 rpm for 5 min. The resultant suspensions were stored at room temperature (25 °C) in airtight polyethylene containers, to avoid contamination, for three months without stirring (static maturation) or manually stirred by means of a glass rod performing planetary movements for 30 min every seven days (dynamic maturation). At time 0, 1, 2 and 3 months, aliquots of the suspensions were taken to be characterized. Name, composition and maturation conditions of each sample are shown in Table 2.

**Table 2**
Components and maturation conditions of the samples.

| Clay mineral | Dispersion medium | Maturation | Peloid |
|---|---|---|---|
| VHV | Distilled water | Dynamic | $VHV/Dist^+$ |
| | | Static | $VHV/Dist^-$ |
| | Graena water | Dynamic | $VHV/Graena^+$ |
| | | Static | $VHV/Graena^-$ |
| SV | Distilled water | Dynamic | $SV/Dist^+$ |
| | | Static | $SV/Dist^-$ |
| | Graena water | Dynamic | $SV/Graena^+$ |
| | | Static | $SV/Graena^-$ |

### 2.2. Water content, pH and particle size

The water content of suspensions was determined by weight loss on drying of 1 g of sample. pH was measured by using a pHmeter Crison (mod. 2001) equipped with a semisolid sensor (5053T). Particle size analysis was performed by a Coulter Counter method (Coulter Multisizer II, Coulter electronics Ltd., UK) with 50 $\mu$m (VHV) and 140 $\mu$m (SV) orifice tube. Prior to the analysis, a few milligrams of each sample were dispersed in isotonic NaCl (0.9% w/v) as conductive medium. Data were on line collected by means of AccuComp Windows Software for Z2 (Beckman Coulter, I) and particle volume equivalent diameters ($d_{10}$, $d_{50}$, $d_{90}$) were calculated. At least three replicates were performed for each test.

### 2.3. Rheological analysis

Rheological analysis was carried out with a Bohlin CS® rheometer (Bohlin Instrument Division, Metrics Group Ltd., UK) connected to a "personal computer" to set analysis parameters, process and record data. A cone/plate combination (CP 4/20) was used as measuring system. Measurements were carried out at room temperature (25 °C) after a rest time of 90 s. Rheological properties of the samples were measured in the range 70–800 $s^{-1}$ following a "constant rate test" procedure. This interval was selected as representative of the stress produced by technological operations and manipulation, like skin spreading (10–200 $s^{-1}$), manual mixing (100–200 $s^{-1}$) and container removal (400–2000 $s^{-1}$) (Schott, 1995).

Rheological characterization included the area of hysteresis between the flow curves obtained with increasing and decreasing shear rate, the yield point and the apparent viscosity of the samples. Six replicates were performed on each sample.

### 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). The comparison of two groups (t Student test) was performed with Statgraphics® 5.0 statistical package (Statistical Graphics Corporation, Rockville, MD, USA). Differences between groups were considered to be significant at a level of $P$ less than 0.05.

## 3. Results and discussion

### 3.1. Water content, pH and particle size

The influence of a possible decrease of water content should be controlled and taken into account for rheological studies. As expected, at time zero water content ranged between 73 and 76% (w/w) (Table 3). Implicit manipulation of the suspensions during maturation did not induced significant changes on water content values. Samples manually agitated showed slight reduction on water content, and therefore increase of solid fraction, related to manipulation, but these changes were in all cases <2% (w/w).

pH of the samples was in the interval 7.42–8.30 (Table 3). These values are included in the region of the isoelectric point of smectite edges (Avena and de Pauli, 1998). It is well known that house-of-cards aggregation of smectite particles requires edge/face contacts between positive charged edges and negative charged faces (van Olphen, 1977). At the measured pH interval face/face contacts should be predominant resulting in formation of three-dimensional band-type networks (Weiss and Frank, 1961; Weiss, 1962). Regarding the sepiolite peloids, at the measured pH and in a similar way to that described for palygorskite suspensions (Neaman and Singer, 2011), the magnitude of the negative surface charge is expected to be low. Consequently, van der Waals attraction predominates over electrostatic repulsion resulting in micro-aggregates of fibrous particles.

