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*The effectiveness of thermal water for skin: Engineering of
peloids based on muds and natural additives for enhanced
applications of thermal waters. A multiscale study.*

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Abstract

This thesis investigates the design, optimization, and functional enhancement of clay-based suspensions and peloids, with the aim of improving rheological performance while promoting the sustainable use of limited thermal resources. VF clay materials was first studied in purified water systems to establish controlled processing–structure–property relationships, reducing reliance on natural thermal muds and mineral waters.

The initial phase evaluates the influence of mixing parameters, including agitator type (axial and radial) and dispersion energy (500 rpm for 5 min, 1000 rpm for 10 min, and 2000 rpm for 15 min), on rheology and spreadability. The results demonstrate that both factors significantly govern suspension microstructure: axial agitators generate higher viscosities, whereas radial systems produce lower-viscosity suspensions. An optimal balance between viscosity and extensibility is achieved under medium energy conditions (1000 rpm, 10 min). A strong inverse correlation between viscosity and spreadability ($R^2 = 0.8065$) highlights the importance of microstructural tuning for application performance.

This framework is extended to peloid systems prepared using purified water and sulfurous thermal water from Terme di Saturnia under high-shear mixing (2000 and 6000 rpm) using 4clay minerals (VF,BC,BB,ILB). Purified water systems consistently exhibit higher viscosities, whereas thermal water reduces viscosity due to ionic strength and divalent cations (Ca^{2+} , Mg^{2+}), which promote electrostatic screening and flocculation. The rheological response is governed by the interplay between smectite content, cation exchange capacity, accessory minerals, and water chemistry, with swelling dominating in low-ionic environments and ion-bridging prevailing in mineral-rich systems.

Building on these optimized systems, the study advances toward enhancing therapeutic functionality through the incorporation of bioactive essential oils. To guide formulation design while minimizing experimental resource consumption, molecular modeling was employed as a predictive tool. Simulations revealed that adsorption is energetically favorable on external clay surfaces, while interlayer intercalation depends on molecular composition and loading conditions. Importantly, modeling of mixed systems (thymol and carvacrol) demonstrated energetically favorable intercalation, in agreement with experimental observations.

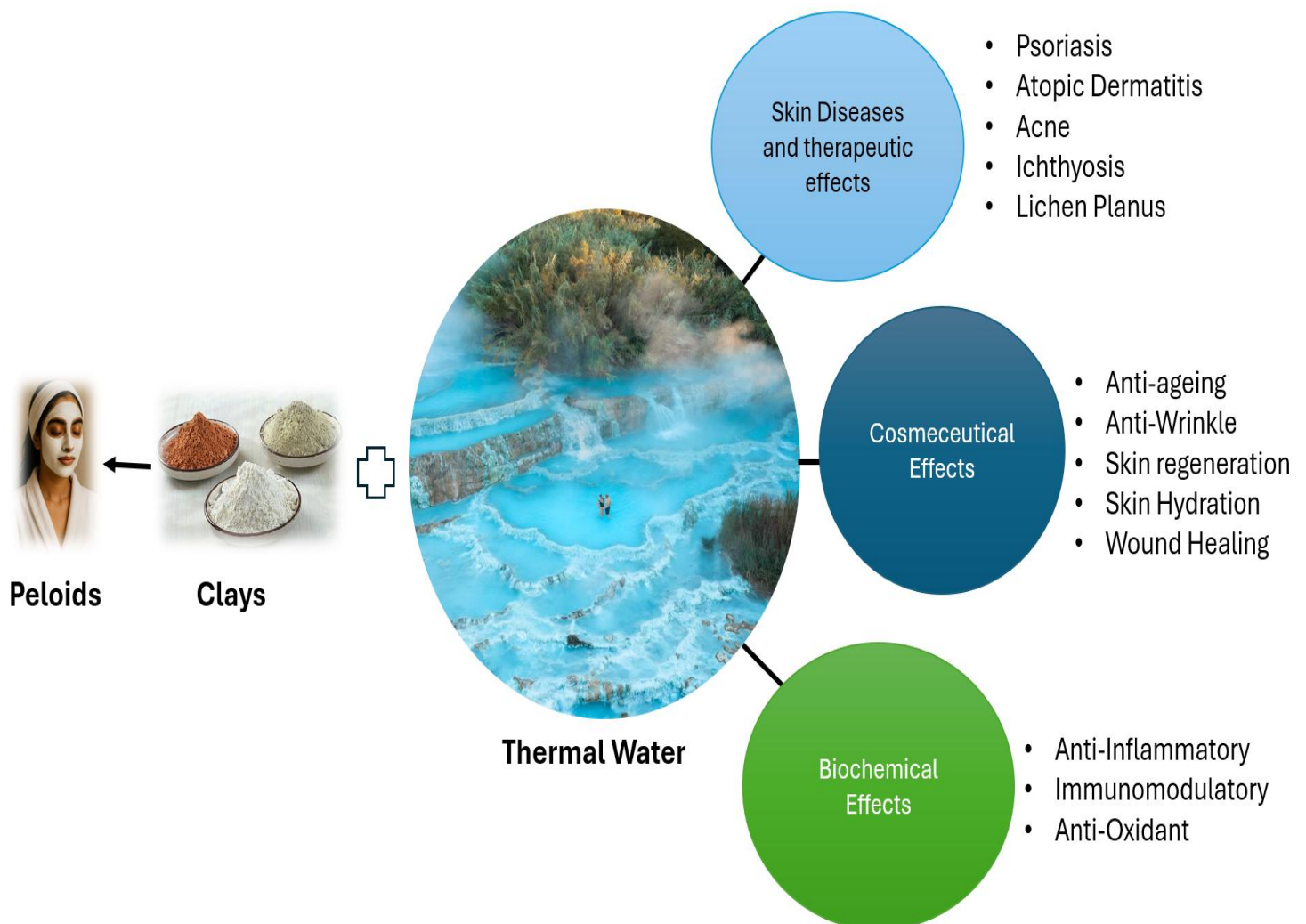
Experimental validation using X-ray diffraction and FT-IR spectroscopy confirmed both surface interactions and interlayer incorporation of essential oil components. A shift in basal spacing from ~ 11.7 Å in pristine clays to ~ 14.7 Å in intercalated systems was observed, exceeding initial theoretical predictions (~ 12.6 Å) due to higher loading conditions. Subsequent simulations at increased molecular concentrations reproduced this expansion (~ 14.9 Å), confirming the consistency between computational and experimental results and enabling estimation of intercalated versus surface-adsorbed fractions.

Overall, this work establishes an integrated multiscale approach linking processing conditions, physicochemical properties, and molecular-level interactions. The findings provide a robust scientific basis for tailoring sustainable, high-performance peloids and advancing clay-based systems for therapeutic and cosmetic applications.

CHAPTER 1
THERMAL WATER IN
DERMATOLOGY



CHAPTER 1



1.1. Thermal water and Skin Diseases (Article 1)

Thermal or mineral spring waters have been used for centuries as natural agents in health and wellness practices, particularly for the treatment of dermatological disorders and in cosmetic skin care formulations. Their therapeutic and cosmetic properties are attributed to the combined effects of their unique mineral composition, temperature, and biological components that act synergistically on the skin's physiology.

Balneotherapy, defined as the therapeutic use of natural mineral waters, gases, and peloids, remains a well-established approach for managing various chronic skin diseases (Protano et al., 2024). Numerous clinical and experimental studies have demonstrated the beneficial effects of thermal waters in conditions such as psoriasis, atopic dermatitis, contact dermatitis, seborrheic dermatitis, and ichthyosis ((Moini Jazani et al., 2023). The improvement in these disorders has been associated with anti-inflammatory, antimicrobial, and immunomodulatory actions of mineral constituents, particularly sulfur, selenium, zinc, and manganese ions (Mourelle et al., 2024).

Thermal waters rich in sulfur have been shown to inhibit pro-inflammatory cytokines and reactive oxygen species, modulate keratinocyte proliferation, and promote re-epithelialization of damaged skin (Carubbi et al., 2013; Kanwal et al., 2025). Furthermore, selenium- and zinc-containing waters demonstrate antioxidant and DNA-protective properties that can mitigate ultraviolet (UV)-induced oxidative stress and photodamage in keratinocytes (Tóth et al., 2019). Clinical evidence indicates that regular balneotherapy sessions not only reduce skin lesions and erythema but also improve skin hydration and patient quality of life (Almeida et al., 2019).

Beyond direct dermatological benefits, emerging research suggests that immersion in thermal waters may influence the cutaneous microbiome, restoring a healthier microbial balance associated with remission in inflammatory skin diseases such as psoriasis(Callewaert et al., 2020; Mourelle et al., 2024). These multifactorial mechanisms collectively support the role of thermal water therapies as a complementary approach to conventional dermatological treatments.

In addition to their therapeutic efficacy, thermal waters are increasingly used as functional ingredients in cosmetic and dermocosmetic formulations. Their ability to soothe irritation, reduce inflammation, and enhance skin hydration makes them valuable in products designed for sensitive or reactive skin. Thermal water sprays, gels, and emulsions are reported to decrease erythema, stinging, and pruritus following dermatological procedures or environmental stress (Gama et al., 2025; Saraiva et al., 2023).

The mineral and trace element composition contributes significantly to these cosmetic benefits. Elements such as silica, calcium, magnesium, and bicarbonate strengthen the stratum corneum barrier, enhance hydration, and improve skin texture and elasticity (Figueiredo et al., 2023). Selenium and zinc ions further provide antioxidant protection, helping to neutralize reactive oxygen species and prevent premature skin aging (Seite, 2013).

Comparative studies of gels and creams prepared with thermal water versus deionized water have demonstrated superior hydration retention, barrier function restoration, and textural

smoothness in formulations containing thermal water. Moreover, thermal waters are considered safe and biocompatible excipients or active agents in cosmetic formulations when physicochemically characterized and microbiologically controlled (Milanković et al., 2025).

The comprehensive review we publish-synthesizes current knowledge on the dermatological and cosmeceutical manifestations of thermal water, drawing from both in vitro and in vivo studies to highlight its therapeutic and cosmetic potentials. The review extensively discusses the impact of thermal water on immune modulation, inflammation reduction, antioxidant defense, and skin barrier restoration, making it a promising multifunctional agent in skin health. Particular emphasis was placed on evaluating its reported efficacy in the management of a range of skin disorders, such as psoriasis, atopic dermatitis, acne, ichthyosis, and lichen planus. In addition to its clinical applications, the review also considered the cosmeceutical benefits attributed to thermal water, including its roles in enhancing skin hydration, promoting wound healing and regeneration, and contributing to anti-aging effects. Furthermore, attention was given to elucidating the underlying biological mechanisms that may account for these dermatological and cosmetic outcomes, with a focus on anti-inflammatory, immunomodulatory, and antioxidant pathways. Finally, the review sought to highlight existing gaps in the current body of research and to suggest directions for future studies, particularly in relation to long-term clinical evaluations and the standardization of treatment protocols.

1.1.1. Methodology Overview

The study synthesizes findings from in vitro, in vivo, and clinical research reported in the literature from 2000 to 2025. Key databases, including PubMed and Google Scholar, were reviewed using keywords related to thermal water, balneotherapy, dermatology, and cosmeceutical applications. Selected studies were evaluated for methodological rigor, relevance, and contribution to understanding the multifaceted effects of thermal water on the skin.

1.1.2.. In Vivo Therapeutic Effects of Thermal Water on Skin Diseases

Thermal water has been widely used in vivo for treating several common skin diseases including atopic dermatitis, psoriasis, inherited ichthyosis, lichen planus, and acne vulgaris. The clinical efficacy of thermal water treatments depends on numerous factors such as the treatment modality, disease severity, presence of ancillary conditions, mineral composition of the thermal water, and treatment duration. Among the most studied thermal springs are La Roche-Posay, Avène, Vichy (France), Vetriolo and Levico (Italy), Cro and Monfortinho (Portugal), Salies de Bearn (France), Uriage (France), Comano (Italy), the Dead Sea (Israel), and the Blue Lagoon (Iceland).

Atopic Dermatitis (AD)

AD is a chronic inflammatory skin disease characterized by lesions predominantly on visible areas and often associated with disturbed sleep and reduced quality of life (Bender et al., 2008; Geat et al., 2021). Both genetic predisposition and environmental factors contribute to AD pathogenesis.

Taieb et al. (2011) reported one of the largest cohorts treated with Avène thermal water hydrotherapy for 3 weeks, documenting a 41.6% reduction in AD severity and pruritus alongside significant improvements in sleep quality and quality of life. Giannetti (2005) observed similar improvements with a 47.8% decrease in the SCORAD index, alongside reductions in insomnia (66%) and pruritus (41%). Merial-Kieny et al. (2011) further confirmed improvements in pruritus, dryness, and SCORAD scores (Fig. II). The therapeutic effect is attributed partly to Avène water's inhibitory effect on IL-8 and reduction of *Staphylococcus aureus* colonization, which mitigates inflammation (Casas et al., 2011).

Piggato (2005) studied Avène thermal water combined with an emollient spray in children with AD and noted meaningful symptomatic relief including reductions in peeling, itching, pruritus, and erythema.

Pharmacological studies suggest Avène water exerts anti-inflammatory effects, modulates the skin microbiome shifting pathogenic to nonpathogenic species, regulates keratinocyte differentiation, and influences cytokine expression (Guerrero & Garrigue, 2017; Merial-Kieny et al., 2011). Enzymes critical to epidermal barrier function in AD— β -glucocerebrosidase, phospholipase A2, and proteases—show normalized activity following 3 weeks of Avène treatment (Redoules et al., 1999; Tarroux et al., 2002).

La Roche-Posay thermal water also improves AD symptoms such as itching, peeling, and xerosis, along with favorable changes in Dermatology Life Quality Index (DLQI) and Eczema Area and Severity Index (EASI) scores (Dikova et al., 2016). Its high selenium content is proposed to underlie antioxidant and immunomodulatory effects related to selenoprotein function (Kouhkan et al., 2006; Roman et al., 2014).

Comano thermal water, rich in bicarbonate, calcium, and magnesium, shows comparable efficacy to corticosteroids in pediatric AD patients, exerting anti-inflammatory effects by downregulating keratinocyte cytokine production such as TNF- α , IL-6, and IL-8 (Chiarini, Dal Pra, Pacchiana, Menapace, et al., 2006, 2006; Chiarini, Dal Pra, Pacchiana, Zumiani, et al., 2006; Farina et al., 2011; Geat et al., 2021).

The Dead Sea's unique mineral and geographic features promote beneficial climatotherapy effects improving SCORAD and Skindex-29 scores in AD patients, linked with shifts in the skin microbiome (Adler-Cohen et al., 2012; Brandwein et al., 2019; Harari et al., 2000). Comparative trials show climatotherapy efficacy comparable to steroid treatment (Marsakova et al., 2020).

Psoriasis

Psoriasis is a chronic inflammatory disorder with a prevalence ranging from 0.5 to 4.6% globally, causing significant physical and psychosocial morbidity (Boros et al., 2013; Qiu et al., 2024). Thermal water treatment represents an effective and safe option (Boros et al., 2013; Seite et al., 2013).

Avène thermal water baths, typically administered at 32°C for 20 minutes, 6 days per week over 3 weeks, combined with emollient and spray application, significantly improved Psoriasis Area and Severity Index (PASI), erythema, and infiltration, with sustained quality

of life enhancements up to 6 months post-treatment (Merial-Kieny et al., 2011; Taieb et al., 2011). Molecular analyses revealed downregulation of inflammatory and pruritus biomarkers (IL-17, IL-8, IL-1 α , TRPV1, IL-31), along with decreased *Staphylococcus aureus* colonization (Casas et al., 2011; Thouvenin et al., 2023).

La Roche-Posay thermal water has demonstrated antipsoriatic properties including microbiome diversity enhancement with increased Xanthomonadaceae colonization associated with keratolytic effects (Martin et al., 2015). Combined interventions over one year reported improvements in stress (PSS), pain (VAS), DLQI, and PASI (Beylot-Barry et al., 2022).

Salies de Bearn thermal water, rich in sodium and magnesium, showed mild improvement in PASI in a randomized controlled trial on psoriasis, with no clear benefit when combined with phototherapy — limitations included adverse events and bias suggesting stress relief rather than direct therapeutic effect (Léauté-Labreze et al., 2001)

Levico and Vetriolo arsenical-ferruginous waters with low pH and arsenic residues demonstrate antiproliferative effects via keratinocyte apoptosis, with histological and immunohistochemical improvements after 12 days of wet packing in patients with mild to moderate psoriasis (Borroni et al., 2013). The impact of iron remains under further investigation.

Comano thermal water baths combined with narrow-band UVB phototherapy improve SkinIndex and SPASI scores, as well as quality of life and mental health (Pagliarello et al., 2012; Tabolli et al., 2009).

Heviz sulfurous thermal water balneotherapy (3 weeks at 36°C, 5 days/week) improved PASI scores and altered skin microbiome, increasing beneficial *Leptolyngbya* genus with antibacterial, anti-inflammatory, antioxidant, and tyrosinase inhibitory effects (Kulisch et al., 2023; Ratnaparkhe et al., 2021).

Blue Lagoon thermal water offers unique mineral and silica brine properties beneficial in psoriasis treatment primarily in combination with phototherapy (Eysteinsdóttir et al., 2014). Immunological biomarkers remain controversial regarding correlation with disease activity, necessitating further large-scale clinical trials.

Inherited Ichthyosis

Inherited ichthyosis characterized by scaling, itching, and inflammation has limited current treatment options primarily consisting of symptomatic management with topical keratolytics and emollients (Oji et al., 2010). Hydrotherapy with thermal water has been explored sparingly. Bodemer et al. (2011) performed an 18-day hydrotherapy trial with 24 adults and 20 children, showing significant and persistent improvements in Dermatology Life Quality Index and clinical ichthyosis scores up to 6 months after treatment.

A case report demonstrated complete but temporary clearance of ichthyosis linearis circumflexa using saltwater bath combined with phototherapy, with relapse managed by intermittent treatment (Gambichler et al., 2000).

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Future research should expand clinical and mechanistic studies of various thermal waters for ichthyosis management.

Lichen Planus

Lichen planus affects mostly middle-aged individuals (prevalence 0.1-2.4%), with pathogenesis involving keratinocyte apoptosis and T-cell dysfunction (Lei et al., 2010; Walton et al., 2010). Standard corticosteroid treatment carries risks limiting long-term use.

An open-label clinical trial with Avène thermal water involving oral baths, vaporization, gingival showers, compresses, and hydropinotherapy for 21 days documented significant symptomatic relief (itching, erythema, erosion) and reduction in need for corticosteroids and analgesics (BOISNIC et al., 2004).

Acne Vulgaris

Acne is a chronic pilosebaceous inflammatory disease prevalent in adolescence, driven by follicular obstruction, abnormal keratinization, excess sebum, and *Propionibacterium acnes* proliferation (Gollnick et al., 2003; Thiboutot et al., 2009). Sulfurous thermal water's keratolytic effects reduce follicular obstruction and bacterial colonization (Soroka et al., 2008).

In a multicenter open-label study, combined treatment with thermal water and retinoic acid for 28 days significantly improved facial acne compared with retinoic acid alone (Alirezai et al., 2000).

Thermal water exerts beneficial effects on acne by modulating follicular obstruction, sebum production, bacterial viability, and inflammation, making it an effective, well-tolerated complementary therapy.

1.1.3. Cosmetic Effects of Thermal Water

Cosmetic products span a broad spectrum, including color cosmetics, hair colorants, skincare formulations, personal care, and body care cosmetics. According to EU Regulation 1223/2009 (Article 2.1.a), cosmetics are defined as any agent or mixture intended for external application to the human body to cleanse, protect, perfume, correct body odor, or alter appearance. Typically, cosmetics comprise active substances alongside ancillary ingredients, with water mainly serving as an ancillary component in lotions, creams, gels, and emollients. Thermal water, with its unique mineral and microbial profile, can play roles both as an active and ancillary ingredient, enhancing the beneficial properties of cosmetic formulations.

In dermatological cosmetics, thermal water has been studied extensively for its benefits in skin regeneration, hydration, wound healing, acne management, and antiaging effects—including anti-wrinkling, soothing, desensitizing, and antioxidant activities. These cosmetic products are commonly formulated as gels, lotions, sprays, and creams (Araujo et al., 2017; Joly et al., 2014; Nunes et al., 2019). The cosmeceutical properties of thermal water depend on both its physicochemical composition and microbial content (Almeida et al., 2019; Joly et al., 2014; Mourelle et al., 2023).

For instance, Vendrely et al. (2022) developed an emollient balm incorporating an extract derived from *Aquaphilus dolomiae* (ADE-G1) found in Avène thermal spring water, demonstrating its therapeutic effect on xerosis in patients undergoing anticancer therapy. The product showed excellent tolerance and a marked reduction in xerosis severity. Similarly, Avène's extract formulated as an emollient exhibited antixerotic and soothing effects in dermatological and systemic disease patients (Deleuran et al., 2020).

The antiaging potential of thermal water-derived algae extracts, and silica mud formulations is notable. Blue Lagoon thermal water creams have been shown to stimulate collagen 1A1 and 1A2 gene expression and promote epidermal differentiation markers such as involucrin, loricrin, transglutaminase-1, and filaggrin, thereby enhancing skin barrier function (Grether-Beck et al., 2008). Moreover, these creams reduced α -melanocyte numbers, contributing to skin-lightening effects (Grether-Beck et al., 2022). Comparable skin tightening and barrier strengthening effects were reported with probiotic fractions from Vichy thermal water, which promoted keratinocyte differentiation, activated antimicrobial peptides, and modulated inflammatory responses via increased IL-10/IL-12 ratios under UV exposure (Gueniche et al., 2022).

Comano thermal water is recognized for its role in accelerating wound healing and skin regeneration, potentially linked to its high bicarbonate content, which promotes vessel formation, moisture retention, and metalloproteinase expression crucial for tissue remodeling (Faga et al., 2012; Liang et al., 2015; Nicoletti et al., 2019).

In clinical settings, a Monfortinho thermal water-based cream applied in psoriasis and atopic dermatitis patients demonstrated significant improvements in skin hydration, with reductions in pruritic areas and erythema by 71%, 86%, and 86%, respectively (Almeida et al., 2019).

1.1.4. Mechanism of Action of Thermal Water

Balneotherapy has been demonstrated to effectively treat various dermatological conditions while also providing notable cosmetic benefits. Although the precise mechanisms by which thermal waters deliver such a wide range of therapeutic effects remain incompletely understood, it is well-recognized that balneotherapy induces neuroendocrine and immune responses contributing to its healing properties. These effects include anti-inflammatory, analgesic, antioxidant, chondroprotective, and anabolic actions, alongside comprehensive neuroendocrine-immune regulation. The specific therapeutic impact of thermal water depends significantly on its mineral composition, which determines targeted actions against different skin diseases (Joly et al., 2014; Takahashi et al., 2008).

Exposure to thermal waters activates the body's immune and antioxidant systems through multiple bioactive components such as magnesium, sulfur, bicarbonates, selenium, and other salts that play pivotal roles in the management of skin diseases (Joly et al., 2014; Takahashi et al., 2008). Magnesium, for instance, enhances polyamine production, which is involved in the progression of psoriasis (Matz et al., 2003). Both magnesium and zinc contribute to strengthening the skin barrier and bolstering the immune system, while the combined presence of magnesium and calcium salts promotes accelerated skin healing.

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In vitro studies have evidenced that selenium and strontium reduce cytokine secretion, particularly interleukin-6 (IL-6), in epidermal cells. Selenium further exerts immunomodulatory effects by suppressing the inflammatory response of Langerhans cells (Wollenberg et al., 1992). Silica and calcium bicarbonate inhibit mast cell histamine release and decrease basophil degranulation, which may disrupt the itch–scratch cycle characteristic of atopic dermatitis (Joly et al., 2000).

Sulfur is one of the most prominent components in thermal waters relevant to skin health, especially for treating chronic inflammatory conditions due to its broad antiproliferative, keratolytic, antioxidant, antifungal, and anti-inflammatory properties. Sulfurous thermal waters can significantly suppress inflammatory cytokines such as IL-2, IL-8, IL-17, and IL-23, alongside inhibiting T lymphocyte proliferation (Lee et al., 2014; Ninković-Baroš et al., 2014). Balneotherapy with sulfurous water has been corroborated to inhibit intercellular adhesion molecule-1 (ICAM-1) and E-selectin expression in endothelial cells, thus dampening disease progression (Lee et al., 2016). Additional in vitro studies support the immunomodulatory role of sulfurous waters, including downregulation of psoriasis-related inflammatory mediators such as interferon-gamma, tumor necrosis factor-alpha (TNF- α), and decreased T lymphocyte proliferation (Castex-Rizzi et al., 2011; Péter et al., 2017).

Sulfur also mediates reduced leukocyte adhesion and cytokine production within the skin, while enhancing β -endorphin synthesis. Thermal waters rich in salts improve microcirculation by regulating elastase enzyme activity, dilating capillaries, and lowering fibrinogen levels. These effects collectively alleviate inflammation, promote skin repair, and improve patient quality of life (Brockow et al., 2007).

Furthermore, the thermal (heat) component of thermal water plays an integral role by increasing endogenous production of cortisol, β -endorphins, prolactin, norepinephrine, and adrenocorticotrophic hormone, all contributing to immune regulation and anti-inflammatory effects. This process also reduces basophil degranulation, mitigating atopic dermatitis exacerbations (Borroni et al., 2013; Sacerdote et al., 2002).

Thermal stimulation induces vasodilation, enhances blood flow, and decreases blood pressure by regulating elastase enzyme production across skin layers, dilating microvessels, and reducing fibrinogen concentrations, potentially reducing psoriasis manifestations (Brockow et al., 2007). Additionally, hyperthermia positively influences granulocyte motility and modulates microbial and enzymatic skin activity. It also improves collagen-rich tissue extensibility, promoting skin health and resilience (Bogdanov et al., 2012; Golušin et al., 2015).

In nutshell, the combined physical and chemical properties of thermal waters are crucial to their therapeutic efficacy. Careful selection of the appropriate thermal water based on its unique mineral and thermal profile is essential when prescribing balneotherapy for the management of various dermatological conditions.

1.1.5 Future Perspectives

Emerging research suggests that targeted exploration of specific mineral and microbial profiles in thermal waters can enhance their therapeutic and cosmetic efficacy. Future studies should focus on:

- to better characterize mineral and microbial profiles of different thermal waters to optimize their incorporation into dermatological treatments and cosmeceutical products.
- Elucidating molecular mechanisms underlying observed dermatological effects.
- Conducting longitudinal clinical trials to determine long-term efficacy and safety.
- Optimizing cosmeceutical formulations for maximum skin benefit.
- Integrating advanced technologies, such as microbiome modulation, into therapeutic and cosmetic applications.
- To expand to underrepresented dermatological diseases such as vitiligo, melanoma, and basal cell carcinoma, exploring potential benefits of thermal water in these contexts.
- integrating advanced technologies like genomics, proteomics, and microbiome profiling may unlock new mechanisms and therapeutic targets.
- Investigating synergistic effects of combined balneotherapy and phototherapy or topical agents

By addressing these areas, thermal water may transition from traditional therapy to scientifically validated dermatological and cosmeceutical interventions, offering safe and effective options for skin care and disease management.

1.2. Sulfurous Thermal Water of Saturnia Spring and Therapeutic Effects

Italy is famous for richest and captivating sources of natural thermal spring waters with 378 thermal spas distributed in 170 municipalities of Italy (Bechari and Quirino, 2012). Thereof, hydrothermal resources are important factor in economy of that municipalities. In Italy, the **Terme di Saturnia**, located in Tuscany, represent one of the most renowned sources of sulfurous thermal water.



Figure 1.1: Saturnia thermal springs situation in southern Tuscany (Italy).

1.2.1. Geographic and Geological Settings

The Saturnia thermal springs are located in southern Tuscany (province of Grosseto, Italy), near the village of Saturnia, within the hydrothermal system associated with the Monte Amiata volcanic complex (Figure 1.1, 1.2, 1.3). The springs discharge at an elevation of approximately 160 m a.s.l. in the Albegna River basin. The geothermal system is structurally controlled by deep fractures that allow meteoric water to circulate through carbonate and evaporitic lithologies at depth, where it is heated by the residual geothermal gradient of the extinct volcanic district.

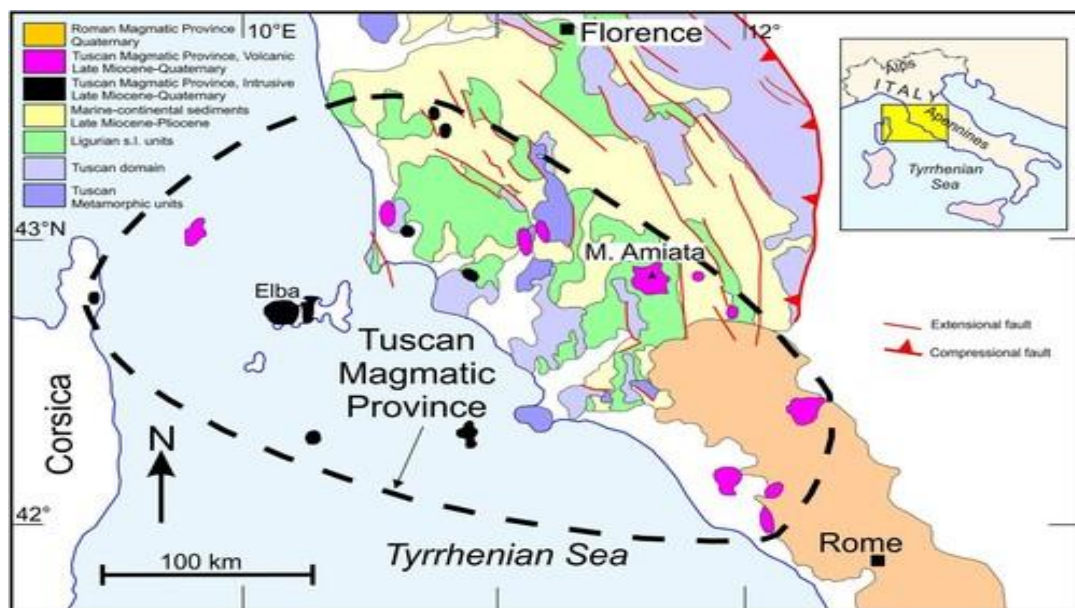


Figure 1.2: Geographical location of the Saturnia thermal springs in southern Tuscany (Italy), showing the regional setting within the Monte Amiata volcanic–carbonate system.

The main spring (Terme di Saturnia) exhibits a remarkably stable temperature of approximately 37.5 °C, with an average discharge rate of $\approx 500 \text{ L s}^{-1}$, corresponding to one of the most productive hydrothermal systems in central Italy. Geochemical and isotopic evidence suggests a long subsurface residence time (~ 40 years) before emergence, during which the water equilibrates with carbonate and sulphide minerals under reducing conditions. The outflow forms extensive travertine deposits, indicating active precipitation of calcium carbonate from degassing of CO_2 -saturated waters.



Figure 1.3: Photo of saturnia baths

1.2.2. Geochemical Characteristics

The Saturnia thermal waters are classified as sulphurous–carbonic, sulphate–bicarbonate–alkaline–earth mineral waters, reflecting both their chemical composition and gas content. The total dissolved solids (TDS) content averages $2.7\text{--}2.8 \text{ g L}^{-1}$, with the dominant ionic species being SO_4^{2-} , HCO_3^- , Ca^{2+} , and Mg^{2+} . Although Saturnia thermal water has been studied previously, the reported chemical and physical parameters show some variation between studies. To provide a consistent and reliable reference, the water was re-characterized in this work. Samples were collected under standardized conditions and analyzed for key physical–chemical parameters, including pH, temperature, mineral composition (cations and anions), and dissolved gases. The resulting values were then compared with earlier reports to evaluate consistency, identify potential sources of variation, and establish an updated profile of Saturnia thermal water. This approach ensures that subsequent experimental or clinical studies using this water are based on accurately defined characteristics, improving reproducibility and the interpretability of biological or therapeutic outcomes. The detailed physio-chemical properties of Saturnia spring thermal water from different sources and present study are given in Table 1.1. The significance of saturnia thermal water is mostly attributed to its highly sulfurous water.

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Trace element analyses have reported the presence of boron ($\approx 4.5 \text{ mg L}^{-1}$), lithium ($\approx 0.18 \text{ mg L}^{-1}$), and strontium ($\approx 8\text{--}9 \text{ mg L}^{-1}$). The relatively high sulphate content reflects oxidation of subsurface sulphides or dissolution of gypsum-bearing evaporites, while the bicarbonate and calcium originate from carbonate dissolution and CO_2 interaction. The presence of dissolved hydrogen sulphide and free CO_2 indicates a chemically reducing, CO_2 -rich environment in the reservoir.

From a hydrochemical perspective, the waters plot within the **Ca–Mg– SO_4 – HCO_3 facies**, consistent with prolonged water–rock interaction in a deep carbonate–evaporitic aquifer overlain by low-permeability clays that maintain temperature stability. The continuous outflow and travertine deposition confirm active degassing and supersaturation with respect to calcite at the discharge point.

Table 1.1: Physical and Chemical Composition of Saturnia Thermal Water

	Unit	Terme di Saturnia website (accessed 26-10-2025)	Benedetti et al., 2010	Barbagli et al., 2013	Ferrara et al., 2025	Ferrara et al., 2025b
Bicarbonate (HCO_3^-)	mg/l	661,2	675	662	667	637
Chloride (Cl^-)	mg/l	74,4	71,4			69
Sulfate (SO_4^{2-})	mg/l	1480	1469	1541	1508	1475
Sodium (Na^+)	mg/l	71,2	63,7	45		53
Potassium (K^+)	mg/l	10	9,3	6		3,8
Lithium (Li^+)	mg/l	traces				0,631
Magnesium (Mg^{2+})	mg/l	129,6	134	92	122	111,5
Calcium (Ca^{2+})	mg/l	561	598	593	518	561
Iron ($\text{Fe}^{2+}/\text{Fe}^{3+}$)	mg/l	traces	<0,01			0,15
Phosphate (PO_4^{3-})	mg/l	0,12				
Nitrate (NO_3^-)	mg/l	>1	<0,1	>0,5		
Nitrite (NO_2^-)	mg/l	absent	<0,01			
Ammonium (NH_4^+)	mg/l	24,9	26,8			
Sulphite (SO_3^{2-})	mg/l	>0,05	14,5			
Total H_2S (H_2S)	mg/l	14	674		14,5	14

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Carbon dioxide (CO ₂)	mg/l	728				
Carbon dioxide (dissolved/loose) (CO ₂ (aq))	mg/l	462				
Strontium (Sr ²⁺)	mg/l			10,879		11,384
Fluoride (F ⁻)	mg/l			0,1879		
Boron (B)	mg/l			18,334		19,315
Diphosphorus pentoxide (P ₂ O ₅)	mg/l		<0,01			
Silicon Oxide (SiO ₂)	mg/l		20,7			
Aluminium (Al)	mg/l					
phosphorus (P)	mg/l					
Sulfur (S)	mg/l					
Silicone (Si)	mg/l					
Fixed residue	mg/l		2990			
Temperature (°C)		37,5	36,9	34,05	37,5	37,5
pH		6,4	6,25		6,3	6,85
Conductivity (μS/cm)			2996			
Hardness (°F)		193	204			

The physicochemical characterization of Saturnia thermal water has been reported in several previous studies; however, the published data show noticeable variability among sources. Variations appear in temperature, pH, conductivity, and the concentrations of major ions such as sulphate, calcium, magnesium, and bicarbonate. These differences may arise from temporal and seasonal changes in the geothermal system, heterogeneity in sampling locations, or methodological divergences related to analytical techniques, calibration standards, and sample handling. Because these parameters are directly linked to the fundamental quality attributes of the thermal water—its *identity*, *purity*, *richness*, *efficacy*, and *stability*—it was scientifically necessary to perform a new and standardized characterization.

Re-evaluation under controlled, reproducible, and well-documented analytical conditions provides an updated reference for the water's identity, defining its unique physicochemical fingerprint. Determination of contaminants and extraneous elements ensures its purity, while quantification of major and trace constituents reflects its richness in mineral and bioactive components. Establishing precise ionic and redox parameters is essential for evaluating the water's efficacy, as these factors influence its potential biological and therapeutic properties. Continuous monitoring of physicochemical parameters further contributes to understanding the stability of the thermal source and the persistence of its quality over time.

Accordingly, the present re-characterization offers a coherent and internally consistent dataset that strengthens the scientific reliability of Saturnia thermal water studies and provides a robust baseline for future comparative and applied research.

1.2.3. Classification of Thermal Waters

The classification of thermal waters is fundamental for understanding their geological origin, physicochemical behavior, and potential therapeutic properties. Such classification is generally based on three principal parameters: temperature, total mineralization (expressed as fixed residue at 180 °C), and dominant chemical composition.

From a thermal standpoint, waters with a temperature below 20 °C are defined as cold, those between 20 °C and 30 °C as hypothermal, between 30 °C and 40 °C as homeothermal, and between 40 °C and 50 °C as hyperthermal.

In terms of total mineral content, measured as the fixed residue after evaporation at 180 °C, thermal waters are categorized as very low mineral content when the residue is < 50 mg/L, low mineral content between 50 mg/L and 500 mg/L, medium mineral content between 500 mg/L and 1500 mg/L, and rich mineral content when the fixed residue exceeds 1500 mg/L. The chemical composition provides a further basis for classification according to the predominant ions or molecular species present. Waters containing more than 600 mg/L of bicarbonate ions are termed bicarbonate waters; those with carbonate concentrations above 300 mg/L are carbonate waters; and waters with sulphate contents greater than 200 mg/L are described as sulphated waters. When reduced sulfur compounds exceed 1 mg/L, the water is referred to as sulphurous. Waters enriched in arsenic and iron compounds (> 1 mg/L) are designated arsenical-ferruginous. If iodide and bromide ions surpass 1 g/L, the waters are classified as iodo-bromo-saline, whereas those with sodium chloride concentrations greater than 200 mg/L are known as saline or sodium-chloride-rich waters. Finally, waters exhibiting radioactivity above 1 nC/L are identified as radioactive thermal waters.

According to the results obtained in the present work, Saturnia thermal water can be classified as a hyperthermal and highly mineralized water. Its temperature, which typically range 37–40 °C, places it in the homeothermal category. The fixed residue value, although variable across literature, is consistently above 1500 mg/L, confirming that it is a rich mineral content water.

From the standpoint of chemical composition, the analyzed sample is characterized by the predominance of calcium (605.6 mg/L), magnesium (59.75 mg/L), sodium (94.77 mg/L), and a substantial level of sulfur species (653.5 mg/L). This ionic profile clearly identifies the water as a sulphurous–calcium–magnesium type, with notable sodium contribution. The relatively high sulfur content, in the form of sulfide and sulfate species, supports its classification among *sulphurous thermal waters*—a group typically associated with recognized therapeutic and antimicrobial properties.

Minor constituents such as potassium (7.69 mg/L), aluminium (0.251 mg/L), phosphorous (0.112 mg/L), and silicon (12.89 mg/L) indicate the presence of trace elements that contribute to the overall mineral richness of the water. The measured concentrations of these elements, together with its expected conductivity and pH values (commonly near neutral to slightly alkaline in previous reports), reflect a stable hydrochemical equilibrium characteristic of mature geothermal systems.

In summary, based on its physicochemical parameters, Saturnia thermal water is classified as a homeothermal, highly mineralized, sulphurous–calcium–magnesium water, exhibiting significant ionic richness and compositional stability—attributes consistent with its long-recognized therapeutic and biological potential

1.2.4. Mechanism of Action of Sulfurous Thermal Water:

Sulfurous thermal water is well reported for various therapeutic effects on skin diseases. The tendency of absorption of sulfur compared to other minerals is manifold high. It is because sulfur is usually present in the form of H_2S in thermal water and it can easily penetrate through skin and mucosa barrier to get in systematic circulation extending its effects not only to skin but also to other organs. In addition to this the pH (<6) of skin also favors the absorption capacity of H_2S in skin. Hence, sulfurous thermal water balneotherapy have been well-known for its therapeutic properties to treat various skin diseases such as atopic dermatitis, psoriasis, acne, contact dermatitis, lesions (Carbajo & Maraver, 2017). It is evidenced that sulfurous mineral water have vasodilation, analgesia, keratolytic, anti-pruritic, anti-inflammatory, anti-fungal and anti-bacterial effects (Carbajo & Maraver, 2017; Matz et al., 2003). Therefore, sulfurous thermal water open wide window of its therapeutic effects to treat various diseases including cardiovascular (Oláh et al., 2011), skin (Amy Huang et al., 2018), arthritis (Verhagen et al., 2015), cancers (Reger et al., 2022), diabetes (Marchenkova et al., 2016), and respiratory diseases (Khaltaev et al., 2020).

Due to miraculous therapeutic abilities of sulfur rich water, they can be used for treatment of many T cell mediated inflammatory autoimmune diseases. Considering range of beneficial (fungicidal, bactericidal, anti-pruritic, keratolytic) properties of sulfurous thermal water they are used in topical formulations and skin care products since many years (Parcell, 2002). Sulfur from water can effectively suppress cytokines (IL-2, IL-8, IL-23, IL-17) production and T-cell proliferation. It also inhibit signalling of mitogen activated protein kinase mechanism to retard keratinocytes proliferation (Gobbi et al., 2009; Y. B. Lee et al., 2014). Even more, sulfurous water also known to act as bactericidal agent against pathogenic microbes such as *Staphylococcus Aureus* generally found in many skin diseases (Akiyama et al., 2000; Scala et al., 2019).

1.2.5. Saturnia Thermal Water and Therapeutic effects Clinical Evidence:

The literature on Saturnia thermal water balneotherapy and therapeutic effects is very scarce. Only 3 studies are reported on balneotherapy application of saturnia thermal spring water.

The biological effects of Saturnia's sulphurous thermal water have been evaluated in a controlled human study conducted by Benedetti et al. (2009), who investigated the impact of a short-term hydropinic regimen on systemic oxidative stress and antioxidant status in healthy adults. The study aimed to determine whether ingestion of the sulphurous mineral water from Saturnia could modulate biochemical markers associated with lipid and protein oxidation, and enhance plasma antioxidant defences.

In this clinical trial, 40 healthy volunteers (18 males and 22 females, aged 41–55 years) were randomly divided into two groups. The intervention group consumed 500 mL day⁻¹ of the sulphurous mineral water directly sourced from Saturnia, characterised by a hydrogen sulphide content of approximately 14 mg L⁻¹, a total mineralisation of ~2.8 g L⁻¹, and a Ca–Mg–SO₄–HCO₃ ionic composition. The control group received an equal volume of a non-sulphurous natural mineral water under identical conditions. The hydropinic treatment lasted for two weeks, during which participants maintained their habitual diet and lifestyle. Blood

samples were collected at baseline (T_0) and after treatment (T_1), and analysed for several oxidative stress biomarkers, including malondialdehyde (MDA), protein carbonyls, and advanced oxidation protein products (AOPP), as well as total plasma thiols ($-SH$) and total antioxidant capacity. Fat-soluble antioxidants (retinol, tocopherols, and carotenoids) were also quantified.

Following two weeks of hydropinic therapy, the group consuming the sulphurous Saturnia water showed a significant reduction in oxidative stress markers, with decreased levels of MDA, protein carbonyls, and AOPP compared to baseline and to the control group ($p < 0.05$). Concurrently, there was a notable increase in total plasma antioxidant capacity and thiol concentrations, indicating an enhancement of endogenous antioxidant defence systems. No significant changes were observed in plasma concentrations of retinol, carotenoids, or tocopherols in either group, suggesting that the observed effects were not attributable to variations in dietary antioxidants.

The authors concluded that the hydropinic use of the Saturnia sulphurous water exerts measurable antioxidative effects at the systemic level, likely mediated by the biological activity of hydrogen sulphide (H_2S), a recognised gaseous signalling molecule (gasotransmitter) with vasodilatory, anti-inflammatory, and redox-modulatory functions. The study supports the hypothesis that regular consumption of sulphurous mineral waters may promote a favourable redox balance, reduce oxidative damage to biomolecules, and enhance cellular antioxidant defence mechanisms, even in healthy subjects.

From a mechanistic perspective, these findings are consistent with the known biochemical actions of H_2S , which interacts with endogenous thiol groups, modulates glutathione homeostasis, and influences enzyme systems involved in oxidative stress regulation. The increase in plasma thiols observed by Benedetti et al. (2009) suggests a reinforcement of sulphur-dependent antioxidant pathways, highlighting a potential link between the geochemical composition of Saturnia water—notably its sulphur and alkaline-earth content—and its biomedical efficacy.

Although the treatment period was limited to two weeks and the participants were healthy adults, the study provides important experimental evidence linking the geochemistry of Saturnia's thermal waters to measurable physiological outcomes. It demonstrates that the ingestion of sulphurous thermal water can elicit biochemical improvements without pharmacological intervention, thereby validating traditional claims of its detoxifying and anti-inflammatory effects. The authors recommended further research involving longer exposure durations and clinical populations to substantiate these preliminary findings and to define optimal dosing regimens for therapeutic use.

Benedetti and colleagues (2010) undertook a controlled clinical investigation to explore the effects of sulphur-based spa therapy in 45 patients with knee osteoarthritis (OA) by assessing biomarkers of oxidative stress, inflammation and cartilage degradation. The study stratified participants into three distinct groups: Group A underwent mud-bath treatments *in combination* with hydropinotherapy (drinking the mineral water), Group B underwent the same mud-bath treatment but *without* hydropinotherapy, and Group C comprised patients who did not receive the spa therapy intervention (control). The interventions were applied and

biomarkers measured at three time-points: before therapy (T0), immediately after therapy (T1), and one month after cessation of therapy (T2). The spa therapy was delivered at the thermal centre of Saturnia (Italy), utilising sulphurous mineral water for baths and mud packs and also including ingestion of the mineral water in the hydropinotherapy arm (Group A). The intervention setting was a sulphurous mineral spa environment, with standardised mud-bath sessions and, for Group A, concurrent ingestion of the mineral water. The primary outcomes comprised plasma/serum biomarkers reflecting oxidative stress (e.g., hydroperoxides), inflammatory mediators (interleukins, adhesion molecules) and cartilage degradation (matrix metalloproteinases, etc.). Although the control group exhibited no significant biomarker changes across T0-T1-T2, Group A demonstrated statistically significant reductions in oxidative, inflammatory and cartilage-destructive markers at T1 which persisted at T2, whereas Group B showed favourable biomarker shifts only at T1 but not maintained to T2. The authors concluded that combining mud-bath therapy with hydropinotherapy appears more effective at sustaining biochemical improvement, suggesting a potential chondroprotective benefit in osteoarthritis management. The possible explanation for these results could be the antioxidant activities of sulfurous thermal water attributed to the ability of sulfur to effectively scavenge different free radicals such as superoxide, hydrogen peroxide, peroxynitrate and at the same time it also have potential to deeply penetrate at cellular level and react to synthesize glutathione-antioxidant (Kimura et al., 2006) and other essential redox regulators such as tioredoxin-1 and increase superoxide dismutase activities (Tyagi et al., 2009). Moreover, Costantino et al. (2021) demonstrated that oral intake of sulfurous mineral water decreased plasma glucose, improved oxidative status, and enhanced quality of life in adult participants after a 2-week intervention. Costantino et al. investigated the effects of hydropinotherapy with sulphurous mineral water on glucose metabolism, oxidative status, and quality of life in patients with chronic gastrointestinal conditions. The study enrolled a total of 125 participants, with 90 receiving a two-week cycle of daily mineral water ingestion (S-HT group) and 35 serving as a control group without hydropinotherapy. The intervention consisted of drinking 1–3 glasses of sulphurous mineral water daily, with the water containing hydrogen sulphide and mineral components such as calcium, magnesium, and bicarbonate. Outcomes assessed before and after the intervention included fasting glycaemia, markers of oxidative stress, and quality of life via the SF-36 questionnaire. Patients receiving hydropinotherapy showed a notable improvement in glycaemic control and a reduction in oxidative stress markers, which were accompanied by enhancements in several aspects of quality of life, particularly vitality, mental health, and emotional well-being. The authors suggest that these benefits may be mediated by the antioxidant properties of the sulphurous and mineral components of the water, which could support glucose homeostasis and overall metabolic health. While the study is limited by its observational design, short duration, and non-randomized assignment, it provides evidence that hydropinotherapy may serve as a useful complementary intervention for improving metabolic parameters and quality of life, supporting further research in larger, controlled trials.

In nutshell, the Saturnia thermal waters represent a rare combination of constant moderate thermal temperature, high mineralisation (especially sulphur, calcium, sulphate, bicarbonate), and trace-gas content that together support a wide range of therapeutic claims spanning

dermatology, rheumatology, respiratory, cardiovascular and digestive systems. Their geological setting—a deep aquifer beneath volcanic-carbonate terrains of southern Tuscany—provides a stable and geochemically active source. While spa traditions and preliminary scientific data are promising, further controlled research is needed to quantify specific clinical outcomes, dose-response relationships (bath time, mineral absorption) and long-term benefits.

1.3. Thermal Water Formulations

The growing interest in natural-based dermatological and cosmetic formulations has driven research toward enhancing the intrinsic therapeutic potential of thermal waters through the incorporation of functional additives such as clay minerals and essential oils. While thermal waters alone exhibit beneficial effects on skin health due to their mineral composition and physicochemical properties, their efficacy can be significantly amplified by combining them with materials capable of improving stability, bioactivity, and controlled delivery of active components. Such hybrid formulations integrate the physicochemical advantages of inorganic matrices with the biofunctional effects of organic actives, leading to multifunctional systems with superior therapeutic and cosmetic performance.

1.3.1. Technological and Biopharmaceutical Additives: Clays

Clays are fundamental biopharmaceutical additives in thermal water formulations, particularly in the preparation of peloids, also called therapeutic muds or thermal muds. A peloid is defined as a matured mud or muddy dispersion with therapeutic and/or cosmetic properties, consisting of a complex mixture of fine-grained natural materials of geological and/or biological origin, mineral water or seawater, and frequently organic compounds derived from biological metabolic activity (Carretero, 2002; Veniale et al., 2007). Peloids are biphasic systems, comprising a solid phase—mainly minerals, particularly phyllosilicates, and organic substances produced through microbial and plant activity—and a liquid phase, typically medicinal mineral water (MMW), seawater, or water from saline lakes (Carretero & Pozo, 2009). Peloids may occur naturally or be artificially prepared from suitable raw materials. Natural peloids form through processes such as sedimentation, flocculation, or precipitation of substances in aquatic environments, leading to their deposition at the bottom of springs, coastal zones, or saline lakes. In other cases, they may arise from the accumulation and humification of plant matter, as observed in peats. Artificial peloids, in contrast, replicate the composition and characteristics of natural peloids, requiring a controlled production process that includes component selection, mixing, homogenization, and maturation. The therapeutic effects of peloids depend on their composition and physical properties. Their performance as thermotherapeutic agents is strongly influenced by cooling kinetics, with slower cooling generally preferred (Ferrand & Yvon, 1991; Veniale et al., 2004). Chemical and mineralogical compositions are important (Carretero & Pozo, 2010; Tateo & Summa, 2007; Veniale et al., 2004), as are physical characteristics such as density, plasticity, abrasivity, specific heat, thermal conductivity, and rheological behavior including sediment volume and viscosity (Barhoumi et al., 2021; Carretero & Pozo, 2010; Ferrand & Yvon, 1991; Rebelo et al., 2011). These properties enhance the mud's molding capacity and ensure a comfortable sensation when applied (Ferrand & Yvon, 1991). Granulometry is also critical, with fine-

grained mud preferred for optimal application (Carretero, 2002; Veniale et al., 2007). Bentonite is considered the most suitable solid phase due to its favorable physicochemical properties, including pH, soluble salts, and exchangeable ions (Carretero & Pozo, 2009; Veniale et al., 2004). Microbiological quality is essential: peloids must be free of pathogens, while the development of specific microbial communities during maturation can contribute to therapeutic effects (Kataržytė et al., 2024).

Clay minerals such as montmorillonite and bentonite have a layered silicate structure with a high cation-exchange capacity and specific surface area that enables absorption, ion exchange, and intercalation of minerals and molecules (Yaghmaeiyan et al., 2022). When suspended in thermal water, clay minerals provide desirable rheological and thermomechanical properties, critical for topical application in balneotherapy. Furthermore, the cation exchange capacity (CEC) of clays enables the reversible binding and release of therapeutic ions present in the thermal water, such as Ca^{2+} , Mg^{2+} , Zn^{2+} , and Se^{2-} , which play essential roles in anti-inflammatory, antioxidant, and regenerative skin processes (Cacciapuoti et al., 2020; Protano et al., 2024). Through these physicochemical mechanisms, clays not only stabilize thermal water systems but also act as delivery matrices, prolonging the contact and diffusion of beneficial ions across the skin barrier during topical application.

The synergy between thermal water and clay underpins the biological activity of peloids. Clay particles interact with skin through adsorptive removal of toxins and microbes while slowly releasing minerals such as magnesium, calcium, and sulfur, which exhibit anti-inflammatory, antioxidant, and keratolytic effects (Guerrero & Garrigue, 2017). The physical heat retention of clay suspensions further enhances these effects by prolonging hydrothermal exposure, which facilitates cutaneous vasodilation and metabolic activation relevant for tissue repair.

Advanced characterization of clays helps tailor formulations by controlling parameters such as mineralogical composition, particle size distribution, plasticity, and thermal conductivity (García-Villén et al., 2020). For instance, montmorillonite clays with expandable layers offer intercalation sites ideal for incorporating active molecules, whereas kaolinite provides a gentler, less reactive matrix beneficial for sensitive skin.

1.3.2. Clinical Additives: Essential Oils and Clay Complexation

Essential oils (EOs) are volatile, lipophilic plant-derived compounds with recognized antimicrobial, anti-inflammatory, and antioxidant properties extensively used in dermatology (Cunha et al., 2022; Gnanewaran et al., 2025). However, their direct application can be limited by volatility, sensitivity to oxidation, and risk of irritancy, requiring innovative delivery systems to maximize clinical benefit and safety.

Intercalation or complexation of essential oils into clay minerals addresses many of these challenges by entrapping EO molecules within the clay interlayer spaces. This incorporation stabilizes EOs against degradation, controls release kinetics, reduces skin irritation, and improves bioavailability (Hongxin et al., 2024; Tretjakova et al., 2024). Molecular modeling simulations, including computational experiments performed at atomic and molecular scale confirm that EO molecules can fit energetically and spatially within clay interlayers, forming

stable organo-clay complexes capable of sustained release (Hongxin et al., 2024; Oliveira et al., 2023).

Clay-EO complexes serve as multifunctional ingredients in thermal water formulations for topical anti-inflammatory, antimicrobial, and antioxidant products (Da Rocha et al., 2022; Duan et al., 2025). Controlled intercalation enhances EO solubility, protects bioactivity, and improves shelf-life and efficacy (Hongxin et al., 2024). Applications include topical anti-inflammatory formulations for atopic dermatitis and acne, antimicrobial products for infected wounds, and antioxidant vehicles in antiaging products (Alves-Silva et al., 2023).

1.3.3. Specifications to be Achieved by Advanced Formulations

Innovative formulations integrating thermal water, clay minerals, and essential oils should aim to meet multifaceted specifications ensuring both clinical efficacy and consumer acceptability:

- **Physical and Chemical Stability:** The suspension or emulsion must maintain homogeneity and prevent phase separation over time. Clay particle dispersion, EO retention, and mineral stability are critical (Joly et al., 2000).
- **Controlled Release Profiles:** Proper modulation of mineral and EO delivery over intended usage periods to achieve sustained therapeutic action without excessive initial burst effects (Saucedo-Zuñiga et al., 2021).
- **Rheological Properties:** Optimal viscosity and spreadability ensure ease of application and adequate residence time on the skin, with thermal muds often exhibiting thixotropic behaviors advantageous in therapy (Maraver et al., 2021).
- **Safety and Biocompatibility:** Low irritation potential, non-sensitizing properties, and maintenance of skin barrier integrity are essential, especially when dealing with volatile actives like EOs (Brockow et al., 2007; Deleuran et al., 2020).
- **Synergistic Therapeutic Efficacy:** Combining anti-inflammatory, antioxidant, antimicrobial, keratolytic, and regenerative activities through the formulation components, supported by clinical data (Castex-Rizzi et al., 2011; Gueniche et al., 2022).
- **Innovative Manufacturing Technologies:** Implementation of advanced encapsulation methods (e.g., micro/nanoemulsions, liposomes), high shear or ultrasonic mixing, and molecular modeling guided design to optimize EO intercalation and mineral bioavailability (Kaur et al., 2025).

Advanced thermal clay formulations also exploit the thermophysical properties of clay-mineral suspensions to deliver controlled thermal energy, stimulating skin microcirculation and metabolism while ensuring bioactive stability (Bogdanov et al., 2012).

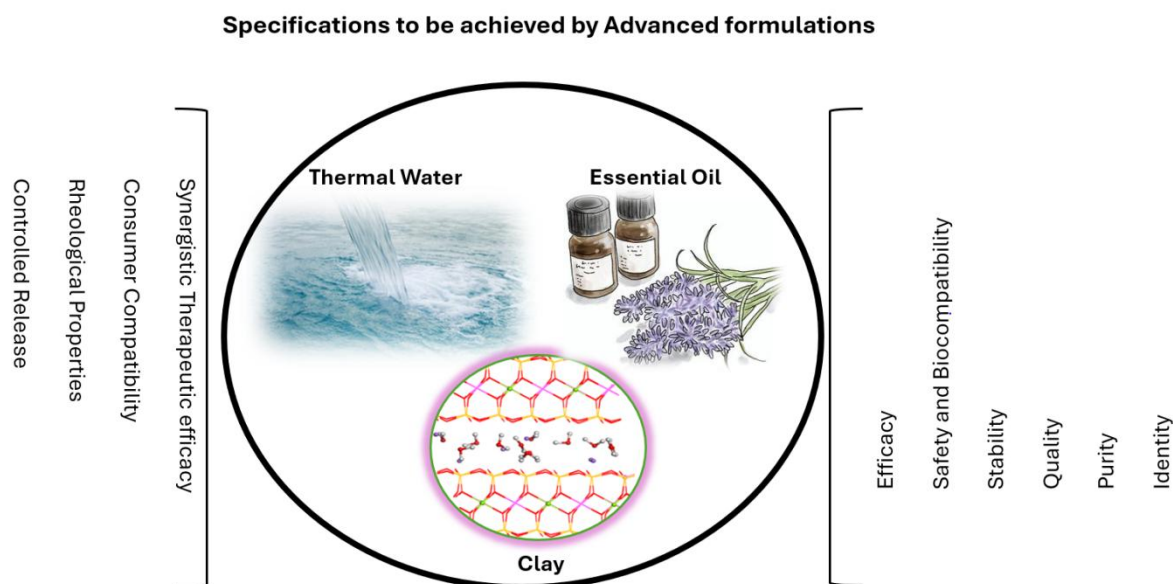


Figure 1.4 Key Specifications and Components in Advanced Formulations of Thermal Water, Essential Oils, and Clays

1.3.4. Emerging Technological Incentives

Contemporary advances in thermal water formulation involve the use of computational modelling and simulation techniques to understand and optimize the interactions between formulation components at the molecular level. In our study, energy optimization and molecular dynamics simulations provided valuable insights into the feasibility and stability of essential oil molecules intercalated within clay mineral interlayers. These approaches enable the rational design of advanced formulations by predicting structural compatibility and energetics, thus guiding experimental formulation development.

Furthermore, ongoing research is exploring novel combinations of thermal water, clays, and natural bioactives to create multifunctional dermatological products with improved efficacy and safety. While biotechnological extracts from thermal water microbiomes represent a promising future avenue, this area remains outside the scope of the current work.

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CHAPTER 1: Introduction

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CHAPTER 2

AIMS AND OBJECTIVES



2.1.Hypothesis

Thermal waters exhibit well-recognized therapeutic and cosmetic properties arising from their distinctive mineral composition and ionic balance, which contribute to skin regeneration, anti-inflammatory activity, and barrier recovery. Nevertheless, their direct application in formulations faces two major challenges: the limited availability of natural thermal sources and the lack of optimized preparation methods for associated peloids or suspensions.

To date, most spa centers prepare peloids following empirical or traditional procedures, often without accurate identification of the clay components or proper control of the clay-to-water ratios. Consequently, many of these preparations are suboptimal, showing variable texture, stability, and therapeutic performance. The clay fraction in peloids is crucial not only for providing the required rheological characteristics—which determine spreadability and adherence to the skin—but also for controlling heat transfer during thermotherapeutic application. As peloids act as thermo-therapeutic agents, their thermal conductivity and heat retention depend strongly on the mineral composition and clay–water ratio. Some mineral phases lack these desired functional attributes, whereas others must be present in specific proportions to achieve effective therapeutic performance.

Hence, it becomes essential to develop a scientifically validated optimization framework for clay–water systems used in pelotherapy. Such optimization ensures that the structural, mineralogical, and thermal characteristics of the clays align with the desired rheological and thermophysical behavior of the formulations, while also minimizing the use of scarce thermal water resources.