Particle size analysis of the suspensions showed trending in aggregation states of the particles as a result of maturation. Initially, VHV suspensions showed $d_{90} \leq 12\ \mu$m, $d_{50} \leq 2\ \mu$m and $d_{10} \leq 1\ \mu$m (Fig. 1). During maturation $d_{90}$ did not show significant changes compared to the initial value. On the other hand, $d_{50}$ progressively increased until 8 $\mu$m and $d_{10}$ increased only at the end of the maturation process. This trend was observed independently of water medium (distilled or Graena water) or maturation conditions (dynamic or static). It must be remarked that the studied systems were very concentrated suspensions and complete swelling of smectite particles in concentrated suspensions requires long period of time (Lapides and Yariv, 2004). Therefore, long maturation time probably induced dimension increase of the aggregates, but also their interaction with individual swelled particles and consequently the number of individual particles decreased, to be less of 10% after three months of maturation. It is well known that ionic strength induces electrostatic changes on the aggregation states of very dilute smectite dispersions (≤1–2% w/v) (Lagaly, 2006). Accordingly, the aggregation behaviour of distilled and Graena suspensions should be clearly different, but it was not the case. It may be assumed that the high particle concentration (25% w/w of solids) reduced their mobility, even when the thickness of the double diffuse layer around the particles decreased as a result of higher ionic concentration (Graena water). Subsequently, the ratio of clay to water in the studied systems overwhelms the ionic content of the Graena water (Table 1). The relative importance of solid fraction in electrokinetic behaviour and aggregation states of these concentrated clay minerals suspensions (≥25%, w/w) has produced some controversy in the last years. An increase of clay fraction in saponite and montmorillonite maturated in seawater was explained as a result of exchange of $Ca^{2+}$ for $Na^{+}$ (Carretero et al., 2007). On the other hand, in other works it was found that maturation of saponite and kaolinite in bidistilled and sulphate mineral water increased the size of particle aggregates and the connexions between them, resulting in enlarged pores (Gámiz et al., 2009). Probably, as suggested by Tateo et al. (2011), after measuring grain size distributions trends on different clays maturated

**Table 3**
Water content and pH of the samples (mean values ± s.e.; $n = 3$).

| Peloid | Water content (w/w %) | | | | pH (20 °C) | | | |
|---|---|---|---|---|---|---|---|---|
| | Time zero | One month | Two months | Three months | Time zero | One month | Two months | Three months |
| VHV/Dist$^{+}$ | 74.30 ± 0.071 | 72.97 ± 0.145 | 72.84 ± 0.855 | 72.67 ± 1.103 | 8.14 ± 0.303 | 7.89 ± 0.032 | 7.83 ± 0.081 | 7.85 ± 0.234 |
| VHV/Dist$^{-}$ | 75.74 ± 0.227 | 75.76 ± 0.155 | 75.73 ± 0.029 | 75.07 ± 0.032 | 8.08 ± 0.303 | 8.06 ± 0.026 | 8.02 ± 0.130 | 7.92 ± 0.169 |
| VHV/Graena$^{+}$ | 73.85 ± 0.816 | 72.74 ± 0.213 | 72.48 ± 0.626 | 72.37 ± 0.947 | 7.55 ± 0.303 | 7.44 ± 0.144 | 7.47 ± 0.090 | 7.42 ± 0.148 |
| VHV/Graena$^{-}$ | 74.20 ± 0.727 | 73.87 ± 0.243 | 73.36 ± 0.100 | 73.44 ± 0.804 | 7.79 ± 0.303 | 7.84 ± 0.070 | 8.08 ± 0.044 | 7.98 ± 0.090 |
| SV/Dist$^{+}$ | 74.40 ± 0.238 | 72.48 ± 1.900 | 72.57 ± 1.292 | 72.59 ± 1.717 | 8.30 ± 0.244 | 8.09 ± 0.049 | 7.88 ± 0.032 | 8.08 ± 0.092 |
| SV/Dist$^{-}$ | 74.55 ± 0.501 | 73.71 ± 0.920 | 73.38 ± 2.135 | 72.95 ± 1.700 | 8.20 ± 0.026 | 8.16 ± 0.118 | 8.15 ± 0.136 | 8.10 ± 0.474 |
| SV/Graena$^{+}$ | 74.24 ± 0.803 | 73.28 ± 0.903 | 72.87 ± 1.279 | 72.56 ± 0.989 | 7.85 ± 0.061 | 7.89 ± 0.030 | 7.87 ± 0.167 | 7.82 ± 0.029 |
| SV/Graena$^{-}$ | 74.63 ± 0.062 | 73.81 ± 0.347 | 73.49 ± 0.806 | 73.01 ± 0.610 | 7.87 ± 0.353 | 7.86 ± 0.076 | 7.81 ± 0.093 | 7.79 ± 0.031 |