Based on these considerations, the central hypothesis of this research is that a multiscale, integrated approach—combining experimental optimization, physicochemical characterization, and molecular modeling—can enable the rational design of advanced thermal water–based formulations with improved textural, rheological, and therapeutic performance. Specifically, it is hypothesized that:

It is hypothesized that:

1. Clay minerals, owing to their layered structure, ion-exchange capacity, and rheological behavior, can serve as functional additives and structural matrices capable of enhancing the stability, texture, and therapeutic efficacy of thermal water formulations.
2. The processing conditions, particularly mixing energy and agitator geometry, significantly influence the microstructure and rheology of clay–water suspensions, thereby affecting their spreadability and application performance. Optimizing these parameters is essential to obtain reproducible and high-quality formulations using minimal thermal water.
3. Thermal water–clay systems (peloids) prepared with optimized clay–water ratios and different clay mineral types will exhibit distinct rheological and sensorial behaviors due to variations in mineralogical composition, cation-exchange capacity, and interaction with thermal ions.

4. Comprehensive mineralogical and chemical characterization of the clays is essential to understand their composition, structural order, and thermal stability, and to correlate these properties with the macroscopic behavior of suspensions and peloids.
5. The incorporation of bioactive natural additives, such as essential oils, into clay–*thermal water* matrices can further enhance the therapeutic and cosmetic value of these formulations by introducing antimicrobial, antioxidant, and soothing activities.
6. Molecular modeling approaches can effectively predict the feasibility and mechanism of essential oil intercalation into clay interlayers and on particle surfaces, providing a rational theoretical basis for experimental formulation design and reducing empirical trial requirements.

Overall, it is hypothesized that a multiscale understanding—linking processing, structure, and functionality—will enable the development of optimized, natural, and efficient dermocosmetic formulations based on thermal water, clays, and essential oils (Figure 2).

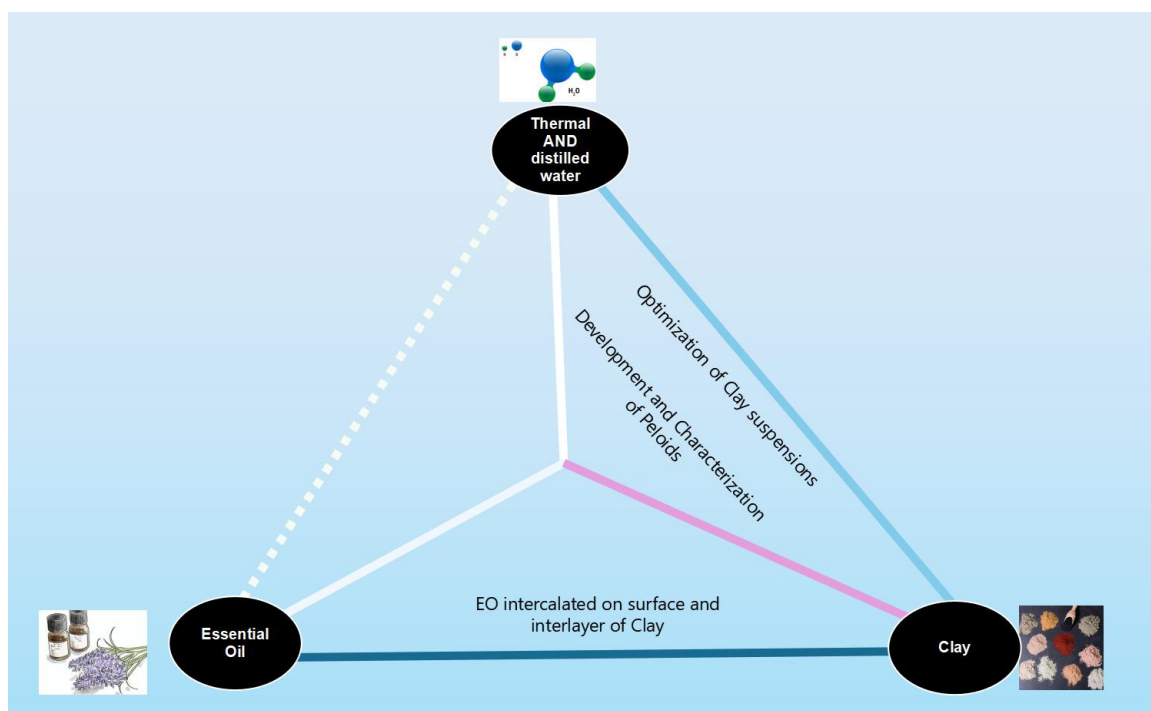


Figure 1.5: *Triangular model integrating molecular and formulation-level studies of clay-based systems.*

2.2. Aims and Objectives:

To validate the above hypothesis, a series of experimental and modeling studies were designed and organized into interconnected research phases, each addressing a specific aspect of the overall investigation.

Objective 1 – Characterization of Raw Material (Chapter III)

CHAPTER 3: Raw Material Characterization

To perform comprehensive physicochemical and mineralogical characterization of the clay minerals (VF, ILB, BB, BC) and Saturnia thermal mud intended for peloid preparation using:

- X-ray diffraction (XRD) to identify crystalline phases and layer structures;
- **X-ray fluorescence (XRF):** to determine the elemental and oxide composition, providing quantitative information on the major and trace elements that define the geochemical fingerprint of each clay;
- Fourier-transform infrared spectroscopy (FTIR) to detect functional groups and surface interactions;
- Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) to evaluate thermal stability, dehydration behavior, and organic/inorganic content.
- Mineralogical Characterization of Saturnia thermal water

This objective provides a structural and compositional foundation for interpreting the behavior of the clay–water and clay–thermal water systems.

Objective 2 – Optimization of Clay–Water Systems (Chapter IV)

To prepare and characterize clay–water suspensions using a single clay mineral type (VF clay) to:

- Optimize the solid–liquid ratio for stable suspension formation prior to thermal water use, thereby conserving limited thermal water resources.
- Evaluate the influence of mixing energy and agitator geometry on the dispersion quality, rheological parameters, and spreadability of VF clay–water systems.
- Establish a baseline understanding of how processing conditions affect the structural and functional properties of clay–water systems.

Objective 3– Development and Characterization of Thermal Water–Clay Peloids (Chapter IV)

To prepare peloids by combining thermal water with four clay minerals types (BB, BC, VF, ILB) using the optimized concentration obtained from Objective 2, and to:

- Compare their rheological and spreadability behavior with corresponding clay–water suspensions.
- Correlate mineralogical composition and thermal water chemistry with the resulting textural and structural properties.
- Identify the most suitable clay–thermal water combination for dermocosmetic applications.

Objective 4 – Theoretical Modeling and Experimental Validation of Essential Oil–Clay Interactions (Chapter V)

To evaluate the molecular feasibility and interaction mechanisms of anti-inflammatory essential oils with clay minerals through computational modeling by:

- Simulating the intercalation and surface adsorption of selected essential oil molecules (thymol, carvacrol, eugenol, menthol) into clay interlayers and surfaces.
- Investigating the energetic stability, orientation, and binding behavior of EO–clay hybrids using molecular modeling approaches.
- Providing theoretical validation for the design of experimental EO–clay formulations.
- Correlating modeling and experimental data to establish structure–property relationships.
- To synthesize clay–essential oil hybrid materials and to characterize their structural, spectroscopic, and thermal properties using XRD, and FTIR analyses.

Objective 5 – Computational Extension to Penetration Enhancer Essential Oils (Chapter V)

To expand the modeling framework to additional naturally Penetration Enhancer essential oils (pinene, ocimene, caryophyllene, limonene) to:

- Assess their intercalation behavior and energetics in clay matrices.
- Predict potential formulation compatibility and stability prior to future laboratory trials.

2.3. Summary

The overarching goal of this research is to establish a scientifically validated framework for designing advanced, natural dermocosmetic formulations based on thermal waters, clays, and essential oils. By integrating experimental rheological studies, comprehensive clay characterization, and molecular modelling, this work seeks to elucidate how material composition, processing conditions, and molecular interactions collectively determine formulation performance. The resulting insights will contribute to the rational development of sustainable, stable, and effective peloids formulations for therapeutic and cosmet

CHAPTER 3

CHARACTERIZATION OF CLAYS, SATURNIA MUD AND SATURNIA WATER



KEY CONCEPTS

- The mineralogical composition shows high smectite clay in following order $ILB > BC > BB > VF = SM$
- The level of carcinogenic elements (quartz and cristobalite) is high in Saturnia mud and VF clay therefore pretreatment to remove these impurities is needed for pharmaceutical grade formulation to use in dermo-cosmetic industry
- For Cation Exchange capacity the trend is $VF > ILB > BB > BC$
- Saturnia thermal water is sulphate– bicarbonate–alkaline–earth mineral waters

3. Characterization of Clays, Saturnia Mud, and Saturnia Water

3.1. Background

Characterization of clays is fundamental in pharmaceutical sciences due to their unique physicochemical properties that directly influence their effectiveness as drug carriers and excipients. Clay minerals, notably montmorillonite, kaolinite, and halloysite, possess a layered structure with high surface area and significant cation exchange capacity (CEC), enabling them to adsorb, intercalate, and stabilize a variety of drug molecules. This ability enhances drug solubility, controls release kinetics, improves stability, and ultimately optimizes therapeutic efficacy while minimizing side effects (Dk et al., 2020; Nomicisio et al., 2023).

These minerals function not only as passive carriers but actively contribute to drug delivery systems by providing controlled and sustained release, increasing bioavailability, and protecting drugs from photodegradation and premature degradation. For example, montmorillonite-based nanohybrids have demonstrated improved solubility and permeability for poorly soluble drugs, and their use reduces toxicity by modulating drug release profiles (Nomicisio et al., 2023). Clay minerals also serve as excipients in pharmaceutical formulations, enhancing rheological properties, acting as emulsifying and thickening agents, and improving organoleptic characteristics such as taste and texture.

Moreover, clay minerals are biocompatible, non-toxic, and abundant, making them suitable for diverse pharmaceutical applications beyond oral delivery. They are used in topical and transdermal formulations to enhance skin permeation and controlled systemic drug delivery, and their bioactive surfaces can promote tissue repair and antimicrobial activity (Dk et al., 2020; Ruiz-Hitzky et al., 2019). The combination of clay minerals with polymers produces nanocomposites that improve mechanical and thermal stability of drug delivery matrices, further enhancing their performance in biomedical applications (Nomicisio et al., 2023).

Comprehensive characterization employing techniques such as X-ray diffraction (XRD) for mineral phase identification, Fourier-transform infrared spectroscopy (FTIR) for functional group analysis, X-ray fluorescence (XRF) for elemental composition, and thermal methods like thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) is essential. These analyses elucidate clay purity, structure, chemical nature, thermal stability, and cation exchange capacity, all critical parameters for optimizing their role in drug formulation and ensuring safety and efficacy (Dk et al., 2020; Nomicisio et al., 2023).

For natural materials such as Saturnia thermal mud, which have a complex mineralogical and organic composition influenced by geothermal processes, such meticulous characterization is even more important. It validates their therapeutic potential, supports pharmaceutical applications, and helps tailor formulations with predictable performance and stability (Dk et al., 2020).

In conclusion, clay mineral characterization stands at the core of pharmaceutical science research involving clays, providing the scientific basis to harness their advantages in drug delivery and therapy. Their multifunctionality, combined with rigorous physicochemical understanding, enables the design of innovative, efficient, and safe pharmaceutical formulations.

3.2. Methodology

3.2.1 Mineralogical and Chemical Composition Analysis

The mineralogical and chemical characteristics of Pharmaceutical-grade clays Berbek CEC (BC), Bentonita Benesa (BB) (from BENESA, Bentonitas Especiales company), Veegum® F (VF) (from Vanderbilt Ltd., USA), and Innogel LB (ILB) (from LB Minerals, Lasselberger group GmbH) and saturnia mud (taken from the themal bath area) were determined to verify sample identity, purity, and composition. X-ray diffraction (XRD) analysis was conducted using a Philips® X'Pert diffractometer (Philips, The Netherlands) equipped with an automatic slit (CuK α radiation, $\lambda=1.5418$ Å, 4–70° 2 θ range, scanning rate 6°/min, 40 kV). Analyses were performed on oriented aggregates (OA). Mineral quantification was based on peak area measurements from diffractograms, adjusted with chemical data using the XPOWER® program (Granada, Spain).

The infrared spectra of the samples were recorded in the mid-IR range (400–4000 cm⁻¹) using a Perkin Elmer 783 Fourier transform infrared spectrometer. Data processing was performed with Origin 6 software.

Major element composition was determined by X-ray fluorescence (XRF) using a Bruker® S4 Pioneer spectrometer (Bruker, Massachusetts, USA) with a Rh X-ray tube (60 kV, 150 mA).

3.2.2. Cation Exchange Capacity

CEC for clays was determined by dispersing BC, BB, VF, ILB clay powder in 25 mL of 1M tetramethylammonium bromide (TMAB) aqueous solution to displace exchangeable cations. The dispersions were shaken over-night at 50 rpm, filtered, and analyzed. TMAB, a quaternary ammonium salt with strong electron-withdrawing methyl groups, preferentially replaces cations by enhancing the positive charge on the nitrogen atom, facilitating bromide ion exchange with clay-associated cations and hydroxyl groups.

The concentrations of displaced cations in solution were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) using an Optima 8300 spectrometer (PerkinElmer, Waltham, USA). CEC was expressed as the sum of exchangeable cations (meq/100 g dry weight). A blank, consisting of TMAB solution without clay, were analyzed in parallel.

3.2.3. Thermal analysis of Clay suspensions and Saturnia Mud

Thermogravimetric (TG) and derivative thermogravimetric (DTG) analyses of studied (BC, BB, VF, ILB) clays and Saturnia mud were conducted using a Mettler Toledo TGA/DSC1 system (Greifensee, Switzerland) equipped with an FRS5 sensor and a microbalance

(precision 0.1 μg) in air atmosphere. Samples were heated from 25°C to 950°C at a constant rate of 10°C/min.

3.2.4. Characterization of Saturnia Thermal Water

The cationic composition of Saturnia thermal water was determined to evaluate its mineral content and ionic profile. Samples were collected in clean, acid-washed polyethylene bottles, filtered through 0.45 μm membranes to remove particulate matter, and stored at 4 °C until analysis. The concentrations of major cations, were quantified using ICP-OES PERKIN-ELMER OPTIMA 8300, following standard protocols. For each analysis, calibration curves were prepared using certified standard solutions of the respective cations. Measurements were performed in triplicate to ensure accuracy and reproducibility. The results were expressed in mg/L of each cation.

3.3. Results and Discussion

3.3.1. Mineralogical and Chemical Characterization of Clays

3.3.1.1. FTIR of Clays

The FTIR results for the four clays (ILB, BB, BC, VF) reveal characteristic spectral features that correlate with mineralogical composition and structure. Each clay exhibits diagnostic peaks in the infrared spectrum, primarily reflecting O–H, Si–O, and Al–OH functional groups, as well as variations in hydration and layer type (Figure 3.1). The main FTIR peaks for each clay are as follows:

(OH-Stretching 3600-3700 cm^{-1}): All samples show strong bands in the 3690–3610 cm^{-1} region, typical of O–H stretching in clay minerals, associated with structural hydroxyl groups. This region is key for distinguishing dioctahedral clays such as kaolinite, illite, and montmorillonite (Caccamo et al., 2020; Ritz et al., 2011). (Water bending 1630-1640 cm^{-1}): Peaks near 1631–1636 cm^{-1} are attributable to H–O–H bending vibrations of adsorbed or interlayer water, a hallmark of hydrated clays. (Si–O stretching 950-1000 cm^{-1}): The bands at 963–984 cm^{-1} are due to Si–O stretching vibrations, significant for identifying silicate frameworks; (Al–OH 911-983 cm^{-1}). Peaks in the 910–983 cm^{-1} region correspond to Al–OH bending, indicative of octahedral coordination and useful for distinguishing montmorillonite and kaolinite. (Low wavenumber peak 400-700 cm^{-1}): The peaks at 401, 438, 441, 450, 505, 510, 517, 652, 655, 688, 697, 793, 797, 875 cm^{-1} are assigned to Si–O, Al–O and coupled vibrations characteristic of clay minerals and minor quartz or feldspar impurities.

Comparative analysis:

Hydroxy Signature: All four clays show peaks in the O–H stretching region, confirming high structural order and the presence of water/structural hydroxyls.

Water Content: The 1630–1640 cm^{-1} band intensity correlates with bound or interlayer water.

Mineralogical Implications: The presence and intensity of Si–O, Al–OH, and O–H bands reflect differences in the clay type:

ILB: Rich suite with intense Si–O and lower-wavenumber features points to a complex structure—possibly montmorillonite or mixed-layer clays.

BB and VF: Fewer, less intense lower-wavenumber peaks may imply purer or less hydrated forms.

BC: High intensity at 401 cm^{-1} and rich in lower wavenumber features—potentially more crystalline or with minor oxide/feldspar contributions.

The FTIR spectra of ILB, BB, BC, and VF show classic bands for clay minerals, with variations reflecting different hydration states and structures. These assignments are supported by the position and intensity of O–H, Si–O, and Al–OH peaks, as well as low-wavenumber fingerprint features.

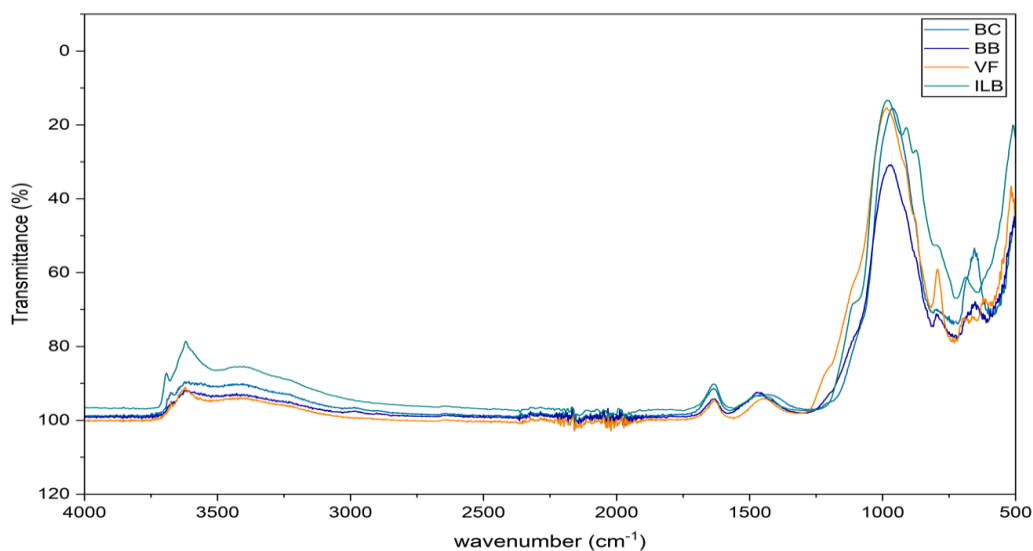


Figure 3.1: FTIR spectra of the four clay samples (BC, BB, VF, and ILB) showing characteristic absorption bands of aluminosilicate minerals in the range $4000\text{--}500\text{ cm}^{-1}$.

3.3.1.2.XRD of Clays

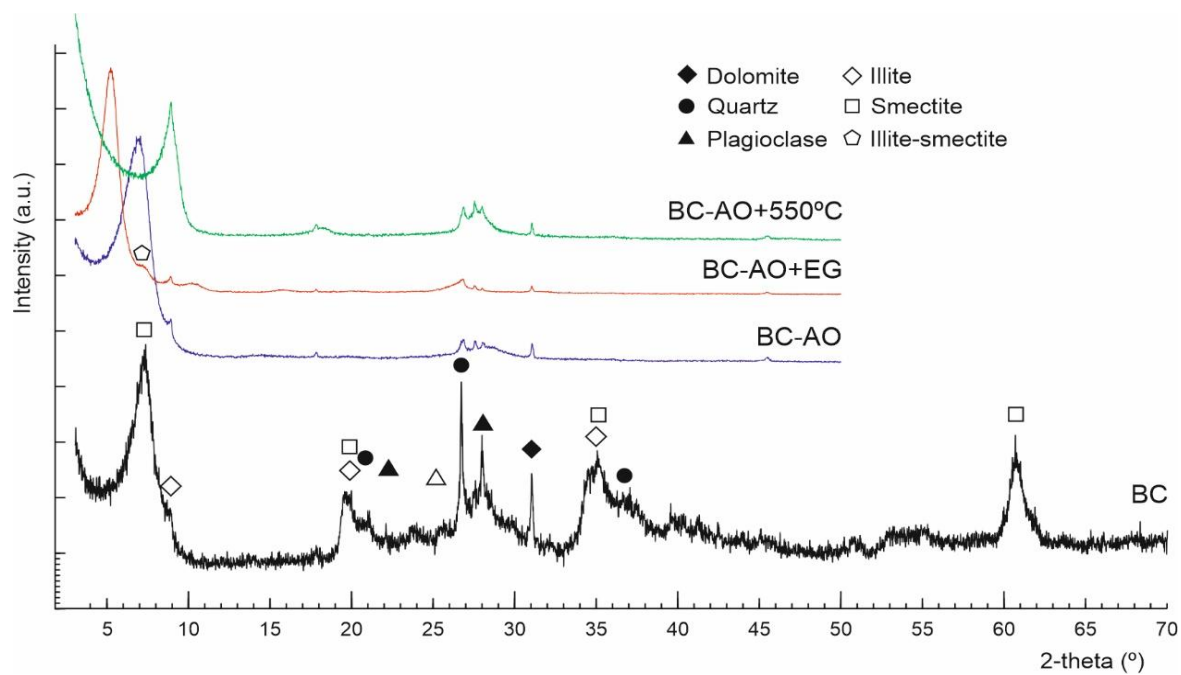
The XRD patterns of BC (bulk, OA, and AO+EG (ethyleneglycol) preparations) show that the sample is dominated by **smectite**, evidenced by an intense (001) basal reflection near ($7^\circ 2\theta$ units) ($d(001) = 12\text{--}15\text{ \AA}$) in the oriented mount that expands upon glycolation with EG ($5.5^\circ 2\theta$ units) ($d(001) = 16\text{ \AA}$). This expansion disappears after heating at 550°C eliminating all EG and water intercalated (Figure 3.2a). This confirms the presence of a highly swelling smectite. Accessory minerals include **quartz**, **plagioclase**, **dolomite**, and minor **illite** indicated by their characteristic mid- and high-angle peaks. The combination of a strong expandable basal spacing and the response to EG treatment confirms BC as a **smectite-rich**

bentonitic clay with significant swelling potential. The mineral phase analysis confirm smectite 75-80% and Illite 5-10% and quartz 4-6% (Table 3.1).

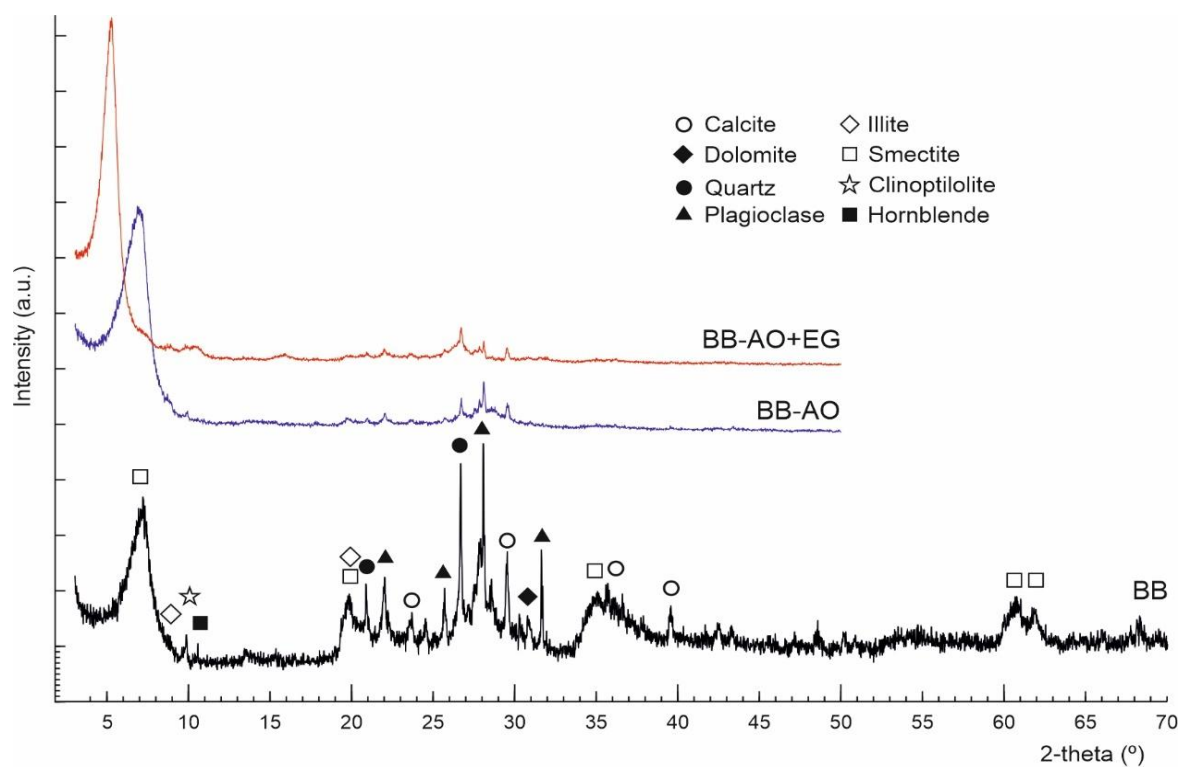
The BB XRD diffractogram (Figure 3.2-b) shows a combination of smectite and illite, with reflections at ($7.0-7.5^\circ$ and 8.8° 2θ units) with moderate $d(001) = 14-12 \text{ \AA}$ and $10 \text{ \AA}$ features indicating a mixed-layer or mixed-mineral assemblage. The smectite reflection partially expands with EG treatment, confirming the presence of swelling layers, although the expansion is weaker than in BC. BB contains substantial quartz and cristobalite, reflected in strong reflections near $26-27^\circ$ 2θ and $21-22^\circ$ 2θ . Minor feldspar is also detected. The elevated silica content and mixed-layer structure suggest limited swelling capacity compared to the more smectitic materials. In general, the BB comprises of 60-65% smectite and <5% illite with 10-15% quartz (Table 3.1).

The XRD pattern of the VF sample shows a basal reflection near $10 \text{ \AA}$ characteristic of illite, accompanied by a broader, weaker smectite-related peak around $12-15 \text{ \AA}$ with possibly different interlayer cations. The diffractogram also includes strong quartz and cristobalite reflections, confirming a high silica content. Overall, the mineralogical profile of VF reflects a low-swelling clay dominated by illite and silica phases, with limited contribution from expandable smectite (Figure 3.3-c).

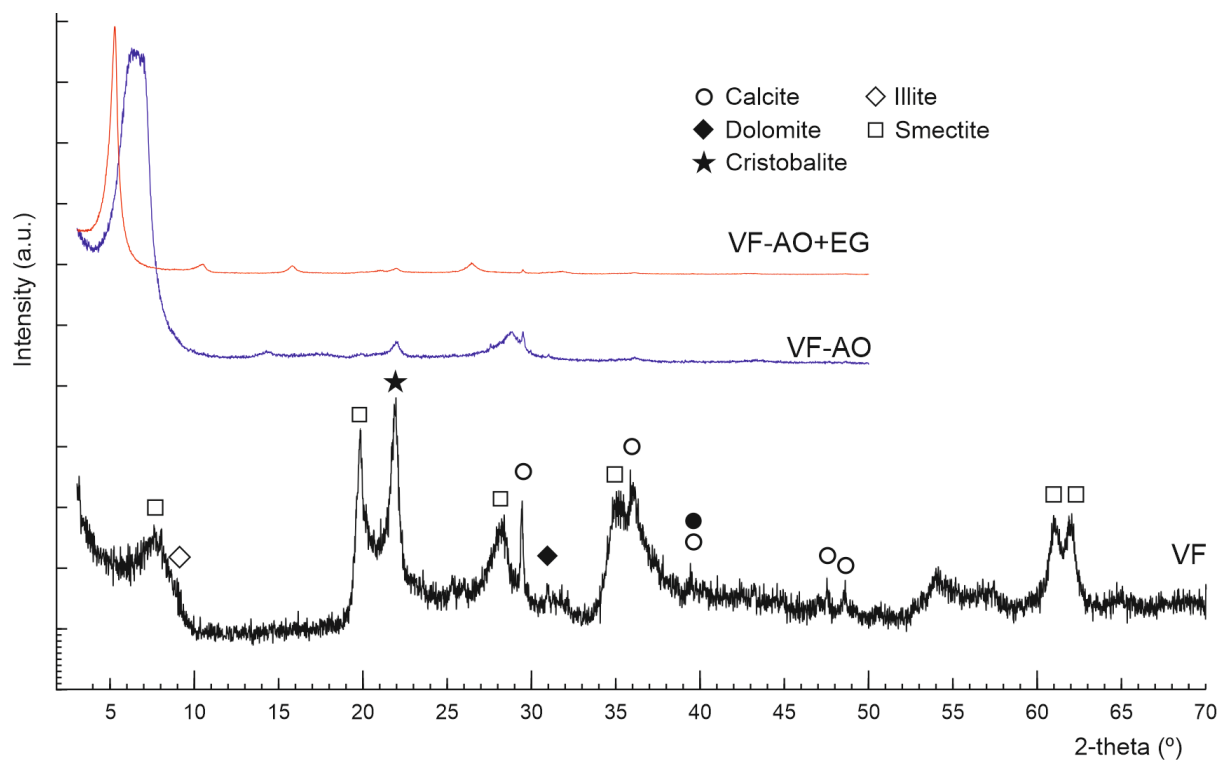
The ILB sample displays XRD features typical of high-purity smectite, with a well-defined basal reflection around $13-15 \text{ \AA}$ in the bulk sample and a clear expansion to larger spacing after EG treatment. The oriented mounts sharpen the basal reflection, indicating good layer stacking and high smectite crystallinity. The presence of quartz and minor feldspar is observed but in lower proportion compared to BC (Table 3.1). Very weak illite reflections may appear but the sample is overwhelmingly dominated by swelling smectite, consistent with a material corresponding to pharmaceutical-grade bentonite. The strong and clean smectite signature indicates excellent swelling and adsorption capability.



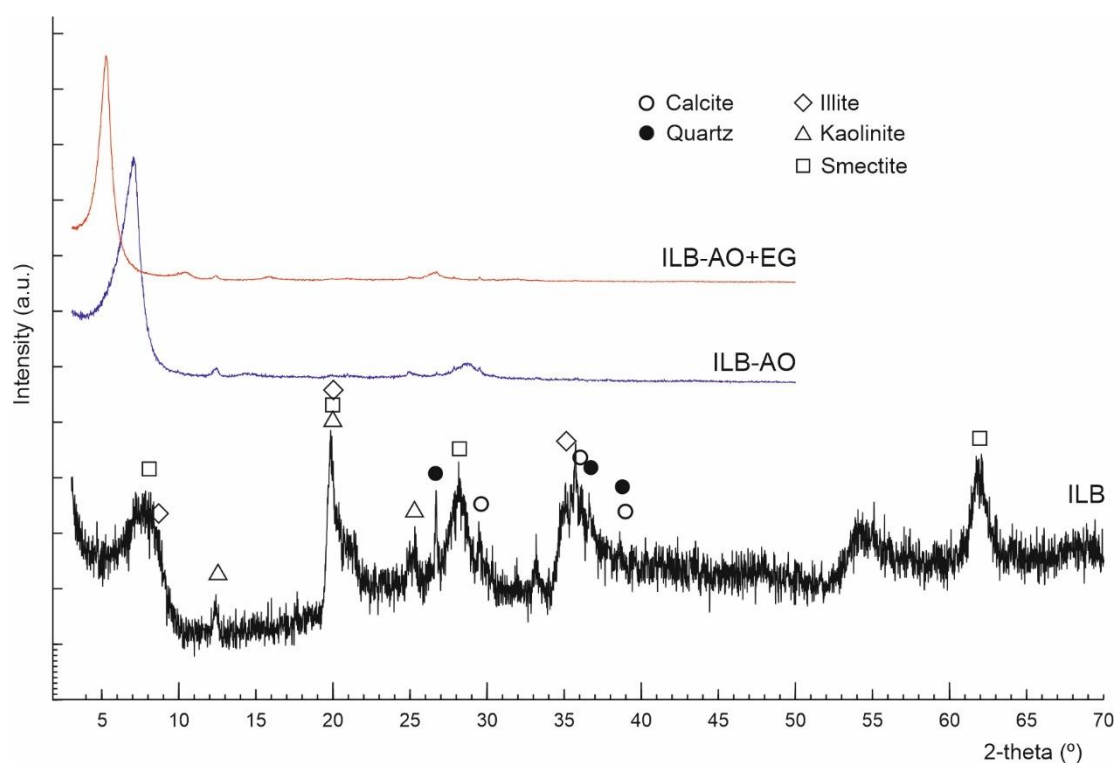
(a)



(b)



(c)



(d)

Figure 3.2: X-ray diffraction (XRD) patterns of the four clay samples (BC, BB, VF, ILB) showing the identified mineral phases. Major reflections correspond to phyllosilicates (smectite/illite), tectosilicates (quartz, feldspars), and carbonates, as labeled on each diffractogram BC(a) BB (b) VF (c) ILB (d)

Table 3.1. XRD-identified mineral phases in clay-rich units

	BC	BB	ILB	VF
Smectites	75-80%	60-65%	80-85%	60-65%
Illite	5-10%	<5%	tr	<5%
Kaolinite	-	-	10-15%	-
Quartz	4-6%	10-15%	<5%	-
Cristobalite	-	-	-	30
Calcite	-	5-7%	<5%	<5%
Dolomite	<5%	trace	-	trace
Plagioclase	5-10%	10-15%	-	-
Clinoptilolite	-	<5%	-	-
Hornblende	-	trace	-	-
Illite-Smectite	<5%			

3.3.1.3.XRF analysis of Clays:

The major and trace element composition of clay samples (ILB, VF, BC, BB), determined by X-ray fluorescence (XRF), reveals significant variations indicative of differing geochemical characteristics and potential mineralogical influences (Table 3.2).

Silica (SiO₂) is the dominant component in all samples, ranging from 49.04% (ILB) to 61.74% (VF), reflecting a predominantly siliceous composition typical of volcanic-derived sediments. The elevated SiO₂ content in VF suggests a more felsic character, while lower SiO₂ in BC corresponds with increased mafic components.

Alumina (Al₂O₃) content is highest in ILB (17.20%), consistent with enrichment in clay minerals, which may contribute to plasticity and cation exchange properties. In contrast, BC has the lowest Al₂O₃ (6.44%) and the highest MgO (21.22%), indicating a more mafic influence, likely from the weathering of Mg-rich silicate minerals.

Iron oxides (Fe₂O₃) are notably elevated in ILB (13.55%), suggesting the presence of iron-bearing minerals such as magnetite or hematite. Conversely, VF, BC, and BB contain significantly lower Fe₂O₃ (<3.5%), highlighting compositional heterogeneity among the samples. Calcium oxide (CaO) varies from 1.26% to 3.78%, with BB being enriched, potentially reflecting minor carbonate contributions.

The alkali oxides, Na₂O and K₂O, are relatively low but consistent with the presence of feldspar or clay mineral phases. Titanium oxide (TiO₂), although minor (0.19–1.55%), along

with trace elements such as Zr, Sr, Cr, Ni, and V, provides insight into provenance and mineral associations. For instance, VF shows high Sr (2606 ppm), likely derived from hydrothermal or volcanic sources, whereas ILB is enriched in Zr and V, indicating accessory zircon and iron-titanium oxide phases.

Loss on ignition (LOI) ranges from 9.26% to 12.12%, reflecting volatile components, including water in clay minerals, organic matter, and minor carbonates. Higher LOI in BC suggests a greater proportion of hydrated or thermally unstable phases.

Overall, the XRF results indicate that ILB and VF are relatively felsic and siliceous, BC is mafic and Mg-rich, and BB represents an intermediate composition. These geochemical variations are consistent with the heterogeneous nature of volcanic-sedimentary mud deposits in the Saturnia area. The observed major and trace element compositions are relevant for potential industrial, cosmetic, and therapeutic applications, as they influence plasticity, mineral release, and thermal behavior.

Table 3.2: Major and trace element composition of Clay samples determined by X-ray fluorescence (XRF). Major oxides are reported in weight percent (wt.%), trace elements in parts per million (ppm), and LOI in weight percent (%).

Sample	Na2O (%)	MgO (%)	Al2O3 (%)	SiO2 (%)	P2O5 (%)	K2O (%)	CaO (%)	TiO2 (%)	MnO (%)	Fe2O3 (%)	Zr (PPM)	Sr (PPM)	Cr (PPM)	Ni (PPM)	V(PPM)	LOI (%)
ILB	2,71	2,26	17,20	49,04	0,14	0,20	1,68	1,55	0,11	13,55	173	242	99	117	362	9,96
VF	2,01	10,97	9,98	61,74	0,02	0,34	2,37	0,19	0,02	1,51	75	2606	23	4	17	9,26
BC	2,37	21,22	6,44	51,15	0,11	1,35	1,26	0,33	0,04	2,09	111	119	42	12	45	12,12
BB	2,57	10,90	11,17	53,93	0,10	1,31	3,78	0,38	0,04	3,16	148	185	39	7	80	10,04

3.3.2. Cation Exchange Capacity

Among the analyzed samples, VF and ILB clays exhibited the highest concentrations of exchangeable cations, particularly Ca^{2+} and Na^{+} , indicating a comparatively greater cation exchange capacity (Table 3.3). In the VF sample, Ca^{2+} and Na^{+} reached 13.08 meq/l and 18.66 meq/l, respectively, while ILB showed similarly high values (11.16 meq/l Ca^{2+} and 20.18 meq/l Na^{+}). These results suggest that both clays possess abundant negatively charged sites, likely due to their layered silicate structures and higher content of expandable minerals such as smectite 60-65% and 80-85% in VF and ILB respectively.

The BB clay displayed intermediate CEC characteristics, with notably high Na^{+} (18.7 meq/l) but lower Ca^{2+} and Mg^{2+} compared to VF and ILB. This suggests that sodium is the dominant exchangeable cation in BB, possibly reflecting the natural ionic composition of its interlayer spaces.

In contrast, the BC clay showed the lowest overall concentrations of exchangeable cations, with Ca^{2+} at 1.606 meq/l and Na^{+} at 0.10 meq/l. This indicates a relatively low cation exchange capacity, which may be associated with a higher proportion of non-expandable minerals (e.g., 5% illite smectite in BC (Figure 3.2a) or reduced surface charge density. Overall, the results show that the CEC of the studied clays follows the approximate order:

VF $\approx$ ILB > BB > BC.

This trend reflects differences in mineral composition, layer charge, and surface area among the samples. The higher CEC values observed for VF and ILB are consistent with the presence of minerals capable of accommodating a greater number of exchangeable cations, which enhances their potential use in adsorption or ion-exchange applications.

Table 3.3 Cation Exchange Capacity of four Clays (BB,BC,VF,ILB)

Sample	Ca	K	Mg	Na	Total CEC
	meq/g	meq/g	meq/g	meq/g	meq/g
TMAB			0,00125	0,0005	0,00175
BC-CONTROL		0,000305	0,02175	0,0075	0,029555
BC	0,04015	0	0,02425	0,0025	0,0669
BB-CONTROL			0,002	0,0025	0,0045
BB	0,01175	0,0045	0,0845	0,4675	0,56825
VF-CONTROL	0,0085125	0,004325	0,11775	0,33455	0,4651375
VF	0,327	0,0136	0,1835	0,4665	0,9906
ILB-CONTROL	0,01072	0,00406	0,1445	0,4975	0,65678
ILB	0,279125	0,01135	0,102	0,50465	0,897125

3.3.3. Thermal Characterization of Clays:

3.3.3.1 Thermogravimetric Analysis (TGA/DTG)

Thermal characterization of the four clay samples (BC, BB, VF, and ILB) was performed by thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG). The DTG curves were plotted as $-d(m/m_0)/dT$ versus temperature (Figure 3.3, 3,4). They highlight the temperatures at which mass loss rates are maximal and thus facilitate identification of dehydration, dehydroxylation, and decomposition events.

All samples exhibit an initial thermal event below ~ 150 °C, attributable to loss of adsorbed and interlayer water. This low-temperature dehydration is present in order of $BC > ILB > BB > VF$ with % mass loss of -6,0903, -4,9977, -4,8615, -4,1822 respectively (Table 3.4).

All four clay samples exhibit a characteristic two-step dehydroxylation pattern in the high-temperature region of the TGA curve, reflecting the presence of hydroxyl groups with different bonding environments in the clay structure.

The first dehydroxylation event, observed between 450–550 °C, corresponds to the removal of edge-site or weakly bonded hydroxyl groups. In this region, the clays show relatively small mass losses: BC (1.683 %), BB (1.5185 %), VF (1.0236 %), and ILB (1.4754 %). These values indicate that VF possesses the fewest weakly bonded hydroxyls, while BC contains the highest amount of such accessible –OH groups.

The second dehydroxylation step, appearing at higher temperatures between 650–750 °C, is associated with the release of structural hydroxyl groups from the octahedral sheets of the clay lattice. This step requires higher thermal energy due to the stronger bonding of structural –OH. In this region, the mass losses increase for all clays except ILB, with values

of BC (2.1997 %), BB (2.4217 %), VF (2.467 %), and ILB (0.3456 %). VF shows the largest structural dehydroxylation loss, suggesting a higher proportion of strongly bonded octahedral hydroxyls, whereas ILB exhibits only a minor loss, indicating a significantly lower structural –OH content or a more thermally stable framework.

Overall, these differences reflect variations in mineral composition and crystallinity among the clay samples, with ILB demonstrating the most pronounced structural transformation in this temperature region. At higher temperatures (roughly 700–850 °C), VF (1,1190%), BC (2.0937%) and BB (0,9951%) show pronounced mass-loss peaks consistent with carbonate decomposition (e.g., calcite/dolomite) and/or structural collapse and recrystallization of dehydroxylated aluminosilicates into spinel/mullite-type phases. The strong high-temperature features in VF, BC and BB imply the presence of carbonate impurities or thermally labile components in those samples. In contrast, ILB displays less pronounced high-temperature decomposition, indicating a comparatively purer or more thermally stable mineral composition.

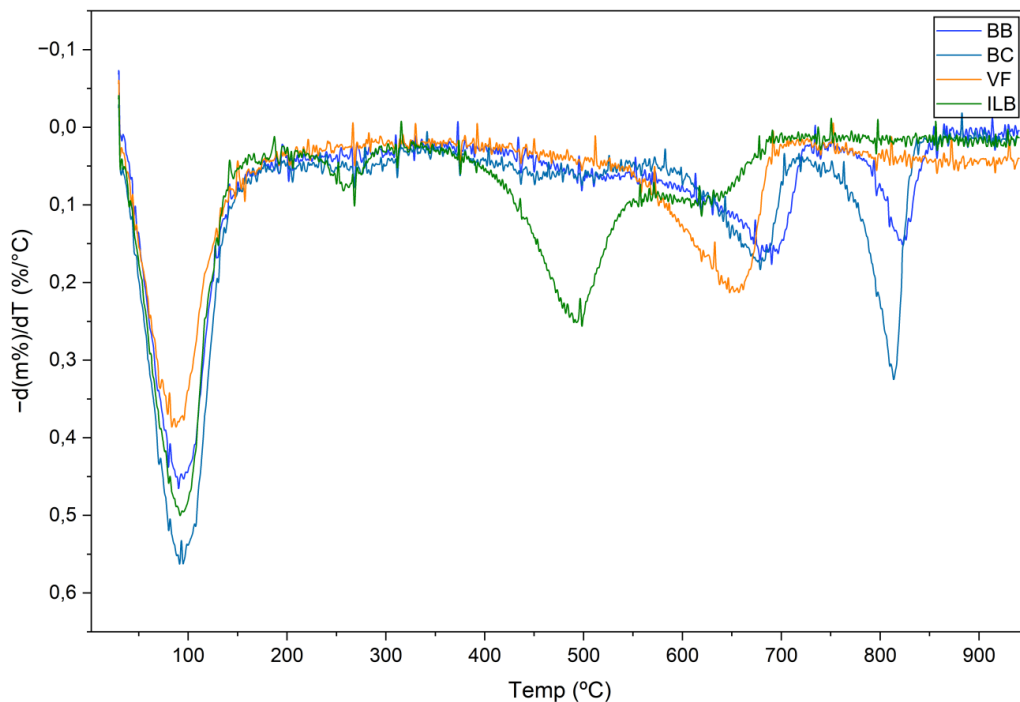


Figure 3.3: Differential thermogravimetric (DTG) curves of the clay samples (BC, BB, VF, and ILB) showing the rate of mass loss

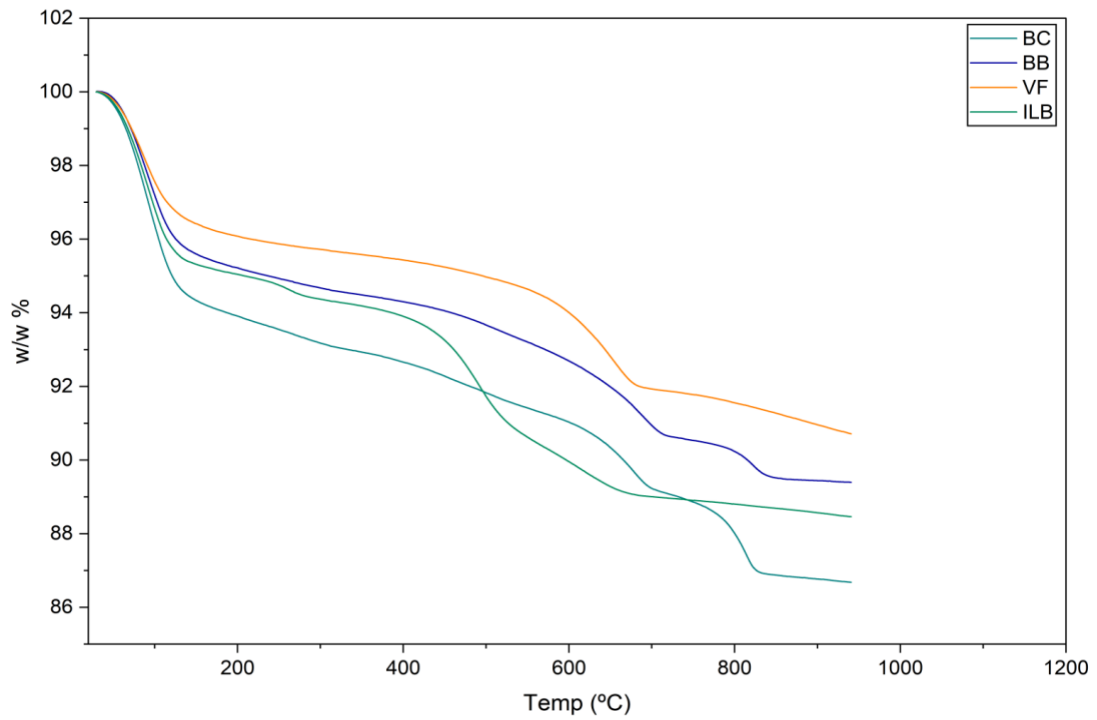


Figure 3.4: TGA of clays BC, BB, VF, ILB

Table 3.4: Weight loss % of Clay suspensions at different temperature range

Temperature range °C	Weight loss %				Description
	BC	BB	VF	ILB	
30-150	6,0908	4,8615	4,1822	4,99 8	Surface water
150-300	13,319 9	10,601 7	9,2807	11,5 4	Tightly bound water
300-350	1,059	0,71		3,88	Organic substances decomposition
450-550	1,683	1,5185	1,0236	1,48	dehydroxylation
650-750	2,1997	2,4217	2,9676		
800-850	2,0937	0,995		0,35	
900	0,1925	0,0007 8	1,119		dry ash

3.3.3.2 Differential Scanning Calorimetry (DSC):

Differential Scanning Calorimetry (DSC) analysis was performed to evaluate the thermal behavior of the four clay samples — BC, BB, VF, and ILB — over the temperature range of approximately 25°C to 950 °C. The DSC curves (Figure 3.5) reveal distinct endothermic and exothermic transitions associated with dehydration, dehydroxylation, and structural changes within the clay minerals.

At lower temperatures (below 200 °C), all samples exhibit a slight endothermic deviation corresponding to the loss of adsorbed and interlayer water. This effect is minor, indicating relatively low moisture content or weakly bound surface water. As the temperature increases to around 200–400 °C, moderate endothermic peaks are observed, which can be attributed to the removal of physically adsorbed water and initial dehydroxylation processes of the clay structure. Between 400 °C and 700 °C, the DSC profiles display prominent exothermic reactions, representing the major dehydroxylation of the clay lattice and the breakdown of hydroxyl groups in the octahedral layers. Among the samples, BB and BC exhibit higher exothermic intensities in this region, suggesting a greater degree of structural hydroxylation or a more thermally stable lattice compared to VF and ILB. The ILB sample shows a broader transition, indicative of overlapping processes or compositional heterogeneity. At higher temperatures (700–1000 °C), all samples undergo strong endothermic events, which are associated with the crystallization of new mineral phases such as mullite ($(3\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2)$ in case of kaolinite) or spinel-type structures (a $\gamma\text{-Al}_2\text{O}_3\text{--SiO}_2$ phase) in case of montmorillonite clay). Notably, VF and ILB exhibit more pronounced exothermic peaks, implying higher reactivity or greater structural rearrangement upon heating. In contrast, BB and BC show slightly delayed and less intense transitions, which may reflect differences in mineral composition (e.g., kaolinite vs. smectite dominance) or impurities influencing the transformation temperature.

Overall, the DSC results demonstrate that each clay sample possesses distinct thermal behavior, reflecting variations in mineralogy and structural water content. The sequence of thermal stability inferred from the DSC profiles follows the order $\text{BB} > \text{BC} > \text{ILB} > \text{VF}$, indicating that BB and BC are more thermally resilient, while VF and ILB undergo more extensive transformations at elevated temperatures.

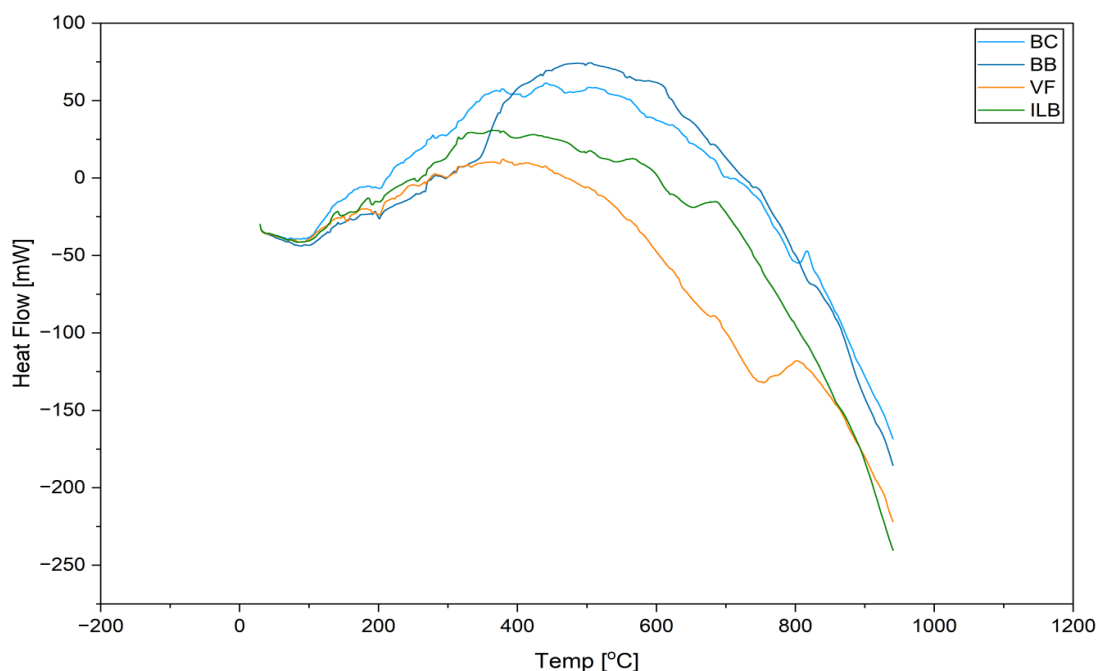


Figure 3.5 : DSC thermograms of BC, BB, VF, and ILB clays illustrating characteristic thermal transitions during heating from 0 to 1000 °C.

3.3.4. Mineralogical and Chemical Characterization of Saturnia Mud (SM)

3.3.4.1. FTIR of SM

The FTIR spectrum of Saturnia thermal mud provides valuable insights into its mineralogical and chemical composition (Figure 3.6).

The broad band observed between 3600–3400 cm^{-1} is attributed to the stretching vibrations of -OH groups, typically associated with both structural hydroxyls in clay minerals and adsorbed water molecules. The peak near 1630–1650 cm^{-1} is indicative of bending vibrations from water molecules, further confirming the hydrated nature of the sample. This feature is common in smectite and montmorillonite clays, which have high water content due to their expansive interlayer spaces. The broad peak at 1400 cm^{-1} corresponds to carbonate groups. In the region around 1100–1000 cm^{-1} , strong absorption bands are observed, characteristic of Si–O stretching vibrations. Such bands are typical markers of silicate frameworks in clay minerals like montmorillonite, illite, or kaolinite. A distinct doublet or shoulders in this region may point to a mixture of layered silicates with some structural variety or minor quartz admixture, which is also present in natural thermal muds. The absorption seen near 800–500 cm^{-1} may correspond to Al–O bending vibrations, confirming the presence of aluminosilicate minerals predominant in muds and natural clays.

These spectral features together point to a composition dominated by hydrated aluminosilicate minerals with minimal organic or carbonate impurities. Such a mineralogical

profile can be useful for pharmaceutical use, conferring adsorption potential, and stability to Saturnia thermal mud, as demanded for advanced drug delivery and topical formulations (Nomicisio et al., 2023; Ruiz-Hitzky et al., 2019).

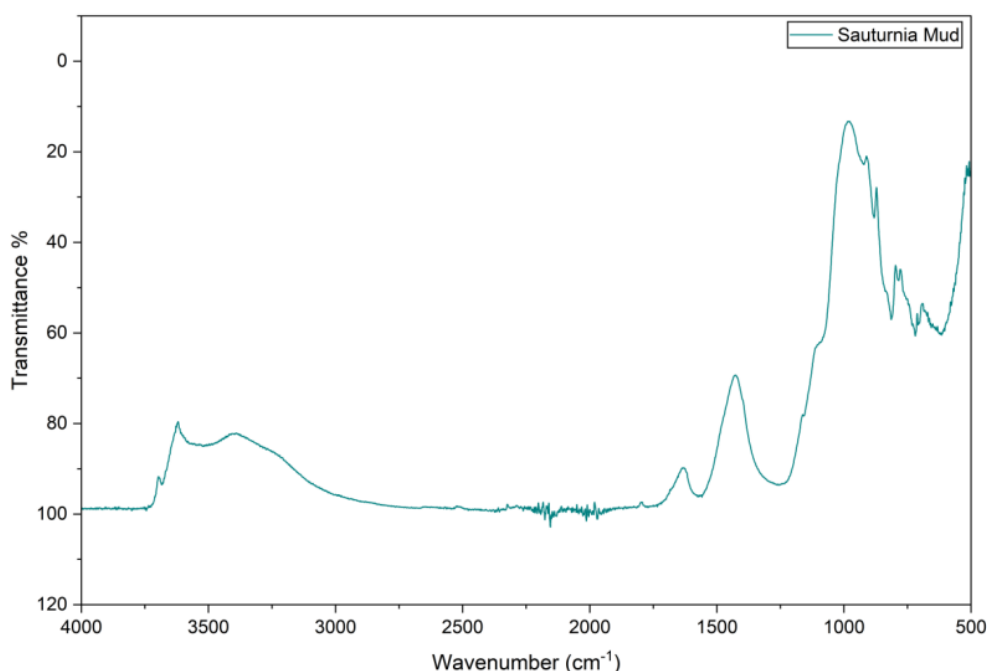


Figure 3.6. FTIR Spectra of Saturnia Mud

3.3.4.2. XRD of SM

The diffractogram of raw Saturnia mud reveals a complex mineral composition, predominantly composed of quartz (SiO_2) and calcite (CaCO_3), as indicated by the intense reflections around $26\text{--}27^\circ$ and $29\text{--}30^\circ$ 2θ , respectively. The content of quartz present in saturnia mud make it less favorable for pharmaceutical application in raw form. It need pretreatment of purification before using for preparation of any formulation for therapeutic or dermo cosmetic purposes. Minor reflections corresponding to plagioclase, illite, kaolinite, and smectite were also detected at lower intensities. The presence of smectite suggests that the mud possesses a potential swelling behaviour, which are desirable properties in therapeutic and cosmetic applications. The coexistence of both crystalline (quartz, calcite) and clay minerals (smectite, kaolinite, illite) indicates that Saturnia mud is a heterogeneous natural clay, combining structural rigidity with surface activity.

Effect of AO Treatment (Saturnia-AO):

The Saturnia-AO sample exhibits a slight reduction in the intensity of peaks associated with clay minerals, while the quartz and calcite peaks remain prominent (Figure 3.7). The partial diminution of kaolinite and smectite peaks suggests that the AO treatment partially modifies the clay lattice with a $d(001)$ spacing $12\text{--}14 \text{ \AA}$ due to the presence of Ca^{2+} in the interlayer space, likely causing minor structural disorder or reduction in crystallinity. Such changes can

enhance the surface reactivity of the clay while maintaining the mechanical stability provided by quartz and calcite.

Effect of AO+EG Treatment (Saturnia-AO+EG):

The Saturnia-AO+EG diffractogram (Figure 3.7) shows further reduction and broadening of the clay mineral reflections, indicating a significant decrease in crystallinity. A slight increase of the d(001) reflection to 16 Å can be observed. The persistence of quartz peaks suggests that crystalline silica remains largely unaffected by the treatment, while the clay fraction becomes more amorphous. This increase in amorphous content can improve adsorption capacity, surface area, and reactivity, which are advantageous for applications such as detoxifying facial masks, thermal mud therapies, and environmental adsorption processes. The combined effect of AO+EG treatment likely disrupts the clay lattice, enhancing the functional properties of the mud without compromising the structural framework provided by quartz and calcite.

Overall Implications:

The XRD results demonstrate that Saturnia mud is naturally composed of both crystalline and clay minerals, which provide a balance between mechanical stability and therapeutic functionality. Processing treatments, particularly AO+EG, modify the crystallinity of the clay fraction, potentially improving its physicochemical and functional properties. The high concentration of quartz 15-20% present in saturnia mud impose an implication to be treat it before using for pharmaceutical formulations. After removal of these impurities it can be consistent with the desired performance of muds in cosmetic and balneological applications, where a higher amorphous fraction can enhance skin adherence, ion exchange, and water retention.

In conclusion, XRD analysis confirms that the mineralogical composition and structural characteristics of Saturnia mud can be tuned through controlled treatments, providing a scientific basis for its use in health, wellness, and industrial applications.

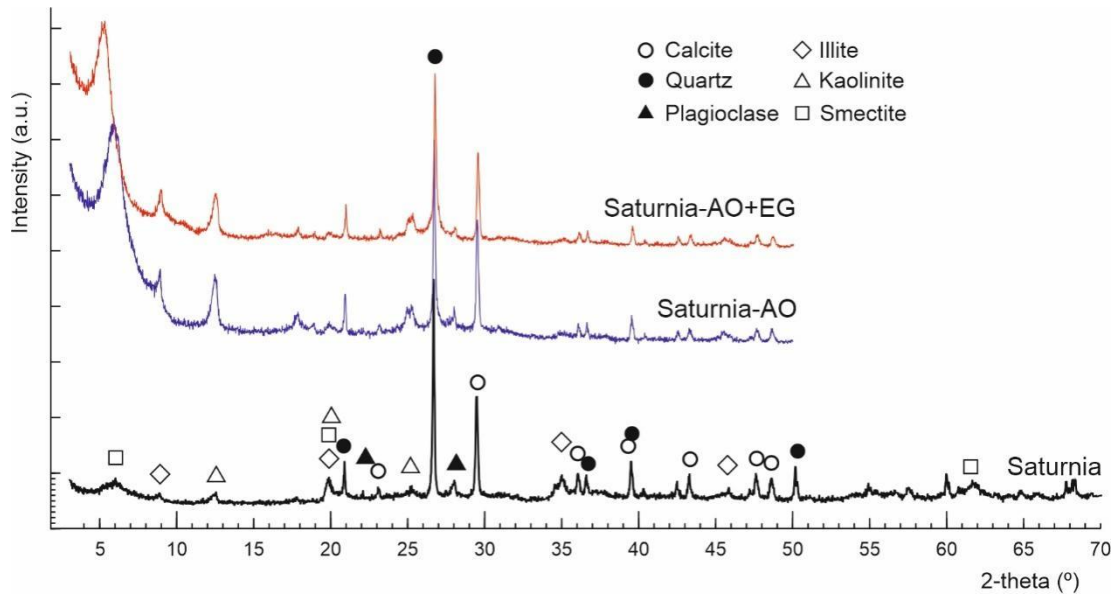


Figure 3.7: X-ray diffraction (XRD) patterns of the Saturnia mud showing the identified mineral phases. Major reflections correspond to phyllosilicates (smectite/illite), tectosilicates (quartz), and carbonates, as labeled on diffractogram.