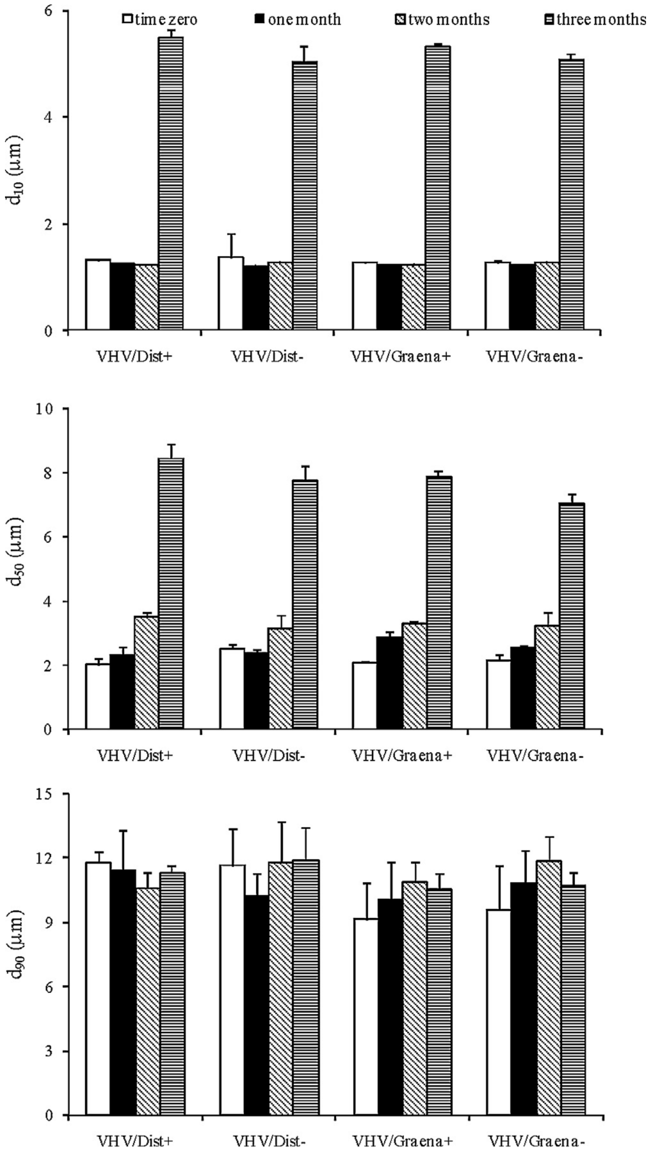

**Fig. 1.** Statistical diameters of VHV suspensions (mean values ± s.e.; $n = 3–6$).

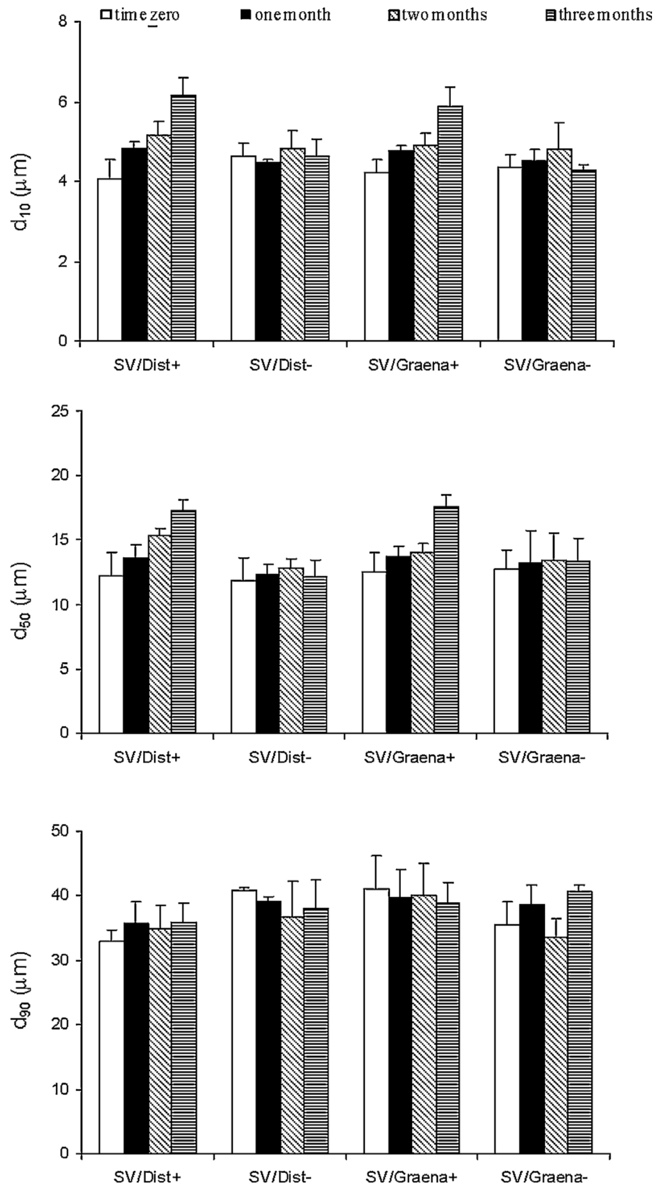

**Fig. 2.** Statistical diameters of SV suspensions (mean values ± s.e.; $n = 3–6$).

in sulphate mineral water, "case by case" studies are needed to describe the behaviour of these systems. In our opinion, the high concentration of solids in these systems (peloids) greatly determines their behaviour. Even in much more diluted systems, it has been observed the influence of solid concentration on the aggregation state and properties of clay suspensions (Lapides and Yariv, 2004).

Particle aggregates in SV suspensions (Fig. 2) showed average diameter at time zero ranging from 4 μm ($d_{10}$) to 40 μm ($d_{90}$), approximately. As a result of maturation, significant differences in statistical diameters (compared to time zero) were only observed in periodically shaken suspensions. In particular, $d_{10}$ values increased from 4 to 6 μm, and $d_{50}$ from 12 μm to approximately 18 μm at the end of maturation. It can be hypothesized that sample agitation led to collisions between particles and small aggregates, promoting their aggregation.

Differences between laminar and fibrous dispersions may be explained on the basis of their aggregation mechanisms (Viseras et al., 1999, 2001b). Rod-like (fibrous) sepiolite particles tend to align the major axis in aggregates, and low shearing promotes sticking of new particles (or aggregates), whereas aggregates of disc-like (laminar) smectite particles do not have a preferential orientation being structurally independent from hydrodynamic effects due to low agitation.