Table:3.5: XRD-identified mineral phases in Saturnia mud

Mineral Phase	percentage
Smectites	25-30%
Illite	15-20%
Kaolinite	10-15%
Quartz	15-20%
Cristobalite	-
Calcite	15-17%
Dolomite	-
Plagioclase	< 5%
Clinoptilolite	-
Hornblende	-
Illite-Smectite	

3.3.4.3. XRF of SM

The XRF analysis of the Saturnia mud provides insight into its major and trace element composition, which reflects its geochemical character and potential mineralogical associations (Table 3.6).

Major oxides indicate that the mud is predominantly siliceous and aluminous, with SiO₂ at 51.21% and Al₂O₃ at 14.83%. This composition suggests the presence of clay minerals and silicate frameworks, which contribute to the mud's plasticity and water retention capacity. CaO is relatively high (10.41%), implying minor carbonate or feldspathic inputs, while MgO (2.73%) and Fe₂O₃ (6.78%) suggest contributions from mafic silicates and iron-bearing minerals such as magnetite or hematite. Alkali oxides are moderate, with K₂O at 2.39% and Na₂O at 0.49%, reflecting minor feldspar or clay mineral phases. TiO₂ (0.82%) and MnO (0.19%) are minor components, likely associated with accessory minerals. P₂O₅ is very low (0.14%), indicating negligible phosphate mineral content.

Trace elements are enriched in several metals, reflecting both the volcanic-sedimentary origin of the mud and potential hydrothermal influences. Notably, Sr (1,081.67 ppm) and Ba (433.67 ppm) are enriched, suggesting contributions from carbonate or strontium-bearing phases. Transition metals including V (166 ppm), Cr (155.33 ppm), Ni (86.33 ppm), Cu (77.33 ppm), and Co (33.67 ppm) likely occur in mafic silicates or iron-titanium oxide minerals. Zr (459 ppm) and Y (75.67 ppm) indicate the presence of zircon or other refractory accessory phases. Other trace elements such as Rb (277.67 ppm), Nb (51.67 ppm), Pb (50 ppm), and Th (33.67 ppm) provide additional geochemical signatures that may be useful for provenance studies or environmental assessments. The absence of detectable Rh suggests that this element is not a component of the mud or is below the detection limit of the XRF analysis.

Overall, the Saturnia mud exhibits a siliceous-aluminous composition with moderate Mg, Ca, and Fe contributions, enriched in several trace metals. These characteristics are consistent with a volcanic-sedimentary origin and indicate potential utility in cosmetic, therapeutic, and industrial applications, where plasticity, mineral release, and trace element content are relevant.

Table 3.6. Major and trace element composition of Saturnia mud determined by X-ray fluorescence (XRF). Major oxides are reported in weight percent (wt.%), trace elements in parts per million (ppm).

Elements	Unit	Concentration
V	ppm	166,00
Cr	ppm	155,33
Co	ppm	33,67
Ni	ppm	86,33
Cu	ppm	77,33
Zn	ppm	240,00
Ga	ppm	28,67
Rb	ppm	277,67
Sr	ppm	1081,67
Y	ppm	75,67
Zr	ppm	459,00

Nb	ppm	51,67
Rh	ppm	-
Ba	ppm	433,67
Pb	ppm	50,00
Th	ppm	33,67
Na ₂ O	%	0,49
MgO	%	2,73
Al ₂ O ₃	%	14,83
SiO ₂	%	51,21
K ₂ O	%	2,39
CaO	%	10,41
TiO ₂	%	0,82
MnO	%	0,19
Fe ₂ O ₃	%	6,78

3.3.5. Thermal Characterization of Saturnia Mud (SM)

3.3.5.1 Thermogravimetric Analysis (TGA/DTG) of SM

The TGA and DTG curves of the Saturnia mud collectively reveal a typical multistep thermal decomposition pattern characteristic of clay-rich natural muds (Figure 3.8 & 3.9). The TGA shows a steep initial mass loss from room temperature to approximately 150 °C, which is mirrored in the DTG by a strong negative peak in the same temperature range. This event corresponds to the removal of physically adsorbed water and interlayer water associated with expandable clay minerals, indicating a high degree of hydration in the raw mud corresponding to 46,659% weight loss in this region (Table 3.7). Between about 150–400 °C, the TGA curve becomes relatively stable with only slow mass reduction, and the DTG signal remains nearly flat, suggesting that only small quantities of low-temperature volatiles or minor organic components are present with loss of only (0,6140%). A broader and more gradual mass decrease (2,0696%) observed between 400–700 °C, accompanied by a shallow DTG inflection, is attributed to the dehydroxylation of the clay minerals, involving the loss of structural hydroxyl groups from the lattice (Centini et al., 2015). At higher temperatures, particularly from ~700 to 900 °C, both curves show a small but distinct mass-loss step, corresponding to the decomposition of carbonate minerals—most likely calcite—resulting in CO₂ release. After this stage, the curves stabilize, indicating completion of the main thermal reactions. Overall, the combined TGA–DTG analysis confirms that Saturnia mud contains significant amounts of bound and structural water, minor organics, dehydroxylating clay phases, and carbonate fraction.

Summarizing findings from Centini et al.(2015), Saturnia thermal muds are confirmed to be composed of hydrated clay minerals with minor organic and carbonate content, and such

compositional features are closely linked to their functional and therapeutic properties in spa and cosmetic applications. Therefore, TGA/DTG results align well with literature reported.

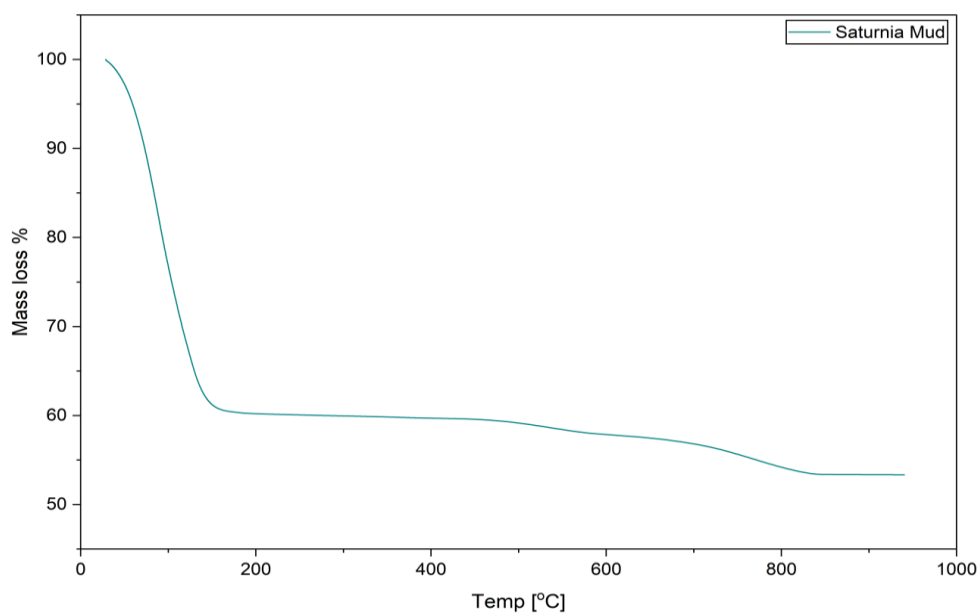


Figure 3.8: TGA of Saturnia Mud

Table 3.7: TGA weight loss in % for Saturnia Mud at different temperatures.

Temperature range °C	Weight loss %	Description
50-100	46,659	Total water
100-550	0,614	Organic substances decomposition
550-750	2,0696	dehydroxylation
750-900	4,1175	dry ash

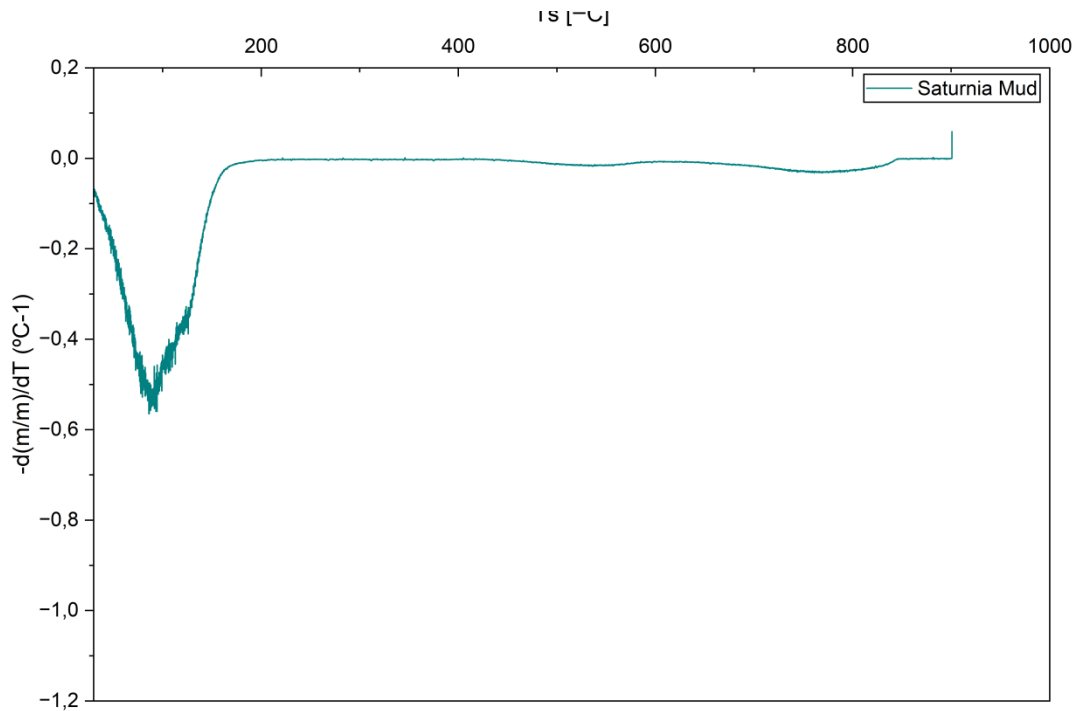


Figure 3.9 Derivative Thermogravimetric Analysis (DTG) of Saturnia Mud

3.3.5.2 Differential Scanning Calorimetry (DSC) of SM

The DSC results for Saturnia thermal mud show strong correlation with the TGA/DTG data, supporting a consistent interpretation of the mud's thermal behavior and composition. The prominent endothermic peak on the DSC curve up to about 150 °C directly aligns with the major mass loss observed on the TGA and a negative DTG peak in the same temperature range, all attributable to the evaporation of physically adsorbed and interlayer water present in hydrated clay minerals. This strong initial signal in both datasets confirms the predominance of swelling clay minerals such as smectite or montmorillonite, known for their hydration capacity (Centini et al., 2015).

The subsequent, broader DSC peak observed between ~400 and 700 °C correlates closely with the slow, continuous mass loss in the TGA/DTG profiles (Figure:3.10). This is attributable to the dehydroxylation of structural hydroxyl groups from the clay lattice, a thermal event characteristic of aluminosilicates and also noted in thermal analyses of similar spa and pharmaceutical muds.,and a partial decomposition of carbonates. At higher temperatures (beyond 700 °C), both DSC (shallow endothermic transition) and TGA/DTG data (minor mass loss) reflect the decomposition of carbonates, likely calcite, with evolution of CO₂ and a formation of new phases being consistent with published studies on Mediterranean and hydrothermal muds.

Overall, the close agreement across DSC, TGA, and DTG analyses affirms that the Saturnia mud is composed mainly of hydrated aluminosilicate clays with a small proportion of

carbonates and only minor organics, as seen in reference profiles documented for natural therapeutic muds. This robust, multi-technique thermochemical characterization underpins its pharmaceutical and cosmetic suitability by confirming a stable, moisture-rich clay matrix with predictable thermal stability (Centini et al., 2015).

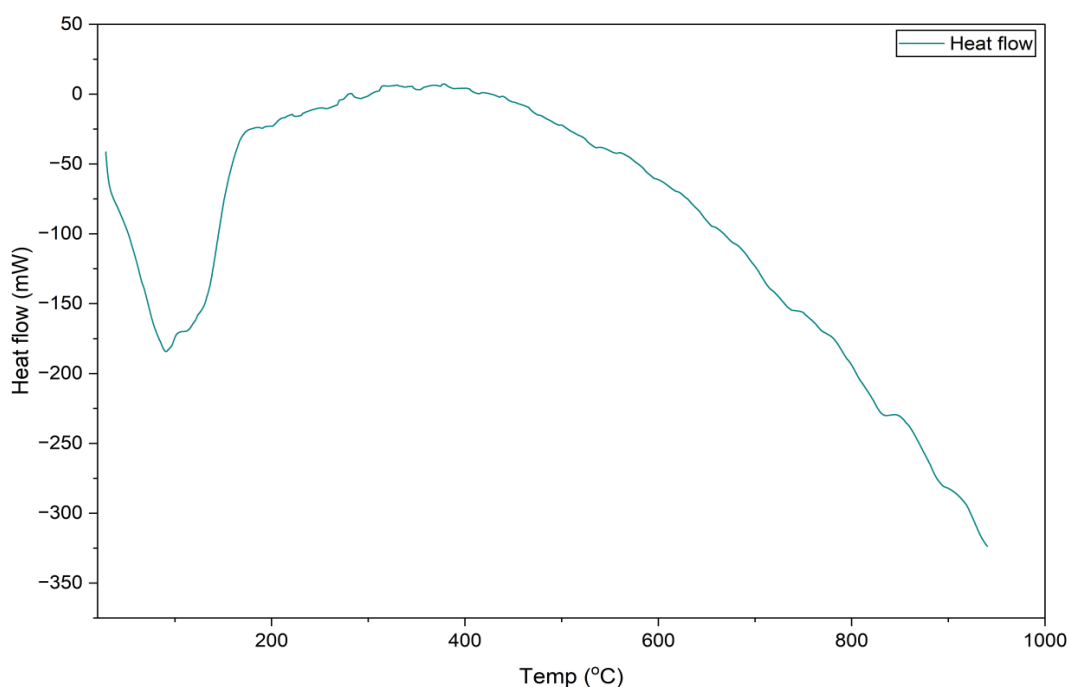


Figure 3.10: DSC of Saturnia Mud

3.3.6. Geochemical Characteristics of Saturnia Thermal water:

The Saturnia thermal waters are classified as sulphurous–carbonic, sulphate–bicarbonate–alkaline–earth mineral waters, reflecting both their chemical composition and gas content. The total dissolved solids (TDS) content averages 2.7–2.8 g L⁻¹, with the dominant ionic species being SO₄²⁺, HCO₃⁻, Ca²⁺, and Mg²⁺. Although Saturnia thermal water has been studied previously, the reported chemical and physical parameters show some variation between studies. To provide a consistent and reliable reference, the water was re-characterized in this work. Samples were collected under standardized conditions and analyzed for key physical–chemical parameters, including pH, temperature, mineral composition (cations and anions), and dissolved gases. The resulting values were then compared with earlier reports to evaluate consistency, identify potential sources of variation, and establish an updated profile of Saturnia thermal water. This approach ensures that subsequent experimental or clinical studies using this water are based on accurately defined characteristics, improving reproducibility and the interpretability of biological or therapeutic

outcomes. The detailed physio-chemical properties of Saturnia spring thermal water from different sources and present study are given in Table 3.8. The significance of saturnia thermal water is mostly attributed to its highly sulfurous water.

Trace element analyses have reported the presence of boron (≈ 4.5 mg/L), lithium (≈ 0.18 mg/L), and strontium ($\approx 8\text{--}9$ mg/L). The relatively high sulphate content reflects oxidation of subsurface sulphides or dissolution of gypsum-bearing evaporites, while the bicarbonate and calcium originate from carbonate dissolution and CO_2 interaction. The presence of dissolved hydrogen sulphide and free CO_2 indicates a chemically reducing, CO_2 -rich environment in the reservoir.

From a hydrochemical perspective, the waters plot within the **Ca–Mg– SO_4 – HCO_3 facies**, consistent with prolonged water–rock interaction in a deep carbonate–evaporitic aquifer overlain by low-permeability clays that maintain temperature stability. The continuous outflow and travertine deposition confirm active degassing and supersaturation with respect to calcite at the discharge point.

The chemical characterization of Saturnia thermal water reveals a mineral profile typical of high salinity thermal sources, with substantial hydration and ion concentration levels. The water contains elevated concentrations of sodium (94.77 mg/L), sulfur (635.5 mg/L), magnesium (59.75 mg/L), and notably high calcium (605.6 mg/L), collectively characterizing it as a calcium-sodium sulfate thermal water (Table 3.8). Such ion dominance is generally associated with deep circulation sources passing through calcareous and sulfated rock formations, consistent with hydrogeochemical models of major European thermal springs (Minissale et al., 2023).

Sulfur and its compounds are significant components, with a high sulfur (S) concentration (635.5 mg/L) and notable magnesium and sulfate levels, indicating strong reducing environments in the aquifer. Sulfate-rich waters are often linked to volcanic or tectonic activity and the reduction of gypsum and sulfide minerals. The presence of hydrogen sulfide (H_2S), sulfur, and sulfite further supports this association, and their amounts often correlate with distinctive therapeutic effects attributed to Saturnia water, such as skin and respiratory benefits documented in balneotherapy literature.

The trace element profile reflects low but measurable concentrations of lithium, strontium, fluoride, boron, phosphorus, and aluminium in past studies (Barbagli et al., 2013; Benedetti et al., 2009). The elevated content of silicon (Si, 12.89 mg/L) aligns with the geological context, suggesting interaction with silicate rocks and potential beneficial properties for connective tissues and skin, as suggested in wellness applications. Minimal iron content (below measurable range) reflects a reducing environment and low oxygenation, common in deep circulating mineral waters.

Such waters are valued for their therapeutic potential but may pose challenges for drinking or agricultural use due to scaling effects.

Overall, the mineral composition and trace element distribution of Saturnia thermal water confirms its geological origin and therapeutic reputation. The balance of major cations and anions, augmented by dissolved gases and trace elements, mirrors the characteristics of classic European thermal waters and supports their use in health resorts and spa medicine.

Table 3.8: Major and Trace Elemental Composition of Saturnia Thermal Water

Ions	Unit	Concentration	LOD
Sodium (Na ⁺)	mg/l	94,77	0,096
Potassium (K ⁺)	mg/l	7,691	0,085
Magnesium (Mg ²⁺)	mg/l	59,75	0,11
Calcium (Ca ²⁺)	mg/l	605,6	0,144
Iron (Fe ²⁺ /Fe ³⁺)	mg/l	<	0,098
Aluminium (Al)	mg/l	0,251	0,089
phosphorous (P)	mg/l	0,112	0,099
Sulfur (S)	mg/l	653,5	0,132
Silicone	mg/l	12,89	0,085

3.4. Conclusion:

Overall, the characterization of the clays, Saturnia mud, and Saturnia thermal water provides a clear picture of the materials involved in the formation of Saturnia peloids. The thermal water is strongly mineralized—rich in calcium and sulfur, with relevant amounts of magnesium, sodium, and trace elements—reflecting the geological processes that shape its composition. The Saturnia mud, consistent with previous studies, is mainly composed of hydrated clay minerals containing structural and bound water, small organic fractions, and some carbonates. These properties underline its capacity to interact with the mineral-rich thermal water during maturation. Together, these findings show how the specific mineralogical structure of the clays and the unique chemical profile of the thermal water combine to create a stable, functional, and therapeutically valuable peloid. This integrated characterization serves as a foundation for understanding the behavior and effectiveness of Saturnia mud in subsequent applications.

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CHAPTER 4

PELOIDS ENGINEERING AND OPTIMIZATION



KEY CONCEPTS

- Agitator geometry and mixing energy are critical determinants of VF clay suspension behavior
- Axial agitators, particularly AG1, AG3, and AG4, promoted higher viscosity structures
- Radial agitator (AG5) generated the lowest viscosity suspensions regardless of energy input
- . The optimal processing condition was identified as medium energy (1000 rpm, 10 min), which provided balanced viscosity, extensibility, and structural integrity.
- the suspensions with Saturnia water (SW) produced less viscosity in comparison with Purified water because the high ionic strength and presence of divalent cations in SW compress the electrical double layer around clay particles, promoting aggregation
- In general, the ILB clay suspensions show high viscosity that could be due to its high cation exchange capacity (CEC)
- BC clay with least CEC showed minimal differences in viscosity in purified and Saturnia water.
- High viscosity suspensions linearly have low spreadability

4a. Optimization of peloids preparation: effect of mixing energies and mixer shape on rheological properties of the resultant systems (Article 2)

4.1 Background

Medical hydrology use spring water as therapeutical agent. The water is orally administered but also inhaled or topically applied. When applied onto the skin, medicinal water is sometimes used as a dispersion medium of inorganic solids. The resultant suspensions are called peloids. A peloid is defined as a matured mud or muddy dispersion with therapeutic and/or cosmetic properties, consisting of a complex mixture of fine-grained natural materials of geological and/or biological origin, mineral water or seawater, and frequently organic compounds derived from biological metabolic activity (C. Gomes et al., 2013). Peloids are biphasic systems, comprising a solid phase—mainly minerals, particularly phyllosilicates, and organic substances produced through microbial and plant activity—and a liquid phase, typically medicinal mineral water (MMW), seawater, or water from saline lakes (Veniale et al., 2007). Peloids may occur naturally or be artificially prepared from suitable raw materials. Natural peloids form through processes such as sedimentation, flocculation, or precipitation of substances in aquatic environments, leading to their deposition at the bottom of springs, coastal zones, or saline lakes. In other cases, they may arise from the accumulation and humification of plant matter, as observed in peats. Artificial peloids, in contrast, replicate the composition and characteristics of natural peloids, requiring a controlled production process that includes component selection, mixing, homogenization, and maturation.

The therapeutic effects of peloids depend on their composition and physical properties. Their performance as thermotherapeutic agents is strongly influenced by cooling kinetics, with slower cooling generally preferred (Cara et al., 2000; Tateo et al., 2009). Chemical and mineralogical compositions are important (Carretero et al., 2007; Da Silva et al., 2015; Karakaya et al., 2010; Pozo et al., 2013), as are physical characteristics such as density, plasticity, abrasivity, specific heat, thermal conductivity, and rheological behavior including sediment volume and viscosity (Casas et al., 2011; Knorst-Fouran et al., 2012; Rebelo et al., 2011a, 2011b). These properties enhance the mud's molding capacity and ensure a comfortable sensation when applied (Rebelo et al., 2011a). Granulometry is also critical, with fine-grained mud preferred for optimal application (Carretero et al., 2007; C. Gomes et al., 2013). Bentonite is considered the most suitable solid phase due to its favorable physicochemical properties, including pH, soluble salts, and exchangeable ions (Carretero et al., 2007; Veniale et al., 2007). Microbiological quality is essential: peloids must be free of pathogens, while the development of specific microbial communities during maturation can contribute to therapeutic effects (Centini et al., 2015; Odabasi et al., 2008; Quintela et al., 2015).

The limited availability of natural peloids restricts their practical use to small patient groups. Consequently, most spas employ artificial peloids, which can be produced in scalable quantities to meet treatment demands (C. Gomes et al., 2013). Till to date, most of spas used clays to prepare these peloids/suspensions, following suboptimal procedures. Main shortfalls are lack of correct identification of the clay components and deficient control of clay/water ratios, resulting in peloids that fall away to be optimized. Clay components are expected to help the water to achieve its therapeutical effect (Bastos & Rocha, 2023a), allowing the suspension to be applied onto the skin (rheological properties) but also (as peloids are thermo-therapeutical agents) controlling the heat transfer to the skin (Maraver et al., 2021). Some mineral components do not feature these properties, whereas others components are

required to be present in relevant proportion. Phyllosilicates, particularly smectites, significantly influence the properties of their aqueous suspensions due to their high swelling capacity and water retention ability, resulting in hydrogels with optimal characteristics for topical skin applications (Legido et al., 2007). Veegum®, a magnesium silicate clay belonging to the smectite group, has gained attention for its role in the development of therapeutic hydrogels. Its general chemical formula is $(\text{Na}, \text{Ca})_{0.33}(\text{Al}, \text{Mg})_2\text{Si}_4\text{O}_{10}(\text{OH})_2 \cdot n\text{H}_2\text{O}$, where n denotes the variable amount of interlayer water (Hosseini et al., 2010; Selvasudha et al., 2020; Viseras & Lopez-Galindo, 1999). These lamellar phyllosilicates feature a 2:1 structure, with an octahedral layer sandwiched between two tetrahedral layers (TOT structure), enabling them to retain substantial water within the interlayer spaces (da Silva et al., 2021; Santos et al., 2020). Research has shown that formulations prepared with Veegum® exhibit favorable rheological behavior and effective moisture retention, making them suitable for skin care, controlled drug delivery, and other topical therapeutic applications (Moraes et al., 2017). The capacity of Veegum® to improve both the stability and efficacy of hydrogels underscores its value in advanced formulations for health and regenerative medicine (Del Mar Ramos-Tejada et al., 2003). On the other hand, it can also happen that unnecessary amount of solid is used to prepare peloids, with three direct consequences; unsustainable use of clay (high quality clays are limited resources), less economic feasibility (they are paying the clay material to be used) and reduction of the dose of the active ingredient applied (medicinal water).

Understanding the influence of processing parameters on their rheological behavior is critical for optimizing peloids formulations stability and performance. The rheological behavior of clay suspensions-peloids—characterized by viscosity, shear stress, and shear rate response—is modulated by a complex interplay of clay mineralogy, particle size, concentration, ionic environment, and shear history (Adeyinka et al., 2009; Viseras & Lopez-Galindo, 1999). Smectite clays like montmorillonite show non-Newtonian behavior, including yield stress and shear-thinning, shaped by network formation and interparticle forces (Sarkar et al., 2023). Viseras et al (1999) demonstrated that shear history significantly affects clay microstructure—e.g., rotor–stator agitation at 1 000–8000 rpm alters viscosity and thixotropy; linear relations between mixing energy and apparent viscosity are reported for bentonite systems. In various other formulations as well (e.g., coatings, drilling muds), mixing energy notably impacts yield stress, gel strength, and viscoelastic response (Torsvik et al., 2014). Despite these insights, the role of specific external processing parameters—such as agitator type, speed, and time (collectively mixing energy)—on rheological properties has received relatively limited attention.

Mixing energy—comprising the agitator type, speed (rpm), and mixing time—directly influences particle dispersion, aggregate breakup, and ultimately the microstructure of the suspension (Christidis et al., 2024; Jo et al., 2023). Several studies underscore that optimized mixing can modulate suspension rheology and improve extensibility, crucial for topical drug delivery systems and other pharmaceutical suspensions (Singh et al., 2022). For instance, variations in shear rate during mixing alter the shear stress distribution within the system, impacting particle alignment and suspension viscosity (Cruz & Peng, 2016). Despite these insights, the effects of specific agitator designs and their operational parameters on phyllosilicate clay suspensions remain underexplored.

The pharmaceutical industry demands formulations with predictable and reproducible rheological and mechanical properties to ensure efficacy and patient compliance. An improved understanding of how mixing energy parameters affect clay-based suspension behavior could enable better control over formulation characteristics, such as extensibility and stability, thereby enhancing drug delivery performance (Krishnaraj et al., 2021; Selvasudha et al., 2020). Furthermore, comprehensive rheological characterization involving shear stress and shear rate profiles helps in predicting flow and deformation behavior under application-relevant conditions.

This research addresses the existing gap by systematically investigating the influence of mixing energy components—agitator type, speed, and mixing time—on the rheology and extensibility of Veegum F (VF) clay suspensions. By varying five different agitators, across a range of rotational speeds (500, 1000, 1500, 2000 rpm) and mixing durations (5, 10, 15 minutes), this study aims to elucidate the relationships between mixing parameters and suspension microstructure reflected through rheological and mechanical properties. The findings not only advance fundamental understanding of clay suspension processing but also offer practical insights for pharmaceutical formulation development where phyllosilicate clay minerals serve as fundamental excipients or functional agents.

In this way, the study aimed to

To evaluate the effect of different types of agitators on the rheological behavior and extensibility of Veegum F clay suspensions.

To investigate the influence of mixing speed and time on shear stress–shear rate characteristics.

To identify optimal mixing conditions for achieving desirable suspension flow and application performance.

To correlate mixing energy parameters with suspension structural properties relevant to pharmaceutical applications.

4.2. Methodology

4.2.1. Development and design of VF suspension system

The bentonite clay Veegum F was studied. For each sample powder the sieve fraction between 50-70 μm was selected by dry sieving and then stored at 40% relative humidity and room temperature (22-25°C) for at least 48h before carrying out study. Suspension of Veegum F were prepared at 20%: 80% w/w in purified water using a axial (AG1,AG3,AG4) and radial rotor stator mixer (AG2,AG5) (Table 4.1). Rotor speeds of 500rpm for 5min, 1000rpm for 10min, 2000rpm for 15min were used. The prepared suspensions were placed at room temperature up to 48h for maturation and followed by pH measurement, rheology and extensibility analysis.

4.2.2. Geometry of different Agitators:

The geometry of an agitator significantly affects clay suspension preparation by influencing flow patterns and shear distribution. Axial agitators (AG1, AG3, AG4) mainly promote top-to-bottom circulation, enhancing overall mixing, while radial agitators (AG2, AG5) generate high-shear zones that improve local dispersion. The specific shape of each agitator further affects particle distribution and suspension properties. The detailed structure and shape of agitators used in this study is well explained here (Table 4.1).

4.2.2.1 -Blade Axial-Hydrofoil (AG1) (TURBINE)

This consists of three pitched blades arranged symmetrically around the shaft. The angled blades promote strong axial flow, generating both upward and downward currents that are particularly effective for solid suspension in moderate viscosity liquids. The axial flow minimizes dead zones and enhances overall circulation. While it does not create high shear, it provides efficient bulk mixing and its axial flow lifts solids upward and redistributes them throughout the fluid.

4.2.2.2. Turbine Vortex Blade (AG2) (DISC)

This radial design resembles an anchor or frame, typically used for viscous media. It scrapes the wall of the vessel while stirring, ensuring good heat transfer and homogeneity in high-viscosity suspensions like gels or creams. It promotes bulk movement over shear, making it ideal for keeping solids suspended and preventing sedimentation at slow speeds.

4.2.2.3. U-Shaped Paddle (Open Slot Design) (AG3) (ANCHOR)

This features two flat vertical blades in a U-shaped or gate-style configuration. It generates radial flow, promoting gentle bulk movement with minimal shear. It is ideal for maintaining uniform suspension of large or fragile solids without damaging them. Due to its low-shear action, it's more suited for blending rather than intense dispersion. However, its open-frame geometry allows solids to pass through freely, preventing clogging in high-viscosity fluids.

4.2.2.4. Umbrella Shaped Flat-Blade (AG4) (PADDLE)

This simple features two flat blades arranged in a T configuration. It offers a mix of axial and radial flow, making it moderately effective for suspending solids and gentle dispersion. It's a general-purpose suitable for low- to medium-viscosity liquids, but not ideal for high-shear tasks like emulsification or fine dispersion.

4.2.2.5. Flat disc with toothed edges (AG5) Homogenizing blade

This is a classic high-shear radial geometry with a toothed disc at the bottom. Its design causes rapid radial flow and vortex formation, effectively breaking down agglomerates and dispersing powders or immiscible liquids (like oil/water). It is excellent for dispersion of fine solids or emulsifying systems requiring high energy input, but not effective for suspending settled solids due to minimal axial movement.

Table 4.1: Characteristics and geometry of five different agitators studied.

Agitator Code	Agitator	Agitator type	Geometry	Flow Pattern	Shear Level	Dispersion capability
AG1		3-Blade axial Flow Agitator (Hydrofoil mixer)	Three angled blades turbine	Axial (Up down flow)	Medium Low	Moderate
AG2		Turbine Vortex Blade	Large frame shape	Radial (Localized)	very High	Excellent
AG3		Anchor U shaped Paddle	Two vertical flat blades (open slot)	Axial (Low velocity)	Low	Low
AG4		Umbrella shaped flat blade Paddle	Two flat blades in a T configuration	Mixed (Axial)	Low	Moderate
AG5		Frame type agitator homogenizing blade	Flat disc with toothed edges	Radial+Tangential	Very Low	Low

4.2.3 Characterization of VF Clay suspension System

4.2.3.1. Cooling Kinetics of Suspensions

Cooling kinetics were evaluated according to the methodology of Cara et al.,(2000) following the procedures of Khiari et al.(2014) and Sánchez-Espejo et al., (2015). In brief, pre-weighed suspension samples were equilibrated at 50 °C in cylindrical polyethylene terephthalate (PET) cells, then immersed in a thermostatic water bath maintained at 25 °C. Cooling was monitored until the sample temperature reached 32 °C (representative of skin temperature), using a thermometric probe positioned at the geometric center of the cell.

The experimental cooling data were modeled using **Newton's law of cooling**, describing heat transfer between two bodies at different temperatures:

$$T - T_{min} = (T_{max} - T_{min}) e^{(-kt)} \quad (\text{Eq.1})$$

where $T_{\min}$ was the room temperature (25 °C), $T_{\max}$ was the initial temperature (50 °C, considering the application temperature), t was the time in min and k was a constant that depend on the material and apparatus, given by:

$$K = \frac{P}{(m \times C_p)} \quad (\text{Eq 2})$$

where P is the instrumental constant of the apparatus, C the heat capacity of the heated material, m represent the heated mass and C_p represent specific heat. The apparatus constant was obtained following Cara et al. (Cara et al., 2000b) by fitting of cooling data obtained with a known amount of a reference water suspension of TiO_2 .

4.2.3.2. pH

The pH of the aqueous VF suspensions was measured under mechanical agitation using a Crison Basic 20+ pH meter equipped with a semisolid sensor (Model 5053 T). The instrument was calibrated using standard buffer solutions at pH 4.0, 7.0, and 9.0, and was operated within its tolerance range of pH 2 to 11. Each measurement was performed in triplicate, and the results were averaged, with a standard deviation of ± 0.1 .

4.2.3.3 Rheological properties

Rheological measurements were performed after 48 h of sample swelling, using a controlled rate viscometer (Thermo Scientific HAAKE RotoVisco 1, Karlsruhe, Germany) equipped with HAAKE RheoWin software (Karlsruhe, Germany). The plate-plate sensor configuration (P20 TiL S) was employed for all clay suspensions. All measurements were conducted at 25 °C after a 90 s rest period, with temperature control maintained via a Peltier-controlled parallel-plate system (TCP/P, HAAKE).

Shear rate sweeps were performed within the range of 40–600 s^{-1} , encompassing conditions representative of skin application (10–200 s^{-1}), manual mixing (100–200 s^{-1}), and product withdrawal from containers (400–2000 s^{-1}). Rheological characterization included flow curves obtained during both increasing and decreasing shear rate flow, as well as determination of apparent viscosity. Each sample was analyzed in quadruplicate.

4.2.3.4. Determination of spreadability (Glass Plate Method)

The spreadability of the clay suspension was evaluated using a parallel plate (glass plate) method, as described in previous studies of semi-solid formulation analysis (Knorst and Borghetti, 2006). Briefly, 2.0 g of the sample was placed on the center of the base glass plate (dimensions: 20 × 20 cm), positioned over grid-lined graph paper to facilitate measurement. A second glass plate of equal dimensions was carefully placed on top of the sample to ensure uniform distribution. After exactly one minute, the extent of spread was measured by determining the diameters of the sample along the horizontal, vertical, and diagonal axes using the underlying grid scale. Subsequently, a series of calibrated weights (500 g, 1000 g, 1500 g, 2000 g, 2500 g, 3000 g, and 3500 g) was incrementally placed on the upper glass plate for one minute each. At each weighting step, the spread diameters were again recorded in three directions. For each applied load, the mean diameter was calculated, and the spread area was determined using the formula:

$$A = \pi(d/2)^2$$

Where “ d ” is the average measured diameter in centimeters. Spreadability “ A ” was defined as the mean area per unit weight and measurements were performed in triplicate for reproducibility

4.3. Results and discussion

4.3.1.Characterization of VF suspensions.

4.3.1.1 Cooling kinetics

All clay suspensions were prepared with a fixed water-to-clay ratio of 80:20% (w/w). The experimental specific heat values (Table 4.2) were comparable to those reported for analogous systems (Cara et al., 2000; Casas et al., 2011; Khiari et al., 2014), where specific heat is primarily determined by water content and, to a lesser extent, by the composition of the solid phase. Since all suspensions contained the same water proportion, specific heat values were essentially identical across formulations.

From a practical perspective, the time required to reach skin temperature (32 °C; t_{32}) and the temperature after 20 min of application (T_{20}) were calculated from fitted cooling curves ($R^2 > 0.9999$ for all cases). For VF–PW, t_{32} was approximately 29.07 min, and T_{20} ranged from 35.25 to 35.41 °C, ensuring effective heat transfer under typical mudpack application conditions.

Table 4.2. Cooling kinetics of the studied VF clay suspension.

Sample	Cp (J/g K)	t_{32} (min)	T_{20} (°C)
VF-PW	3.21 ± 0.069	29.07 ± 0.46	35.41 ± 0.011

Note: $\bar{x}$ means values $\pm$ s.d.; n=2.

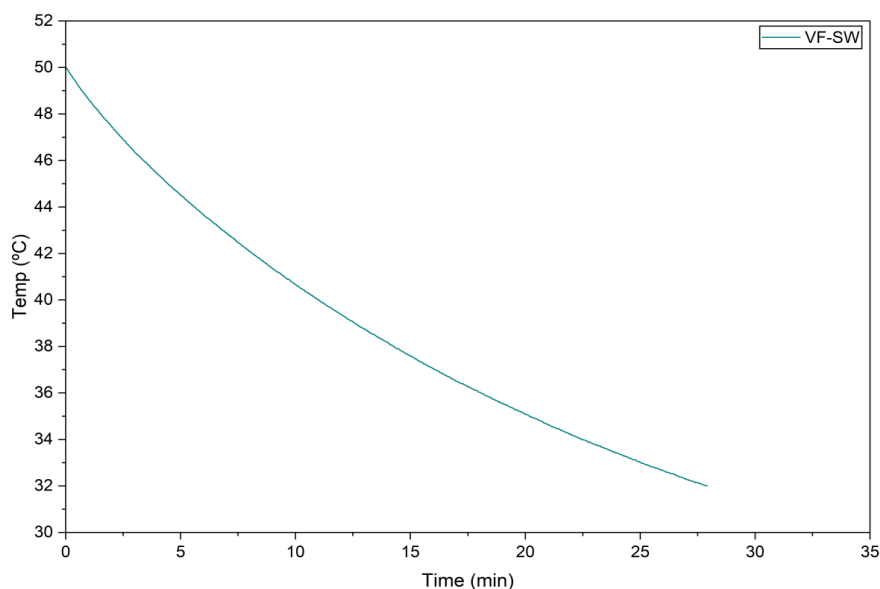


Figure 4.1: Cooling rate of VF clay suspension

4.3.1.2. pH

The pH of the clay suspensions at 25°C are shown in Table 4.3. Those values were in the range from 8.5 to 8.6 (Table 4.3). The composition of clay used for preparation of

different clay suspension at different energies is the same therefore the variation in pH is narrow.

Table 4.3: The pH of clay suspensions prepared at different mixing energies and agitators

<i>Mixing energy</i>	<i>Agitator codes</i>	<i>pH¹±SD</i>
Low Energy	AG1	8,5±0,02
	AG2	8,6±0,03
	AG3	8,6±0,02
	AG4	8,6±0,02
	AG5	8,5±0,03
Medium Energy	AG1	8,6±0,05
	AG2	8,6±0,03
	AG3	8,6±0,03
	AG4	8,5±0,03
	AG5	8,5±0,03
High Energy	AG1	8,7±0,05
	AG2	8,6±0,06
	AG3	8,6±0,06
	AG4	8,6±0,06
	AG5	8,6±0,05

¹means values ± s.d.; n=4.

4.3.1.3 Rheological properties

4.3.1.3.1. Effect of different agitator types and mixing energies on viscosity and adhesiveness of suspensions.

To investigate the impact of agitator type and mixing energies on the rheological behavior of clay suspensions, viscosity versus shear rate profiles were obtained for five different agitators (AG1–AG5) at three constant mixing energy levels: 500 rpm (5min), 1000 rpm (10min), and 2000 rpm (15min) (Fig 4.2). The observed trends consistently showed that agitators AG3 and AG4 produced higher viscosities, whereas AG5 resulted in the lowest viscosities across all energy levels.

These trends correlate closely with the **flow characteristics** generated by each agitator. Specifically:

AG3 and AG4 are characterized by predominantly **axial flow patterns**, which direct fluid motion vertically along the vessel axis.

AG5, in contrast, generates primarily **radial flow**, promoting outward dispersion from the agitator into the bulk medium.

The axial-flow agitators (AG1, AG3, AG4) generally yielded higher viscosities, especially at low and medium energy inputs, highlighting the role of circulation in maintaining a more structured suspension. This fact could be attributed to the axial flow tendency to promote **bulk circulation and particle clustering** rather than intense localized shear. This flow regime facilitates the development or retention of clay microstructures such as edge-face associations or flocculated networks, which increase internal resistance to flow. At lower mixing energies (500rpm, T5), the lower shear intensity of axial flow lead to insufficient disruption of particle aggregates. At medium energies (1500rpm, T10), these agitators may intensify floc interactions or induce shear-induced structure formation through frequent particle collisions and re-agglomeration, sustaining elevated viscosities. Whereas, at high energy (2000rpm, 15min) the applied shear is sufficient to disrupt these particle networks, leading to dispersion of the aggregates into smaller units. Consequently, the breakdown of flocs reduces the overall structural integrity of the suspension, decreasing both viscosity and adhesiveness (Fig 4.3 & 4.4). These findings are consistent with the shear-sensitive behavior of clay suspensions, where moderate shear encourages network formation while strong shear leads to structural breakdown and flow facilitation.

AG4 showed strong viscosity retention, aligning with its superior adhesiveness performance. AG2, despite being radial, produced higher viscosity at medium energy, suggesting that moderate shear intensity can promote better dispersion and apparent thickening of clay particles. However, at higher energies, viscosity dropped, indicating breakdown of particle aggregates.

In contrast, the radial-flow agitator (AG5) consistently produced the lowest viscosities across all energy levels, reinforcing that its homogenizing geometry promotes strong dispersion and reduces inter-particle structuring. Radial flow agitators impose stronger shear gradients in the horizontal plane and produce higher turbulence near the agitator region. This flow regime enhances the breakup of clay agglomerates, promotes de-flocculation, and leads to a more homogeneously dispersed suspension. As a result, the internal structure is minimized, reducing the viscosity across the shear rate range. The consistent ranking of AG5 as the lowest-viscosity system across all mixing energies supports its superior dispersive efficiency.

Similarly for adhesiveness, axial flow agitators (AG1, AG3, AG4) generally promoted higher values compared to radial flow agitators (AG2, AG5) (Fig 4.4). Among them, AG4 (umbrella type blade) showed particularly high adhesiveness at low and medium energy levels, suggesting that its geometry enhances axial circulation and wall-surface interactions, which promote particle bridging and network formation. Similarly, AG1 (hydrofoil) and AG3 (U-shaped anchor) maintained moderate to high adhesiveness across energy levels, indicating that axial pumping helps sustain cohesive particle interactions.

In contrast, AG2 (turbine vortex blade, radial type) exhibited the highest adhesiveness at medium energy but a decline at higher energy, likely due to excessive shear disrupting clay particle flocculation. AG5 (dispersing homogenizer with anchor frame) consistently showed the lowest adhesiveness, which may be attributed to its radial flow pattern and strong shear that favors dispersion rather than network development.

Overall, these findings emphasize that axial agitators are more effective in maintaining suspension structure (higher adhesiveness and viscosity), while radial agitators tend to favor dispersion with lower structuring effects, especially under high energy conditions. The results highlight the importance of matching agitator geometry and energy input to the desired balance between dispersion and network integrity in clay-based suspensions. Previous studies have shown that, in single-agitator suspension systems, mixing

quality deteriorates when the stirrer operates at speeds higher than the optimal level (Hosseini et al., 2010; Mishra & Ein-Mozaffari, 2016, 2017; Tahvildarian et al., 2011). Similar trends were reported by Mishra and Ein-Mozaffari (Mishra & Ein-Mozaffari, 2021b) for dense systems, and by Mishra and Ein-Mozaffari (2021a) for complex fluid systems under high-speed operation.

These findings underscore the significant role of agitator-induced hydrodynamics in governing the microstructural evolution and flow behavior of structured fluids such as clay suspensions. These findings emphasize that axial agitators are more effective in maintaining suspension structure (higher adhesiveness and viscosity), while radial agitators tend to favor dispersion with lower structuring effects, especially under high energy conditions and reduces viscosity. Importantly, the relative ranking of viscosities among agitators shifts subtly with increasing energy, reflecting the complex interplay between shear forces, particle interactions, and energy dissipation mechanisms that vary with both agitator design and operational speed. The overall agreement between adhesiveness and viscosity across agitators and energies highlights the intrinsic link between bulk flow resistance and interfacial bonding strength in clay suspensions, and underscores the critical role of mixing conditions in tuning their rheological and adhesive performance.

This knowledge is critical for formulation and process optimization in industries where specific rheological properties are desired (e.g., controlled spreading, suspension stability). Selection of the appropriate agitator must therefore consider not only the desired energy input but also the directionality and distribution of shear forces imparted to the system.

CHAPTER 4: Clay water system and Peloids optimization

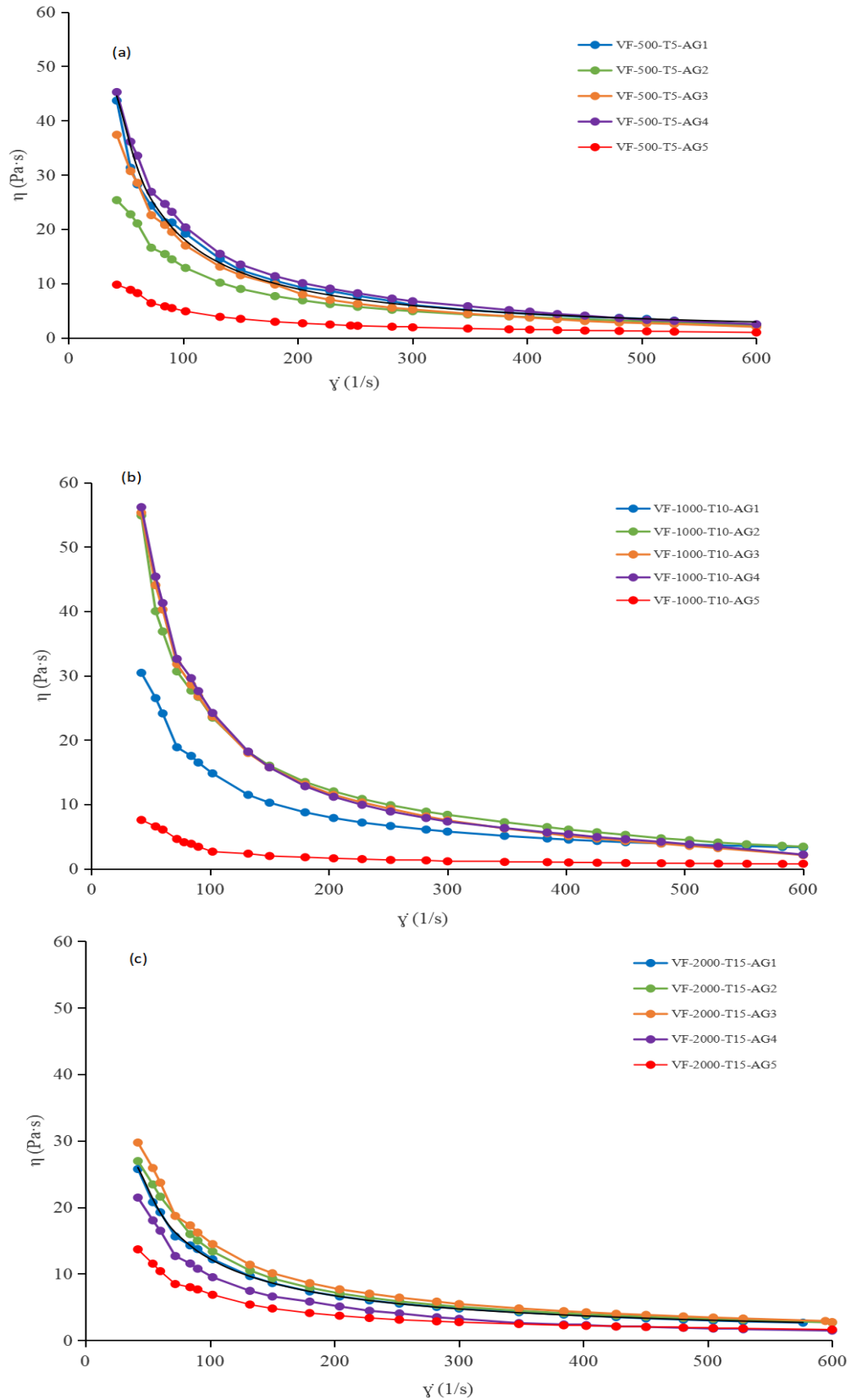


Figure 4.2: Effect of different agitators on correlation with apparent viscosity measured at (a) 500rpm T05, (b) 1000rpm T10, (c) 2000rpm T15min.

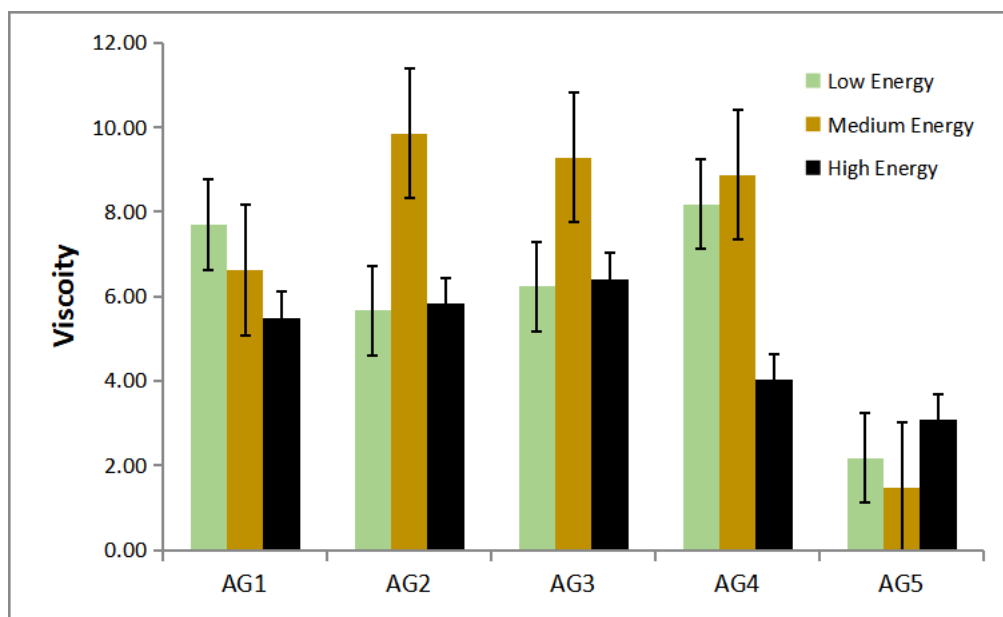


Figure 4.3: The effect of different agitators geometry on clay suspensions viscosity at different mixing energies.

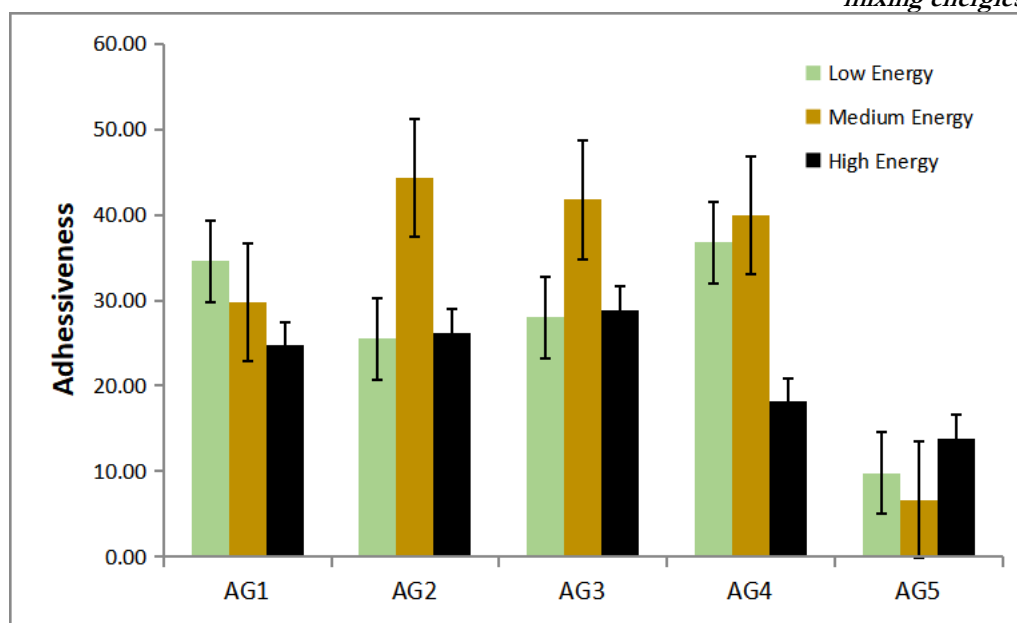


Figure 4.4: Effect of different geometries of Agitators on adhesiveness of clay suspensions at different mixing energies.

Table 4.4. Apparent viscosities (252 Pa·s at 25 °C) of the samples.

Mixing energies	Agitator type	Apparent η'	Theoretical η'
Low energy (500rpm, T5)	AG1	7.695	9.855
	AG2	5.673	2.937
	AG3	6.234	8.939
	AG4	8.181	1.301
	AG5	2.176	5.146
Medium energy (1000rpm, T10)	AG1	6.62	11.95
	AG2	9.85	8.733
	AG3	9.28	8.043
	AG4	8.88	0.843
	AG5	1.48	0.843
High energy (2000rpm, T20)	AG1	5.49	3.720
	AG2	5.83	3.497
	AG3	6.41	3.408
	AG4	4.03	4.221
	AG5	3.08	3.006

Note: ¹means values $\pm$ s.d.; n=4.

4.3.1.3.2. Bonding strength and adhesiveness

In this work, the theoretical bonding strength coefficient (BSC) of clay suspensions was derived by combining the exponential relationship between viscosity and extensibility with the viscosity–BSC ($\eta=0.044 e^{0.179}$, $R^2=0.901$) correlation proposed by Tian et al.,2022

The results showed that the apparent viscosity increased with increasing the bonding strength coefficient. Regression analysis indicated that bonding strength coefficient (BSC) had a good correlation with apparent viscosity, with R^2 values of 0.9715 (Fig 4.5). In particular, apparent viscosity was defined as the ability of system internal structure to hinder its flow. The bonding strength coefficient was a parameter that characterizes the strength and size of the flocculation structure, so it had a better correlation with the apparent viscosity.

Although Tian et al. (2022) developed their model using cement pastes with a water:cement ratio of 0.4, its application to our clay suspensions with a water:clay ratio of 4 demonstrates that the approach can be extended to more dilute and structurally different systems. Despite the significantly higher water content in our suspensions, the calculated BSC values showed clear correspondence with adhesiveness trends, both decreasing as the suspensions became less viscous and the particle network weakened (Fig 4.6). The theoretical BSC and adhesiveness calculated values are shown in Table 4.5 The BSC of systems depends on the amount of water used to prepare system. Higher the water: solid content less will be BSC therefore the BSC values in our study are negative but in general the correlation trend is similar to previously reported study. This consistency highlights the usefulness of BSC as a theoretical indicator of network strength even across different classes of particulate systems. Our findings therefore suggest that, while originally established for cementitious materials, the viscosity–BSC relationship provides a valuable framework for interpreting the microstructural bonding and macroscopic adhesive behavior of clay-based suspensions.

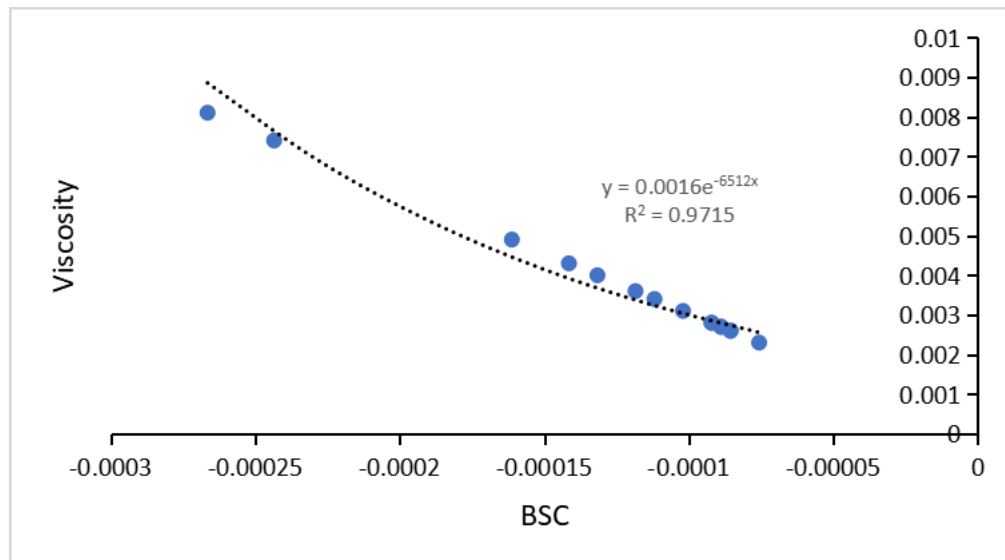


Figure 4.5: Bonding strength coefficient correlation with viscosity.

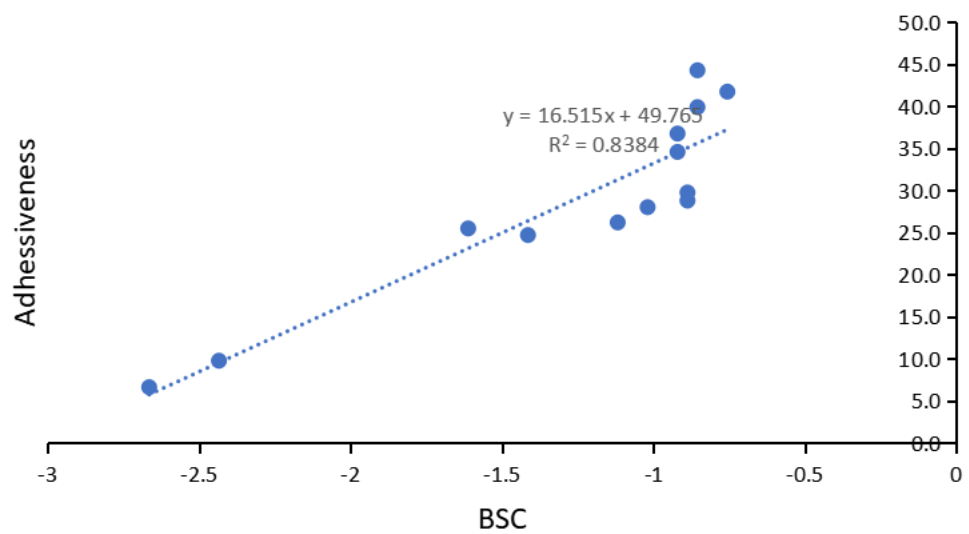


Figure 4.6: Bonding strength coefficient (BSC) correlation with adhesiveness.

Table 4.5: Theoretical bonding strength coefficient (BSC) and adhesiveness values of clay suspensions prepared with different agitators with low energy (LE), medium energy (ME) and high energy (HE).

Agitator type	BSC (LE)	BSC (ME)	BSC (HE)	Adhesiveness (LE)	Adhesiveness (ME)	Adhesiveness (HE)
AG1	-0,9212	-0,8883	-1,4147	34,60435485	29,7816441	24,72060825
AG2	-1,6121	-0,8554	-1,1186	25,51067844	44,30733411	26,21935836
AG3	-1,0199	-0,7567	-0,8883	28,03422648	41,76579927	28,81620261
AG4	-0,9212	-0,8554	-1,1844	36,78660336	39,9261993	18,13249308
AG5	-2,4346	-2,6649	-1,316	9,78436953	6,63757887	13,85388303

4.3.1.3.3. Correlation between Extensibility and Viscosity

The relationship between viscosity and extensibility of Veegum® F clay suspensions prepared under varying mixing energies and agitator types shows a strong inverse correlation, as described by the power-law model $y=0.0102x^{-0.625}$ $y > y=0.0102x^{-0.625}$ ($R^2=0.8065$) (Fig 4.7). This indicates that as viscosity increases, the extensibility of the suspensions decreases markedly.

At lower viscosities (approximately 2–3 Pa·s), extensibility values are highest (~0.007–0.008 cm²), suggesting that reduced internal resistance to flow facilitates spreading behavior. This behavior is consistent with less compacted or less structured clay networks, which are likely generated by lower shear rates and shorter mixing durations (e.g., 500 rpm for 5 min). Conversely, higher viscosities (>8 Pa·s) are associated with minimal extensibility (~0.002–0.003 cm²), reflecting more densely packed clay platelets and stronger interparticle interactions, which resist deformation under applied stress.