### 3.2. Rheological properties

The studied peloids showed, whatever the maturation time and dispersion medium, typical non-Newtonian viscoplastic (i.e. pseudoplastic with yield points) flow curves, as expected with concentrated suspensions of flocculated clay particles (van Olphen, 1977). As representative example, flow curves (shear stress vs. shear flow) at time zero are shown in Fig. 3, in which the progressive decline in shear stress slope as shear rate increased is characteristic of pseudoplastic systems, that can be accurately described by the Bingham model (Güven, 1992). Laminar particle gels (VHV) showed spur peaks (typical of very concentrated suspensions (Barry, 1974)) and thixotropic behaviour whereas rod-like particle gels (SV) did not show any initial spur and were rheopectic. In thixotropic VHV peloids, application of shear rate determined a reversible rupture of the internal network, the recovery of which gradually went on once the applied stress was removed or reduced. Negative thixotropy

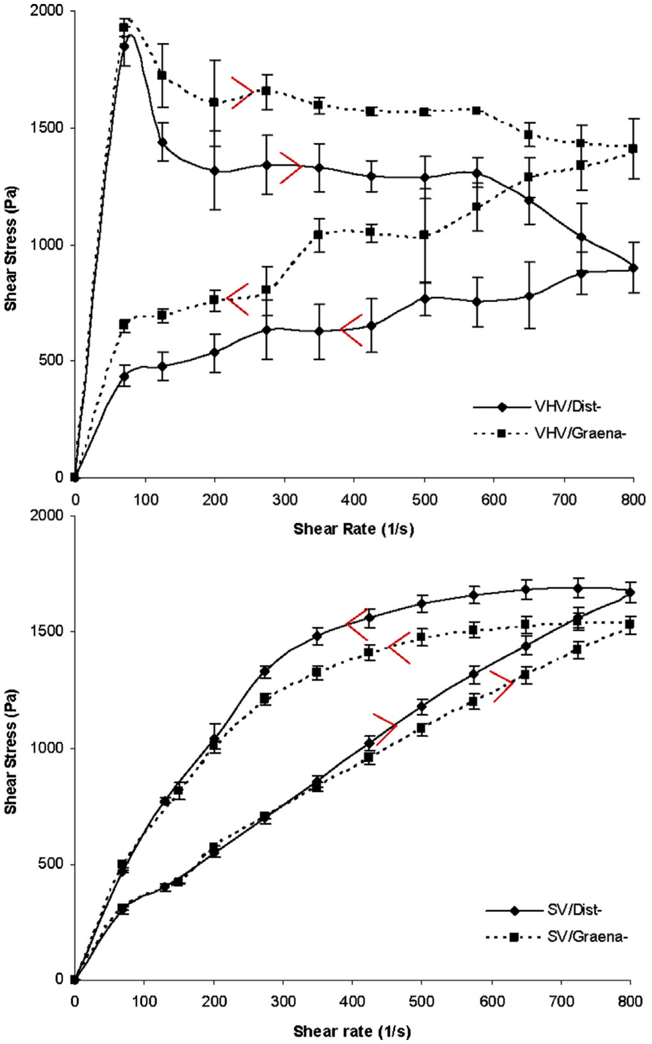

**Fig. 3.** Flow curves of VHV and SV suspensions obtained at time zero and static maturation. Up: VHV; down: SV (mean values ± s.e.; $n = 6$).

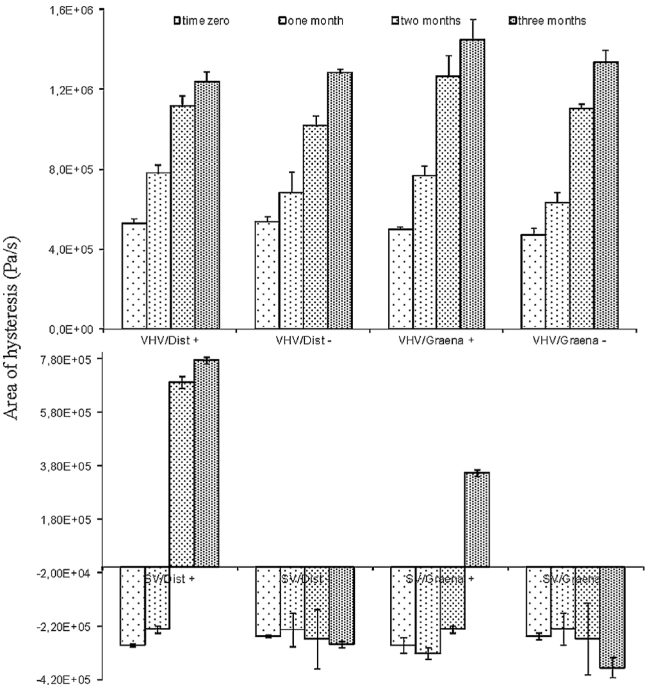

**Fig. 4.** Area of hysteresis of the samples. Up: VHV; down: SV (mean values ± s.e.; $n = 6$).

observed in SV peloids represents a reversible increase, rather than a decrease, of the consistency of the sample in the curve of return. It could be said that the structure of negative thixotropic materials is formed during the shear and vice versa weakens when the stress stops. Orientation of the fibres during the flow align them respect to their major axis, increasing their contact area and resulting in an augment of required energy to flow in the return curve of the rheogram.

From the flow curves, it was possible to obtain the apparent viscosities (at 350 $s^{-1}$), yield values (characteristic value of shear stress necessary to break the attractions between adjacent particles, representative of the flow resistance of the formed gel) and hysteresis area (correlated to thixotropy). Yield values of SV were calculated according to the Bingham model as the intercept of the linear portion of the flow curve with the stress axis. In the case of VHV, yield stresses were directly obtained from the shear stress value corresponding to the experimental spur point (Barry, 1974).

The influence of maturation process in apparent viscosities and yield points of the studies gels are shown in Table 4. Maturation in laminar gels resulted in significant increase of apparent viscosity values, independently from agitation conditions. In particular, after three months of maturation the level of significance (with respect to time zero) was 99.9% in all cases ($P < 0.001$, 1-way ANOVA, post hoc Scheffé test, "Simple Contrast between Means"). Fibrous gels only showed minor increases of apparent viscosities in samples that had been maturated with agitation.

Changes of yield values followed the same pattern that apparent viscosities. These results indicated that maturation increased the degree of structure of the laminar systems (as a result of delamination and swelling). Yield values in systems constituted by flocculated particles in concentrated suspensions are associated to attractions between adjacent particles established by virtue of van der Waals forces, which must be broken before the material can start to flow. In the case of VHV, these links were particularly intense, and flow curves showed spurs typical of structured systems with high flow resistance (Barry, 1974; Martin, 1993; Mewis and Wagner, 2009).

Regarding time-dependent characteristics of the gels, area of hysteresis of VHV peloids increased with progressing maturation time (Fig. 4). These results suggested that samples became more structured, in line with the observed increase in apparent viscosities and yield values.

Fibrous peloids of SV showed negative thixotropy, as it has been also described for aqueous suspensions of palygorskite (Neaman and Singer, 2000). It has been hypothesized that, similarly to what is observed in a system subjected to mechanical stirring, shearing may increase the frequency of collision between suspended particles, promoting their aggregation and the formation of a three-dimensional system structured (Martin, 1993; Barnes, 1997; Mewis and Wagner, 2009). When the flow stops, the structure weakens and the system gradually recovers to its initial state. This hypothesis agrees with the theory that attributes the formation of gels of sepiolite mainly to hydrodynamic factors and not to electrostatic interactions (Viseras et al., 1999, 2001b; López-Galindo et al., 2011). In the case of dynamic maturation, the time dependent behaviour of SV gels, initially anti-thixotropic, changed significantly, to become thixotropic after 2–3 months of maturation (Fig. 4). Probably, the periodic agitation of the samples, by promoting the assembly of fibres, produced a stable structured system over time, capable of manifesting a thixotropic behaviour in flow conditions. To support this idea, the observed time-depended

**Table 4**
Apparent viscosities (Pa s; 350 $s^{-1}$; 25 °C) and yield values (Pa) of the samples (mean values ± s.e.; $n = 6$).