From a formulation standpoint, this inverse viscosity–extensibility trend highlights a trade-off between structural stability and ease of application. For topical pharmaceutical or cosmetic uses, intermediate viscosity values (4–6 Pa·s) may provide an optimal balance between suspension stability and acceptable extensibility, depending on the intended application and user preference.

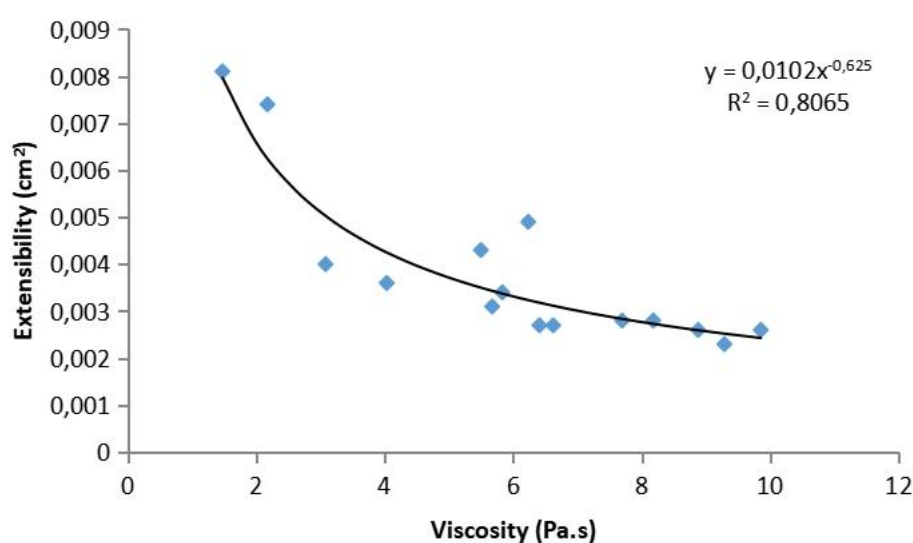


Figure 4.7: Relationship between viscosity and extensibility of Veegum® F clay suspensions prepared under different mixing energies and agitator types.

4.4. Conclusion

The present study demonstrates that agitator type and mixing energy are critical determinants of VF clay suspension behaviour. Axial agitators, particularly AG1, AG3, and AG4, promoted higher viscosity structures, whereas AG5 generated the lowest viscosity suspensions regardless of energy input. The optimal processing condition was identified as medium energy (1000 rpm, 10 min), which provided balanced viscosity, extensibility, and structural integrity. The strong inverse relationship between viscosity and extensibility, along with the clear correlations of BSC with both viscosity and adhesiveness, underscores the importance of controlling mixing parameters to achieve desired suspension properties. These findings not only highlight the role of agitation mechanics in tailoring clay-based systems but also establish a basis for optimizing VF suspensions for practical applications requiring specific flow and adhesion characteristics.

4b. Influence of Sulphate-Rich Saturnia Thermal Water on the Rheological and Extensibility Properties of Prepared Peloids (Article 3)

4.5. Background:

The term “**peloid**” was originally defined by the International Society of Medical Hydrology (1949) as a natural product composed of mineral waters—such as seawater or water from salt lakes—combined with organic or inorganic matter produced through geological and/or biological processes, and applied therapeutically in baths or poultices. Later definitions describe peloids as semi-liquid therapeutic or thermotherapeutic agents, obtained by mixing a liquid phase (mineral water, seawater, or saline lake water—such as sulphur, sulphate, chloride, sodium, or bromine waters) with a solid organic and/or inorganic component formed through biological and geological activity, and employed for local or systemic treatments (Veniale et al., 2004). Naturally occurring peloids are uncommon, but they can be artificially prepared using medicinal mineral waters (MMWs).

Traditionally, peloids have been applied locally for the treatment of musculoskeletal and dermatological conditions (Fioravanti et al., 2011; Fraioli et al., 2018; Sukenik et al., 1990). More recently, their use has expanded to include cosmetic, wellness, and anti-stress therapies (Mourelle et al., 2023, 2025). A critical aspect in pelotherapy is the need to tailor peloid formulations for specific therapeutic purposes. While conventional preparations are mainly intended for rheumatologic, musculoskeletal, and dermatologic treatments, cosmetic uses demand formulations with distinct features. This has led to the concept of artificial/synthetic peloids, described as the emerging “new frontier” in pelotherapy (Veniale et al., 2007). To achieve this goal, systematic studies are required to establish the optimal properties of clay–water suspensions according to their therapeutic application. The characteristics of the water used in peloid formation play a decisive role in its chemical profile, influencing factors such as pH, salinity, and ionic exchange between the solid and liquid components (Gámiz et al., 2009)). Trace elements contributed by the water, including iron and hydrogen sulphide, can influence the therapeutic action of the peloid, such as providing analgesic effects (Quintela et al., 2012)). Natural saline waters and brines, enriched in elements like bromine, iodine, boron, fluorine, lithium, and strontium (Glavaš et al., 2017), are also thought to contribute potential therapeutic benefits (Halevy et al., 2001). Consequently, the type of MMW selected determines the properties and therapeutic application of the resulting peloid (Veniale et al., 2004). . The effectiveness of peloids is further influenced by the mineralogical and chemical composition of their solid fraction (Carretero et al., 2007). Clay minerals such as bentonites and kaolins are regarded as especially suitable for peloid production due to their favorable physicochemical characteristics (Carretero et al., 2007; Veniale et al., 2004, 2007). Numerous works have examined peloid properties and their constituents, focusing on mineralogical and chemical characteristics (Cara et al., 2000; Carretero & Pozo, 2010; Karakaya et al., 2010; Rebelo et al., 2011a, 2011b; Tateo et al., 2009; Tateo & Summa, 2007; Veniale et al., 2004), thermal performance (Cara et al., 2000; Casas et al., 2011; Ferrand & Yvon, 1991; Knorst-Fouran et al., 2012) and biochemical or microbiological aspects (Ferrand & Yvon, 1991) . Given that peloids are semisolid systems, their rheological behavior must be carefully characterized (Rebelo et al., 2011c).). Rheology governs product handling during mixing, filling, packaging, and application, while also determining how the peloid performs (Bastos & Rocha, 2023b). Formulations with high viscosity and thixotropic properties are considered most suitable, since they combine structural stability at rest with progressive softening under

stress and subsequent recovery when stress is removed (Mewis & Wagner, 2009). Peloids naturally exhibit these characteristics because they are concentrated clay suspensions that form gel-like structures (Rebelo et al., 2011a; Viseras et al., 2007; Viseras & Lopez-Galindo, 1999). Such gelling behavior arises from aggregation mechanisms among clay particles, which vary depending on mineral type, pH, and ionic strength (Lagaly & Bergaya, 2013). Clay powders themselves are composed of single mineral particles, aggregates, and higher-order assemblies of aggregates. Whereas, less attention has been given on influence of mixing energy and rheological properties.

Therefore this study aim to:

- ✓ Identify sulfuric saturnia thermal water effect on properties of prepared peloids.
- ✓ Comparison of rheological behaviour of peloids prepared with saturnia thermal water and purified water.
- ✓ Effects of mixing energies on rheological and extensibility properties of prepared peloids.
- ✓ To investigate the differences in properties of peloids prepared with different clay samples: Berbek CEC (BC), Bentonita Benesa (BB) (from BENESA, Bentonitas Especiales company), Veegum® F (VF) (from Vanderbilt Ltd., USA), and Innogel LB (ILB) (from LB Minerals, Lasselberger group GmbH)

4.6. Materials and methods

4.6.1 Materials

The VF, BB, BC, and ILB clay samples are pharmaceutical-grade magnesium aluminium silicate clay commercially used as an excipient. These materials comply with the specifications of the European Pharmacopoeia (8.0) and United States Pharmacopoeia (USP 32) monographs.

4.6.2. Development and study of suspension.

The bentonite clays BC, BB, VF, and ILB were studied. For each sample powder the sieve fraction between 50-70 μm was selected by dry sieving and then stored at 40% relative humidity and room temperature (22-25°C) for at least 48h before carrying out study. Suspension of each clay was prepared at 20%: 80% w/w in purified water using Silverson high shear rotor stator mixer model L5T. Rotor speeds of 2000 rpm for 5min, 6000 rpm for 5min were used. The prepared suspensions were placed at room temperature up to 48h for maturation and followed by pH measurement, cooling kinetics, cation exchange capacity, zeta Potential, rheology and extensibility analysis.

4.6.3. Characterization of clay suspension system.

4.6.3.1 Cooling kinetics

Cooling kinetics were assessed following the methodology described by Cara et al. (2000), in accordance with the procedures reported by Khiari et al. (2014) and Sánchez-Espejo et al. (2015). Briefly, pre-weighed suspension samples were equilibrated at 50°C in cylindrical polyethylene terephthalate (PET) cells and subsequently immersed in a thermostatic water bath maintained at 25 °C. The cooling process was monitored until the sample temperature decreased to 32 °C, corresponding to typical skin temperature, using a thermometric probe positioned at the geometric center of the cell.

CHAPTER 4: Clay water system and Peloids optimization

The experimental cooling profiles were analyzed based on **Newton's law of cooling**, which describes heat transfer between two bodies maintained at different temperatures:

$$T_{min} = (T_{max} - T_{min})^{(-kt)} \quad (\text{Eq. 1})$$

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

$$K = \frac{P}{(m \times C_p)} \quad (\text{Eq 2})$$

where P denotes the instrumental constant of the apparatus, C is the heat capacity of the heated material, m represents the heated mass, and C_p corresponds to the specific heat. The instrumental constant (P) was determined following the procedure described by Cara et al., (Cara et al., 2000) through fitting of cooling data obtained using a reference aqueous suspension containing a known mass of TiO_2 .

4.6.3.2. pH

The pH of the aqueous clay suspensions (VF, BC, BB, and ILB) was determined under mechanical agitation using a Crison Basic 20+ pH meter equipped with a semisolid electrode (Model 5053 T). The instrument was calibrated with standard buffer solutions at pH 4.0, 7.0, and 9.0, and operated within its specified tolerance range (pH 2–11). Each measurement was carried out in triplicate, and the mean values were reported with a standard deviation of ± 0.1 .

4.6.3.3. Rheological properties.

Rheological measurements were carried out after allowing the samples to swell for 48 hours, using a controlled-rate viscometer (Thermo Scientific HAAKE RotoVisco 1, Karlsruhe, Germany) equipped with HAAKE RheoWin software (Karlsruhe, Germany). A plate–plate sensor configuration (P20 TiL S) was employed for all clay suspensions. All tests were performed at 25 °C following a 90 s rest period, with temperature regulation ensured by a Peltier-controlled parallel-plate system (TCP/P, HAAKE).

Shear rate sweeps were conducted over the range of 40–600 s^{-1} , covering conditions representative of skin application (10–200 s^{-1}), manual mixing (100–200 s^{-1}), and product withdrawal from containers (400–2000 s^{-1}). Rheological characterization included flow curves obtained during both the ascending and descending shear rate phases, as well as determination of the apparent viscosity. Each sample was tested in quadruplicate.

4.6.3.4. Spreadability Analysis.

Spreadability was evaluated following the procedure described by Knorst and Borghetti, (2006). A circular glass mold plate (20 cm in diameter, 0.2 cm thick) containing a central opening (1.2 cm in diameter) was positioned on top of a glass support plate (20 × 20 cm). A predetermined volume of the sample was placed into the central opening, after which the mold was gently lifted away. A glass plate of known weight was then placed on the sample. After 1 minute, the diameter of the resulting spread area was recorded. Additional glass plates were added at one-minute intervals to progressively increase the applied weight. Spreadability (E_i) for each applied weight was calculated using Equation (3):

$$E_i = \frac{d^2 \pi}{4} \quad (\text{Eq. 3})$$

where E_i is the spreadability of the sample at weight i (mm²), d is the average diameter of the spread area (mm), and $\pi = 3.14$. All measurements were performed in triplicate, and the results are reported as mean values.

4.7. Results and discussion

4.7.1 Clay Suspensions Characterization

4.7.1.1 Cooling Kinetics

All clay suspensions were prepared with a fixed water-to-clay ratio of 80:20% (w/w). The experimental specific heat values (Table 4.6) were comparable to those reported for analogous systems (Aguzzi et al., 2007; Cara et al., 2000), where specific heat is primarily determined by water content and, to a lesser extent, by the composition of the solid phase. Since all suspensions contained the same water proportion, specific heat values were essentially identical across formulations. From a practical perspective, the time required to reach skin temperature (32 °C; t_{32}) and the temperature after 20 min of application (T_{20}) were calculated from fitted cooling curves ($R^2 > 0.9999$ for all cases). The specific heat capacities (C_p) of the suspensions ranged from 3.06 to 3.27 J·g⁻¹·K⁻¹, showing only minor variation among the different clay types (BC, BB, ILB, and VF) and water sources (purified water, PW, and Saturnia water, SW). The experimental cooling times to reach 32 °C (t_{32}) varied between 26.9 and 28.7 min, while the times to reach 20 °C (t_{20}) were slightly higher, ranging from 34.7 to 35.3 min. These results indicate that, under the same clay-to-water ratio, the type of clay exerts a relatively small influence on the overall cooling kinetics. The small differences observed between suspensions prepared with PW and SW suggest that dissolved minerals or salts in natural water slightly enhance heat transfer, resulting in marginally faster cooling rates (Figure 4.8).

Table 4.6 Cooling rates of BC, BB, VF, and ILB clay suspensions measured at 32 °C and 20 °C

Sample	C_p (J/g K)	$t_{32^\circ\text{C}} (\text{min})^1$ Exp	$t_{32^\circ\text{C}} (\text{min})^1$ Theor	$t_{20^\circ\text{C}} (\text{min})^1$ Exp	$T_{20 \text{ min}} (^\circ\text{C})^1$ Theor
BC-PW	3.24±0.120	27.7±0.283	27.93±0.386	34.99±0.0078	34.94±0.08
BB-PW	3.124±0.041	28.60±0.247	28.67±0.280	35.23±0.085	35.21±0.08
ILB-PW	3.197±0.104	27.98±0.141	28.20±0.146	35.11±0.035	35.08±0.042
VF-PW	3.21 ± 0.069	28.75 ± 0.46	28.95±0.381	35.33±0.113	35.31±0.042
BC-SW	3.062±0.248	26.90±0.891	27.13±0.913	34.76±0.318	34.73±0.312
BB-SW	3.268±0.087	28.55±0.189	28.64±0.05	35.23±0.014	35.19±0.004
ILB-SW	3.260±0.018	28.07±0.092	28.22±0.104	35.12±0.042	35.08±0.035
VF-SW	3.123±0.047	28.74±1.153	28.83±1.089	35.31±0.311	35.27±0.333

Note: ¹ means values ± s.d.; $n=2$. Exp. = measured viscosity; Theo. = model-predicted viscosity.

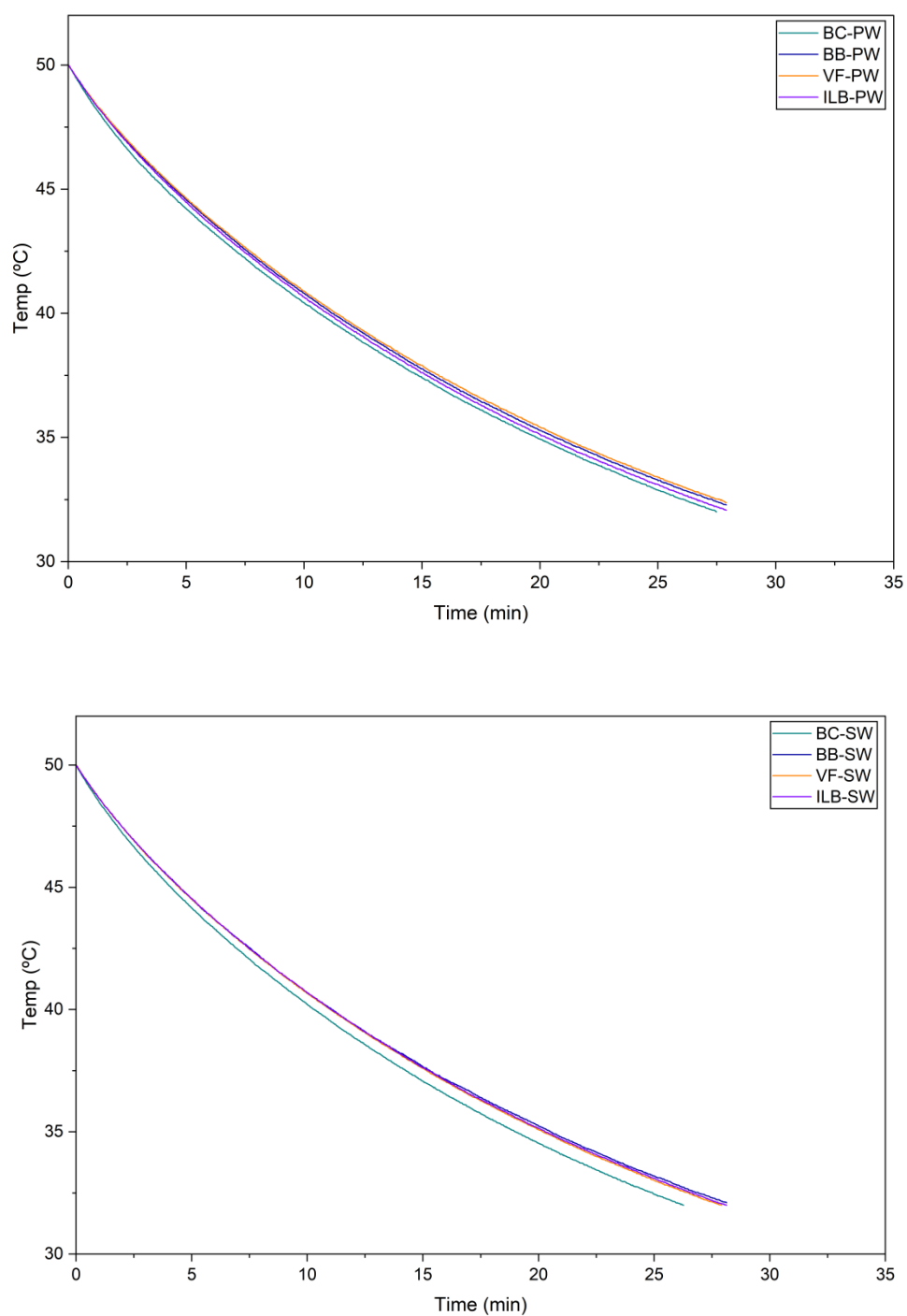


Figure 4.8: Cooling rate of studied clay suspensions with PW and SW.

4.7.1.2. pH measurements

The pH of the clay suspensions prepared using purified water (PW) and Saturnia thermal water (SW) showed clear differences among the four clay types (BC, BB, VF, and ILB) (Table 4.7), reflecting both the intrinsic mineralogical properties of each clay and the chemical characteristics of the water used for dispersion.

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For the suspensions prepared with purified water, all clays exhibited alkaline pH values, although with noticeable variation among samples. BC-PW and BB-PW showed the highest pH values (10.51 and 10.29, respectively), indicating a strong alkaline response. This is typically associated with clays containing exchangeable alkaline cations (e.g., Na^+ , K^+) and the presence of negatively charged layers capable of hydrolyzing water. ILB-PW also showed a moderately alkaline pH (9.30), while VF-PW presented the lowest value within this group (8.72), suggesting a reduced alkalinity compared to the other clays, possibly due to differences in layer charge or mineral impurities affecting surface reactions.

When suspensions were prepared with Saturnia water, the pH values decreased for all clays. For example, BC-SW decreased from 10.51 (PW) to 9.55 (SW), BB-SW from 10.29 to 8.90, VF-SW from 8.72 to 7.76, and ILB-SW from 9.30 to 8.20. This consistent reduction indicates that Saturnia water—characterized by its mineral content, including bicarbonates, sulfates, and dissolved CO_2 —exerts a buffering effect that shifts the suspensions toward a less alkaline pH. The presence of dissolved CO_2 in particular promotes the formation of carbonic acid, which can partially neutralize the alkalinity generated by the clay surfaces.

Among the clay–Saturnia water suspensions, VF-SW shows the lowest pH (7.76), indicating that VF has the weakest tendency to increase alkalinity when interacting with mineral-rich water. In contrast, BC-SW remains the most alkaline among the SW suspensions, suggesting that BC retains stronger basic surface properties even in the presence of the natural buffer system of Saturnia water.

Overall, these results demonstrate that:

- ✓ All clays produce alkaline suspensions in purified water.
- ✓ Saturnia water leads to a systematic decrease in pH, likely due to its dissolved ions and natural buffering capacity.
- ✓ The magnitude of pH reduction varies by clay type, reflecting differences in surface chemistry, cation exchange behavior, and layer charge.

Table 4.7: Measurement of pH of clay suspensions prepared with Saturnia water and purified water at high and low energies.

Mixing energy	Sample code	pH ¹ ±SD
High energy 6000rpm,5min	BC-PW	10,32±0,003
	BB-PW	10,22±0,004
	VF-PW	8,75±0,002
	ILB-PW	8,34 ± 0,003
	BC-SW	9,25±0,002
	BB-SW	8,66±0,003
	VF-SW	7,68±0,003
	ILB-SW	8,45 ± 0,002
Low energy 2000rpm,5min	BC-PW	10,51±0,003
	BB-PW	10,29±0,004
	VF-PW	8,72±0,001
	ILB-PW	9,30±0,001
	BC-SW	9,55±0,004
	BB-SW	8,90±0,004
	VF-SW	7,76±0,007
	ILB-SW	8,20±0,002

Note: † means values $\pm$ s.d.; n=4.

4.7.1.3. Rheological properties

4.7.1.3.1. Viscosity/Flow behavior and mixing energy.

Across all formulations and conditions, the viscosity–shear rate curves show clear **shear-thinning** (pseudoplastic) behavior: viscosity (η) decreases monotonically with increasing shear stress ($\dot{\gamma}$) and tends toward a low-shear plateau at high $\dot{\gamma}$ (~ 400 – 600 s⁻¹). In general, the clay suspensions prepared with purified water (PW) show increase in viscosity from low (2000 rpm, 5min) to high energy (6000 rpm, 5min) mixing conditions. In case of PW suspensions, the most pronounced increase in viscosity at high energy was observed for ILB clay suspension that show increase in viscosity up to ~ 25 – 27 Pa·s at ~ 50 s⁻¹ from ~ 1.5 – 2 Pa·s at ~ 500 s⁻¹ indicating a strong particle network compared to other clay suspensions (Figure 4.9). In general, the viscosity of all clay suspensions prepared at high energy (H.E) were high than low energy (L.E) suspensions. Hence, the response to shear rate is more sharp for suspensions prepared at high energy whereas for low energy prepared suspension it is gradual (Fig 4.9,4.10).

Similarly, in case of saturnia water (SW), BC-SW and VF-SW suspensions also exhibited same trend of increase in viscosity at high energy. Whereas, ILB suspensions show decrease in viscosities at 6000 rpm and BB suspensions show comparatively less significant change in viscosity at both energies (Fig X).

The effect of increasing mixing speed (rpm) leading to higher viscosity is typical for clay suspensions as aggregation states are disrupted and particle alignment changes, but overall viscosity remains lower in high-mineral-content waters due to the above mechanisms (Carty et al., 2000). The increase in viscosity at high mixing energy is well supported in literature—higher mixing speeds generally increase suspension viscosity by disrupting aggregates and realigning particles, as shown by studies on clay suspensions (Ngo et al., 2009).

Our findings are consistent with prior research showing that mineral water composition strongly influences the physical properties of peloids used for pelotherapy, with sulphate-rich waters reducing viscosity compared to purified/diluted systems (C. S. F. Gomes et al., 2015).

The persistent SW < PW gap at both energies indicates potential influence of water chemistry, more than mechanical energy, is the primary factor controlling rheology of these suspensions. For easy spreadability of these peloids, PW formulations may need lower solids or higher energy to match SW's ease of application; conversely, SW systems may require slight thickening (e.g., higher clay fraction or supportive colloids) if a firmer structure is desired.

The rheological behaviour of the four clay suspensions is controlled by their smectite content, cation-exchange capacity (CEC), secondary minerals, water chemistry, and dispersion energy. All systems show shear-thinning, but the magnitude of the viscosity and its sensitivity to water type varies strongly with clay composition.

In purified water (PW), swelling and hydration dominate. ILB (CEC = 35.88 meq·g⁻¹, 80–85% smectite) (Chapter 3, Table 3.1, 3.3) consistently shows the highest viscosity—especially at 6000 rpm—indicating extensive platelet delamination and formation of a strong, swollen network. Its 10–15% kaolinite likely reinforces this network, producing higher low-shear

viscosities than the other clays. VF, despite having the highest CEC ($39.62 \text{ meq}\cdot\text{g}^{-1}$), produces lower viscosities than ILB, suggesting that calcite and possible textural differences limit the development of a continuous swollen structure. BB (CEC = $22.73 \text{ meq}\cdot\text{g}^{-1}$) shows moderate viscosities consistent with its lower smectite fraction and dilution by quartz. BC, with an unusually low CEC ($2.67 \text{ meq}\cdot\text{g}^{-1}$) (Chapter 3, Table 3.3), shows the lowest viscosity in PW, indicating limited swelling and weaker hydration-driven interactions.

In Saturnia mineral water (SW), the system shifts from swelling-dominated to ion-bridging/flocculation-dominated behaviour. Dissolved ions compress the double layer and promote cation-mediated aggregation. Under these conditions—and especially at high dispersion energy—BC becomes the most viscous clay. This reversal suggests that BC's surface chemistry, mixed-layer components, or layer charge distribution make it highly responsive to cation bridging in SW, allowing rapid re-aggregation into rigid flocs once tactoids are broken down at 6000 rpm. ILB, which dominates under swelling conditions, shows reduced viscosity in SW because double-layer compression suppresses its swelling advantage. BB and VF remain comparatively low in viscosity in SW due to either lower smectite content (BB) or the presence of calcite and other modifiers that limit strong network formation (VF).

Overall, the rheology is not predicted by smectite percentage alone. High viscosity in PW correlates with high CEC and strong swelling capacity (ILB, VF), whereas high viscosity in SW is governed by how surface charge and mixed-layer character respond to dissolved ions (BC). Thus, swelling-driven and ion-bridging-driven thickening mechanisms dominate under different water chemistry.

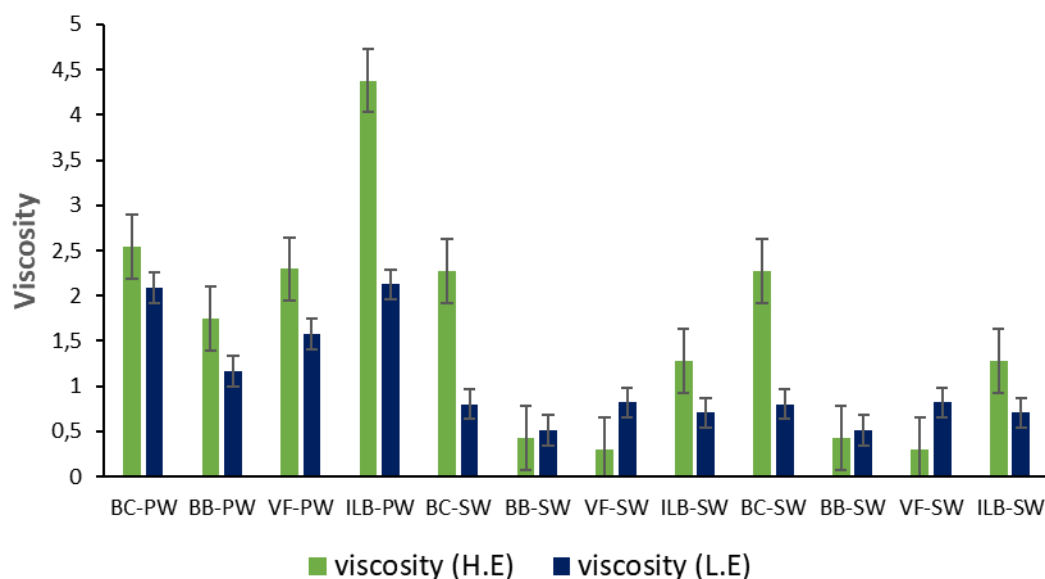


Figure 4.9: Comparison of high energies (H.E) and Low energy (L.E) on viscosity of suspensions.

4.7.1.3.2. Influence of water chemistry on viscosity

For every clay, **SW** < **PW** in viscosity across the measured $\dot{\gamma}$, the same trend holds at both processing energies (2000 rpm and 6000 rpm) reflecting the disruption of clay particle networks by the ions and minerals present in Saturnia water. Increasing energy from 2000 to

6000 rpm (5 min) increases viscosity in all systems but the **PW > SW** gap persists for all clay types indicating a more structured or gel-like network for purified water suspensions.

The main explanation for the observed lower viscosities in clay suspensions prepared with Saturnia thermal water compared to purified water is the presence of high anions, cations and minerals in saturnia water sulphates, calcium, magnesium and other ions. These dissolved salts and minerals interact with clay particles and influence the structure, aggregation state, and networking within the suspension, leading to modifications in the rheological behaviour. Minerals and electrolytes in saturnia water screen the charges on clay particle surfaces, reducing the thickness/rigidity of the interparticle network and reducing electrostatic repulsion that result in particle aggregation or flocculation. Such aggregation generally leads to more compact structures with lower viscosity, as opposed to the more dispersed and swollen systems formed in purified water.

The findings are consistent with previous reported rheological studies on clay suspensions in distilled versus mineral-rich waters (Carty et al., 2000). Clays with high cation exchange capacity, particularly those rich in smectite or montmorillonite components like many bentonites, swell more and form thicker hydration layers around their particles when dispersed in water, leading to higher viscosity in suspensions (Morais et al., 2019). Studies have shown that, when sodium exchange is optimized in bentonite, an increase in CEC corresponds directly to greater hydration, swelling, and thus increased viscosity (Zhang & Peng, 2015). For example, the swelling ability (and consequent viscosity increase) in sodium-rich clays arises from strong interlayer hydration and repulsion, which is most apparent in pure water conditions (Leiva et al., 2025).

Differences in water chemistry significantly affect clay behavior. In pure water, the dispersivity and swelling of clays—especially those with high CEC—are maximized because there are few competing cations to suppress interlayer expansion (Morais et al., 2019). In mineral or thermal waters, such as Saturnia water, high ionic strength and the presence of divalent or multivalent cations like Ca^{2+} , Mg^{2+} , etc., can neutralize charges on clay surfaces, suppress swelling, promote flocculation, and thus decrease viscosity, even for clays with high CEC (Leiva et al., 2025). Saturnia thermal water contains dissolved electrolytes (e.g., Na^+ , Ca^{2+} , Mg^{2+} , anions), they can form bridges between clay particles and can **exchange** with native interlayer cations (especially in smectitic components), decreasing interlayer swelling and promoting compact tactoids rather than fully dispersed platelets. Less swelling and fewer extended face–edge contacts translate to **low-shear viscosity** and a weaker yield structure. Studies show that peloid muds matured with sulphate-rich and Ca-Mg-sulphate waters exhibit reduced viscosity, plasticity and water retention compared to those made with distilled/purified water (Aguzzi et al., 2013).