| Maturation time (months) | Viscosity | Yield value | Viscosity | Yield value | Viscosity | Yield value | Viscosity | Yield value |
|---|---|---|---|---|---|---|---|---|
| | VHV/Dist⁺ | | VHV/Dist⁻ | | VHV/Graena⁺ | | VHV/Graena⁻ | |
| 0 | 4.04 ± 0.060 | 2037 ± 125.0 | 3.78 ± 0.260 | 2009 ± 223.4 | 4.18 ± 0.180 | 2177 ± 170.0 | 4.01 ± 0.145 | 2065 ± 95.0 |
| 1 | 6.67 ± 0.493 | 2860 ± 80.0 | 6.46 ± 0.288 | 2558 ± 173.2 | 6.29 ± 0.250 | 2658 ± 67.4 | 5.11 ± 0.137 | 2464 ± 178.1 |
| 2 | 7.45 ± 0.257 | 3005 ± 52.0 | 7.28 ± 0.352 | 2970 ± 74.0 | 6.70 ± 0.609 | 3120 ± 147.0 | 6.80 ± 0.045 | 2004 ± 151.0 |
| 3 | 8.00 ± 0.200 | 3108 ± 256.4 | 7.60 ± 0.479 | 3050 ± 87.2 | 8.04 ± 0.465 | 3450 ± 99.2 | 7.77 ± 0.140 | 2560 ± 88.2 |

| Maturation time (months) | Viscosity | Yield value | Viscosity | Yield value | Viscosity | Yield value | Viscosity | Yield value |
|---|---|---|---|---|---|---|---|---|
| | SV/Dist⁺ | | SV/Dist⁻ | | SV/Graena⁺ | | SV/Graena⁻ | |
| 0 | 2.46 ± 0.074 | 160 ± 18.4 | 2.48 ± 0.058 | 218 ± 19.4 | 2.60 ± 0.104 | 151 ± 24.6 | 2.38 ± 0.051 | 209 ± 15.2 |
| 1 | 2.78 ± 0.131 | 190 ± 12.7 | 2.54 ± 0.212 | 189 ± 28.7 | 3.25 ± 0.081 | 211 ± 26.3 | 2.77 ± 0.199 | 222 ± 19.1 |
| 2 | 3.32 ± 0.037 | 1165 ± 24.2 | 3.11 ± 0.492 | 225 ± 21.5 | 3.16 ± 0.001 | 336 ± 11.4 | 2.69 ± 0.067 | 232 ± 32.5 |
| 3 | 3.41 ± 0.008 | 1208 ± 43.5 | 2.83 ± 0.094 | 215 ± 18.2 | 3.40 ± 0.238 | 957 ± 25.9 | 3.23 ± 0.084 | 231 ± 25.6 |

behaviour changes concurred with high increases in yield point values (Table 4) observed at 2–3 months of maturation on periodic agitated samples.

## 4. Conclusions

Particle and aggregate sizes, as well as rheological properties of concentrated suspension of laminar and fibrous clay mineral particles depended on the networking mechanisms; laminar clay gels, at the studied pH, were made by "face–face" interactions between individual particles resulting in band-type networks, whereas fibrous clay gels were formed by hydrodynamic interparticle contacts. Maturation time increased dimensions of laminar aggregates, whereas fibrous aggregates only enlarged under agitation. Rheological properties of these systems resulted from their aggregation states; apparent viscosities and yield points increased with particle aggregation. Consequently, maturation of clay suspensions resulted in improved rheological properties, as peloids should easily flow when applied and posses sufficient consistency at rest. In the case of laminar clay suspensions the systems became more structured with time of maturation, whereas for fibrous clay systems it was also necessary to agitate the suspensions periodically during maturation process to obtain thixotropic gels. The observed changes in the properties of the systems as a result of maturation were independent from the water type (distilled or Graena). Consequently, as concern the studied properties, the physical stability of the suspensions was not impaired by the specific composition of the Graena medicinal water.

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