In contrast, clay suspensions with purified water remain more swollen, with higher viscosity, due to the lack of ionic screening and greater repulsion between particles (Veniale et al., 2004). The lower viscosity of saturnia water suspensions can be attributed to the high ionic strength and specific interactions of dissolved ions with clay mineral surfaces, leading to increased aggregation and less water absorption.

Exceptions can occur if a clay with lower CEC (like BC) has surface properties or mineralogical characteristics that promote greater structure-building (e.g., due to specific interactions with ions in thermal water), which can lead to higher viscosity compared to higher CEC clays under these conditions (Barhoumi et al., 2021). In this study, we observed that differences of viscosity of Bentonite Benesa (BC) suspension prepared with PW and SW at high energy were less pronounced (Figure 4.11) it is attributed to the fact that BC have low CEC. On the other hand, highest viscosities were observed for ILB-PW suspensions it

is because ILB have highest CEC capacity and therefore in PW it build the strongest network with highest viscosities (Leiva et al., 2025; Morais et al., 2019; Zhang & Peng, 2015). In SW, electrolyte-induced compaction disproportionately reduces viscosity (η) for swelling clays (ILB), while a less-swelling clay (e.g., BC) retains comparatively more of its structure, becoming the most viscous within the lowered-viscosity set (Barhoumi et al., 2021; Leiva et al., 2025).

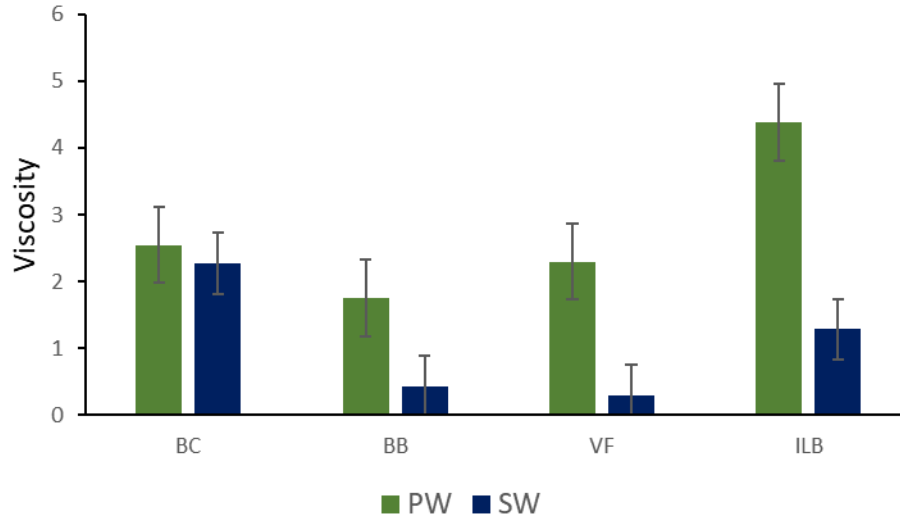


Figure 4.10: Influence of water chemistry on viscosity of Clay suspensions.

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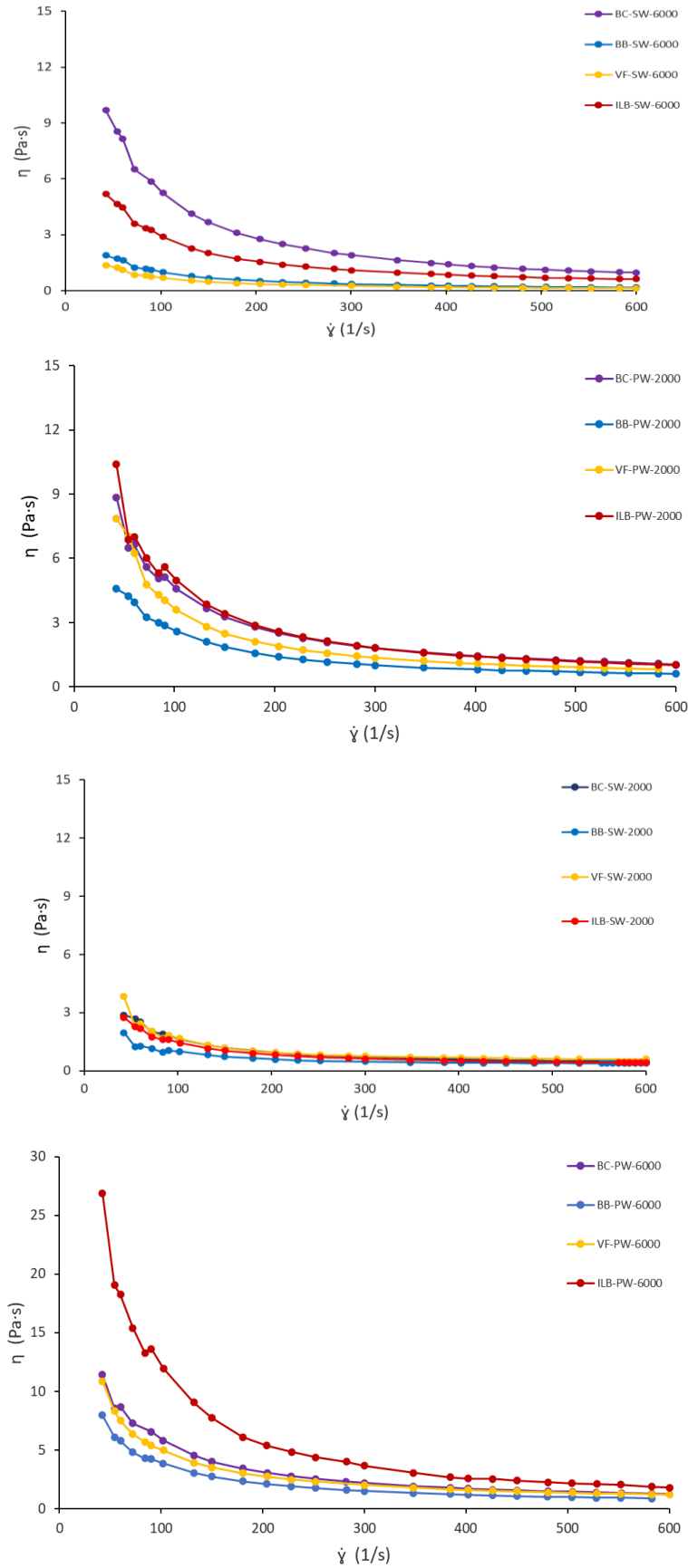


Figure 4.11 "Shear viscosity (η) as a function of shear rate (γ) for four clay suspensions prepared with purified water (PW) and Saturnia thermal water (SW) at high mixing energy (6000rpm, 5min) and Low mixing energy (2000rpm, 5min) .

4.7.1.3.3 Influence of pH on viscosities of suspensions

The lower viscosities observed for suspensions prepared with Saturnia water (SW) can be attributed to the combined effect of lower pH and higher ionic strength/mineral content of the thermal water. The reduced pH of SW suspensions partially neutralizes surface charges on clay particles (reducing deprotonation of surface sites), and the dissolved ions — particularly polyvalent cations — compress the electrical double layer and promote particle aggregation (cation bridging) (Cao et al., 2024). Both effects reduce electrostatic repulsion and interparticle hydration/swelling, resulting in poorer dispersion and lower suspension viscosity compared with the more alkaline purified-water (PW) systems. These observations are consistent with the measured pH shifts and the known influence of ionic strength and divalent cations on clay rheology (Amorim et al., 2007).

4.7.1.3.4 Relation between TGA/DTG characteristics and viscosity behavior.

The thermal (TGA/DTG) behavior of the four clays provides valuable insights into the factors governing their suspension viscosities. The DTG curves show distinct peaks corresponding to specific thermal events, such as dehydration (water loss at low temperature) and dehydroxylation (loss of structural hydroxyl groups at higher temperatures).

ILB clay stands out in both viscosity and TGA/DTG profiles. The ILB samples studied show the most pronounced dehydroxylation peak at higher temperatures (~400–600°C), indicating a higher content of expandable clay minerals-smectite 80-85% as supported by XRD results (Chapter 3, Figure 3.2d and TABLE 3.1). Such minerals have higher CEC and greater water retention/swellability (Chapter 3 Table 3.3), which directly explains why ILB-PW suspensions exhibit the highest viscosities—more interlayer hydration leads to increased swelling and greater resistance to flow. This links the thermal evidence of more tightly bound water and larger exchangeable cation capacity seen in TGA/DTG data with the higher suspension viscosity in PW.

Daneluz et al. (2020a) reported similar results that demonstrate-clays with more intense thermal events between 60°C and 220°C (surface water release) and 500°C–650°C (dehydroxylation of interlayer hydroxyls) showed higher viscosity and swelling in hydrophilic gels, since these properties represent a greater ability to retain and exchange water molecules and cations. As observed in ILB clay, both high dehydroxylation peaks and elevated viscosity in PW suspension indicate high CEC and swelling ability. Similarly, Kök et al. (2021) confirmed that different clay minerals exhibit distinct thermal behavior in TGA/DSC directly related to surface area and activation energy, which affects colloidal stability and rheology in suspension systems.

In contrast, BC shows a relatively less intense mass loss in the same TGA/DTG region and a smaller overall change, pointing to lower CEC and a reduced capacity for swelling and water retention (Chapter 3 Figure 3.2b, Table 3.1). This matches the smaller difference in viscosity observed between BC-SW and BC-PW suspensions. The TGA curves for BB and VF clays fall between ILB and BC, consistent with their intermediate viscosity behaviors; their moderate dehydroxylation peaks and mass losses suggest moderate CEC and swelling capacity.

In summary, clays exhibiting greater mass losses (particularly at the water loss and dehydroxylation steps in TGA/DTG) tend to show higher viscosity in water, particularly in purified water systems, due to increased swelling and hydration. The mineralogical factors

revealed by thermal analysis (such as the nature and content of hydrophilic clay minerals) thus reinforce and explain the observed rheological trends in suspensions.

4.7.1.3.5 Correlation between extensibility and viscosity

The relationship between viscosity and spreadability observed for the prepared clay suspensions and peloids can be interpreted as a manifestation of the general balance between internal structure strength and ease of deformation in semisolid systems. In the present work, combining all formulations into a single plot showed that formulations with higher apparent viscosity exhibited lower extensibility on the glass-plate test, yielding a clear inverse linear trend with a high coefficient of determination ($R^2 = 0.8234$). This finding indicates that, despite differences in clay type, aqueous phase (sulfurous Saturnia water versus purified water), and mixing energy, the macroscopic spreading behavior is mainly governed by the rheological resistance to flow (Garg et al., 2002).

Comparable trends are reported for cosmetic formulations structured by clays or clay-like particles. In the study, on clays sourced from sand-extraction residues were incorporated into gels and emulsions, and the resulting formulations were characterized in terms of viscosity, adhesion, and spreadability. The clay that produced stronger structuring (higher viscosity and adhesion) led to more cohesive, less easily spread gels, whereas the clay with the largest specific surface area improved balance between adhesion and spreadability, highlighting that tuning clay-induced viscosity is crucial for obtaining good application properties (Da Silva Favero et al., 2025a).

Similarly, a study on hydrophilic cosmetic formulations containing a natural clay reported pseudoplastic behavior and associated the rheological profile with high but controlled spreadability on the skin. The authors showed that formulations with suitable, intermediate viscosities displayed homogeneous spreading without phase separation, while excessively viscous or highly structured systems were less easy to distribute, again supporting that spreadability decreases when viscosity becomes too high. Technical literature on organoclay-based cosmetic gels also notes that these clays are selected to provide thixotropic viscosity that is high enough for stability but low enough for “easy dispersal and spread-ability” of the product (Daneluz et al., 2020b).

The present results extend these concepts to therapeutic peloids and model clay–water suspensions prepared under controlled mixing conditions. Saturnia-water peloids, containing dissolved electrolytes and sulfur species, generally showed lower viscosity and higher extensibility than analogous systems prepared with purified water, suggesting that the ionic composition weakens or rearranges the clay network, facilitating layer sliding and lamellar rearrangement during spreading. In contrast, suspensions made with purified water, especially at high shear rates, developed stronger colloidal networks (higher apparent viscosity and consistency), which resisted extension during the glass-plate test and therefore produced lower extensibility values (Burger et al., 2025).

By combining the rheological data of all formulations into a single power-law description and correlating viscosity with extensibility (Figure 4.12), this study quantitatively demonstrates a principle long noted qualitatively in cosmetic semisolids: increasing viscosity and structural strength enhances physical stability and skin adherence but simultaneously reduces spreadability. The high R^2 obtained for the inverse viscosity–extensibility relationship indicates that, within the explored formulation space, spreading behavior can be reliably predicted from rheological parameters. This provides a practical framework for the rational

design of future clay-based peloid formulations, enabling optimization for both handling characteristics and therapeutic performance (Da Silva Favero et al., 2025b).

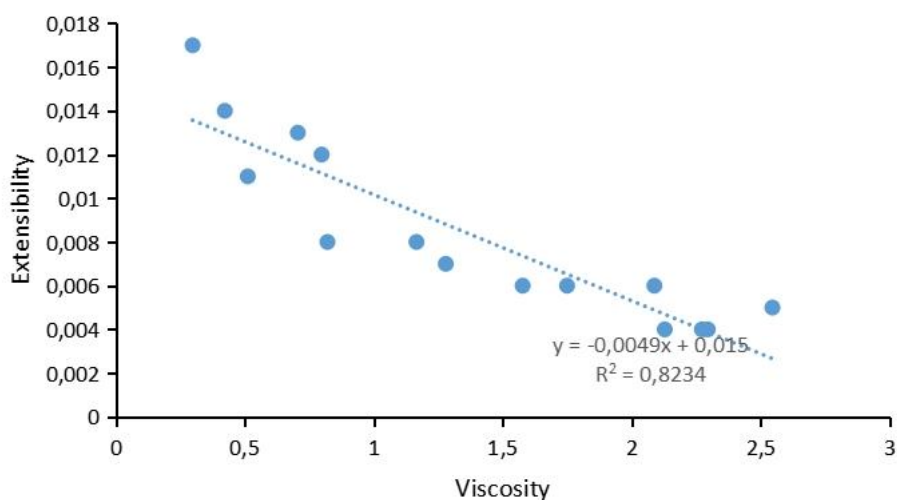


Figure 4.12: Relationship between apparent viscosity and extensibility for all prepared clay-based suspensions and peloids.

4.8. Conclusion

The rheological and extensibility properties of peloids are strongly influenced by the composition of the liquid phase. Suspensions prepared with Saturnia water (SW) consistently exhibited lower viscosities compared to those prepared with purified water (PW), which can primarily be attributed to the higher concentration of dissolved ions in SW. The elevated ionic strength and presence of divalent cations in SW compress the electrical double layer around clay particles, reducing electrostatic repulsion and promoting aggregation. Additionally, the lower pH of SW suspensions partially neutralizes the surface charges on clay particles, further decreasing particle swelling and dispersion stability, thereby reducing viscosity. Among the clay types studied, ILB showed the highest viscosities when suspended in PW, which aligns with its higher cation exchange capacity (CEC). The greater CEC implies a higher capacity for ion adsorption and swelling, enhancing the viscosity of ILB-PW suspensions due to increased interparticle hydration and repulsive forces. In contrast, the BC clay, with the lowest CEC among the samples, showed minimal differences in viscosity between BC-SW and BC-PW suspensions. This limited variation is consistent with the comparatively lower swelling capacity and ion exchange potential of BC, making it less sensitive to changes in water chemistry. These findings underscore the interconnected roles of mineral water composition, pH, and clay CEC in controlling the viscosity and rheological behavior of clay suspensions used in therapeutic peloids. The complex interplay of these factors must be considered when designing and optimizing such systems for specific applications.

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CHAPTER 5

PELOIDS ENHANCEMENT WITH CLINICAL ADDITIVES



KEY CONCEPTS

- Essential oil molecules intercalation into the confined interlayer space of montmorillonite was energetically less favourable due to the hydrophilia of the interlayer space.
- However, the adsorption of terpenes on the external surfaces is more favourable owing to the hydrophobicity of the silanol layers of the external surface of montmorillonite
- Adsorbates can be adsorbed in the phyllosilicate clay mineral by using non-polar solvents or without solvents
- Diffusion of organics is one order of magnitude slower than water in the interlayer space of MNT, predicting the high stability of these guest compounds into the confined interlayer space of MNT
- Drug release is easy therefore they can act as drug-delivery bioactive compounds for therapeutic skin-treatment, especially for specialized thermal baths

5. Biopharmaceutical Additives-Terpenes as Penetration Enhancers-Mechanistic and Molecular level insights

The outermost layer of the skin, the stratum corneum (SC), is the principal barrier to transdermal and topical delivery of drugs. The SC consists of corneocytes embedded in a lipid-rich intercellular matrix predominantly composed of ceramides, free fatty acids, cholesterol and their esters (Cal, 2006). Disruption or modulation of this barrier is the focus of chemical penetration enhancers (CPEs). Among these, naturally-derived terpenes and essential oil constituents have gained significant attention owing to their favourable safety profiles and demonstrated permeation enhancement properties (Longgos et al., 2024). Here we specifically focus on mechanistic and molecular-level evidence for terpenes—including limonene, α -pinene, β -caryophyllene and others—as penetration enhancers, with emphasis on lipid-membrane interactions, molecular dynamics (MD) simulations, structural perturbation of the SC and structure–activity relationships.

5.1. Mechanisms of Action at the Molecular Level

Terpenes appear to enhance skin penetration by several inter-related mechanisms: disruption or fluidisation of SC intercellular lipids, extraction of lipid components, increased partitioning of the drug into the SC, and in some cases modulation of keratin or protein domains (Sapra et al., 2008; Williams & Barry, 1991). A widely-cited three-step mechanistic model is: (1) partial disordering of the lipid alkyl chains of the SC, (2) interaction of terpenes with lipid head groups (e.g., ceramides, cholesterol) and disruption of hydrogen bonding networks, and (3) increased hydration and formation of new polar pathways within the SC (Jiang et al., 2017).

5.1.1. Lipid disruption and fluidisation

A core mechanistic effect is the ability of terpenes to partition into the SC intercellular lipids, disturb the ordered lamellar lipid structure and thereby reduce the energy barrier for drug diffusion. Many systematic reviews confirm that the enhancement ratio (ER) of terpenes correlates with their lipophilicity (cLog P) and ability to disrupt SC lipids, supporting that hydrophobic terpenes favour interaction with the lipid tails (J. Chen et al., 2016; El-Kattan et al., 2001).

5.1.2. Interaction with lipid head groups and hydrogen-bonding networks

Beyond mere tail-disordering, terpenes may interact with polar head groups of ceramides or cholesterol and disrupt hydrogen bonds, altering the lipid packing and increasing permeability. Terpenes such as geraniol partition into SC lipids and interact with the lipid matrix to promote permeation, as demonstrated by molecular dynamics simulations of chemical enhancers in skin barrier models (Rocco et al., 2017).

5.1.3. Partitioning and drug co-solvation

Terpenes can also increase drug partitioning into the SC by acting as co-solvents or carriers. As reported by Chen & Pan (2016) the terpenes possess high enhancement activity for both hydrophilic and lipophilic drugs even at low concentration. Terpenes interact with SC components by more than one mechanism, although their interactions with SC intercellular lipids could be the key mechanism. An important molecular safety aspect is that many terpenes appear to cause reversible perturbation of the SC lipids, rather than permanent damage. For example, β -caryophyllene was shown to reduce skin electrical resistance and increase TEWL (transepidermal water loss) in a reversible manner, with recovery of barrier function, while synthetic enhancer azone caused irreversible increases in erythema.

5.2. Overview of terpene-based penetration enhancers

5.2.1. D-Limonene

D-limonene (a monocyclic monoterpene abundant in citrus oils) is one of the most studied hydrocarbon terpenes. In in vitro skin models it **increases permeation of lipophilic drugs** (e.g., butylparaben) more than of hydrophilic markers, consistent with increased partitioning into lipid domains. Limonene has been used in formulation approaches (nanoemulsions, carriers) to enhance delivery of compounds such as curcumin. Many early and recent studies confirm its enhancer activity, but irritation at higher concentrations and formulation-dependent variability have been reported (Koyama et al., 1994)

5.2.2. α -Pinene

α -Pinene (a bicyclic hydrocarbon monoterpene found in pine oils) has shown penetration-enhancing potential in comparative experiments with other terpenes (e.g., Almirall et al. comparative skin transport studies). Besides penetration effects, α -pinene has been examined for wound-healing and anti-inflammatory activities that may be beneficial in topical therapeutics. Enhancement magnitude is often lower than for some oxygenated terpenes, but α -pinene's low molecular weight and high volatility make it useful in certain formulations. (Almirall et al., 1996).

5.2.3. β -Caryophyllene

β -Caryophyllene is a sesquiterpene (larger, more lipophilic) that has recently emerged as a promising penetration enhancer — notably showing strong interference with SC lipids, higher SC retention, and superior enhancement compared with 1,8-cineole and even azone (a classical synthetic enhancer) in Franz cell. The experimental study demonstrated β -caryophyllene improved permeation of hydrophilic macromolecules and altered SC lipid order (ATR-FTIR / SER evidence), suggesting sesquiterpenes can be highly effective, possibly with lower irritation risk than some synthetics. This marks an important extension of the terpene toolbox beyond small monoterpenes (Tang et al., 2023).

5.2.4. Ocimene

Ocimene (an acyclic monoterpene found in various floral oils) is less well studied specifically as a penetration enhancer. Reviews of acyclic monoterpenes note that this class can influence skin penetration and that biological/toxicological profiles are highly structure-dependent; however, direct experimental data explicitly evaluating ocimene's permeation-enhancing potency are scarce in the peer-reviewed literature. Thus ocimene remains under-characterised and would benefit from targeted in vitro Franz cell and mechanistic studies. (Dascalu et al., 2023).

5.2.5. Elemene

“Elemene” typically refers to a group of sesquiterpenes (e.g., β -elemene) studied mainly for anti-cancer activity. Several formulation studies have investigated transdermal delivery systems for elemene (i.e., elemene as the drug rather than as an enhancer), and some works explore combinations of matrices and classical enhancers to optimise delivery. There is limited evidence that elemene itself acts as a penetration enhancer for other drugs; most data concern its own topical/transdermal administration. More direct head-to-head enhancer studies are needed (Zeng et al., 2011).

5.3. Structure–Activity relationships and formulation considerations

A number of factors influence the enhancer activity of terpenes at the molecular level:

- **Lipophilicity (cLog P):** Higher log P of the terpene often correlates with higher enhancement ratios in human skin ($r \approx 0.67$) (Prasanthi & Lakshmi, 2012)
- **Molecular size/weight and saturation:** Smaller terpenes may diffuse more quickly into the SC lipids; larger sesquiterpenes may have stronger retention and sustained disruption.
- **Functional groups:** Oxygenated terpenes (e.g., cineole, terpineol) show enhanced interaction with polar headgroups and may be more effective for hydrophilic drugs, whereas hydrocarbons (e.g., limonene) favour lipophilic drugs (Kaspute et al., 2025)
- **Concentration:** Typical effective concentrations lie between 1–5% w/w; however, the relationship between concentration and flux enhancement is not always linear.
- **Vehicle/Excipient interactions:** The vehicle influences the partitioning of terpene into the SC and thus its effective concentration at the barrier
- **Reversibility of disruption:** At molecular level, reversible perturbation (rather than permanent extraction of lipids) is desirable for safety; MD studies show reversible structural changes in lipid order upon terpene treatment (Tang et al., 2023; Uchino et al., 2024).

At the molecular level, terpenes and essential oil constituents act as penetration enhancers primarily by interacting with and perturbing the ordered lipid matrix of the SC—disordering the alkyl chains, disrupting hydrogen bonding headgroup networks and increasing

CHAPTER 5: Design of Essential oil Formulations with Clays

partitioning and diffusivity of drug molecules. Recent molecular dynamics simulations (e.g., of limonene, cineole and β -caryophyllene) provide atomistic insight into how terpenes penetrate into cholesterol-rich domains (Yu et al., 2024), alter energy barriers and thereby enhance permeation. Structure–activity relationships highlight lipophilicity, functional group and molecular size as key determinants of efficacy. For translation into topical/transdermal products, mechanistic understanding must be coupled with formulation design, safety/irritation profiling and reversible barrier modulation. As the field advances, extending mechanistic modelling to under-studied terpenes, combining enhancers synergistically and correlating molecular perturbation data with in-vitro/in-vivo flux metrics will strengthen the scientific basis for terpene-based penetration enhancement.

5.4. Molecular modeling of the adsorption and delivery of α -pinene and similar terpenes of essential oils on montmorillonite surfaces. (Article 4)

Essential oils are complex mixtures of volatile and aromatic compounds produced by plants, and they have been recognized for their therapeutic properties, particularly their anti-inflammatory, antimicrobial, and antioxidant activities (AMIRI et al., 2015). Among the numerous constituents of essential oils, monoterpenes such as limonene, β -ocimene, β -elemene, and α -pinene, and the sesquiterpene β -caryophyllene, have attracted significant attention due to their potent anti-inflammatory effects. These molecules have the methyl-ethenyl group as the common structural feature.

Limonene (Figure 5.1), a monocyclic monoterpene found abundantly in citrus oils, has demonstrated anti-inflammatory activity by downregulating pro-inflammatory cytokines such as TNF- α and IL-6 and inhibiting the activation of the NF- κ B pathway (Santana et al., 2020). The β -ocimene (Figure 5.1), another monoterpene commonly found in basil and lavender oils, has been used for inhibiting nitric oxide production and reducing inflammatory responses in vitro, although its mechanisms are less well defined compared to other terpenes (Laraib et al., 2025). The α -pinene, a bicyclic monoterpene (Figure 5.1) widely distributed in pine and coniferous essential oils, exhibits both anti-inflammatory and bronchodilatory effects by suppressing COX-2 expression and prostaglandin E2 production (Qiu et al., 2024). The β -caryophyllene, a bicyclic sesquiterpene (Figure 5.1) present in clove and cannabis oils, acts as a selective agonist of the cannabinoid receptor type 2 (CB2), thereby exerting immunomodulatory and anti-inflammatory effects without psychoactivity (Guo et al., 2025). Similarly, β -elemene (Figure 5.1), a naturally occurring sesquiterpene commonly extracted from plants like *Curcuma wenyujin*, has demonstrated substantial anti-inflammatory and anticancer activities. β -Elemene inhibits the production of inflammatory mediators such as TNF- α , IL-1 β , and IL-6 by interfering with NF- κ B and MAPK signaling pathways (Liu et al., 2015). β -Elemene is a candidate of interest in various pharmaceutical formulations due to its multifaceted bioactivity, including those targeting chronic inflammation and tumor microenvironments (Xie et al., 2020).

Despite the pharmacological promise of these compounds, the clinical application of essential oils is limited by several physicochemical constraints. Notably, their high volatility, low aqueous solubility, and chemical instability under environmental and physiological conditions restrict their bioavailability and therapeutic efficacy (Baptista-Silva et al., 2020). Upon exposure to air or elevated temperatures, essential oil components readily evaporate, and many of them are susceptible to oxidation or degradation, making formulation into stable and controlled delivery systems a persistent challenge.

To address these limitations, various encapsulation and adsorption techniques have been explored to enhance the retention and bioavailability of volatile therapeutic compounds. Among these, the use of phyllosilicate clay minerals, particularly MNT, offers a promising strategy. Montmorillonite (MNT) is a 2:1 layered aluminosilicate with a high surface area, cation-exchange capacity, and interlayer spacing that can accommodate a range of guest molecules (Aguzzi et al., 2007a). Its structural features—comprising negatively charged

tetrahedral-octahedral-tetrahedral (T-O-T) layers—make it well-suited for adsorption and intercalation of small organic compounds, thereby improving their thermal stability, volatility, resistance to oxidation, and controlled release profiles (Tenci et al., 2017; Yang et al., 2019).

Recent studies have demonstrated the viability of clay–essential oil nanohybrids for this adsorption-desorption purpose (Berraaouan et al., 2025; Ghrab et al., 2018). For instance, Aguogui et al. (2022) studied the adsorption of limonene and carvone from caraway oil onto Tunisian bentonite, showing that clay significantly delayed volatilization and improved chemical stability. Their results highlighted the MNT ability to be used as an effective pharmaceutical excipient for essential oil-based therapies. Similarly, Essifi et al. (2022a) developed montmorillonite nanoclay formulations capable of selectively retaining and releasing volatile essential oil compounds, depending on the molecular polarity and structural compatibility of the oil component with the clay matrix.

Moreover, Saucedo-Zuñiga et al. (2021a) investigated the use of laminar nanoclay and porous halloysite nanotubes in multilayered film composites for essential oil encapsulation. Their work confirmed the role of clays in retarding diffusion and prolonging release, thus enhancing the functional shelf life of active compounds in food and pharmaceutical applications. In a related application, Tsagkalias et al. (2021) explored Na- and organo-modified MNT/essential oil nanohybrids and demonstrated their influence not only on release behavior but also on the kinetics of in situ polymerization, indicating the versatility of such hybrids in biomedical and materials science.

Although experimental studies have provided valuable macroscopic insights, however a molecular-level understanding of the adsorption mechanisms of essential oil compounds onto clay surfaces remains limited. Computational tools applying molecular modeling with computational chemistry techniques, such as Force Fields (FF) and Density Functional Theory (DFT), offer a powerful approach to study clay mineral properties intermolecular interactions, adsorption energetics, and spatial configurations at atomic resolution (Francisco-Márquez et al., 2017). These techniques allow us for the prediction of vibrational binding modes, adsorption and desorption energies of bioactive organic compounds onto clay mineral surfaces at atomic scale, providing critical information for the rational design of clay-based drug delivery systems (Cygan et al., 2004; Meruvia-Rojas et al., 2025a). These insights are particularly critical for the optimization of formulations involving highly volatile or reactive molecules such as essential oils.

In this study, we apply molecular modeling methodology for investigating at atomic scale the crystallographic and spectroscopic properties of the crystal structure of α -pinene and the adsorption behavior of five anti-inflammatory terpenoids essential oil components— α -pinene, limonene, β -ocimene, β -caryophyllene, and β -elemene (Figure 5.1) on the MNT surfaces. Adsorption and desorption energies were calculated to assess the thermodynamic stability of each molecule in different adsorption states. The results of this study provide molecular-level insights into the feasibility of using MNT as a carrier system to enhance the stability, reduce volatility, and improve the delivery potential of essential oil-based anti-inflammatory agents on skin treatments.

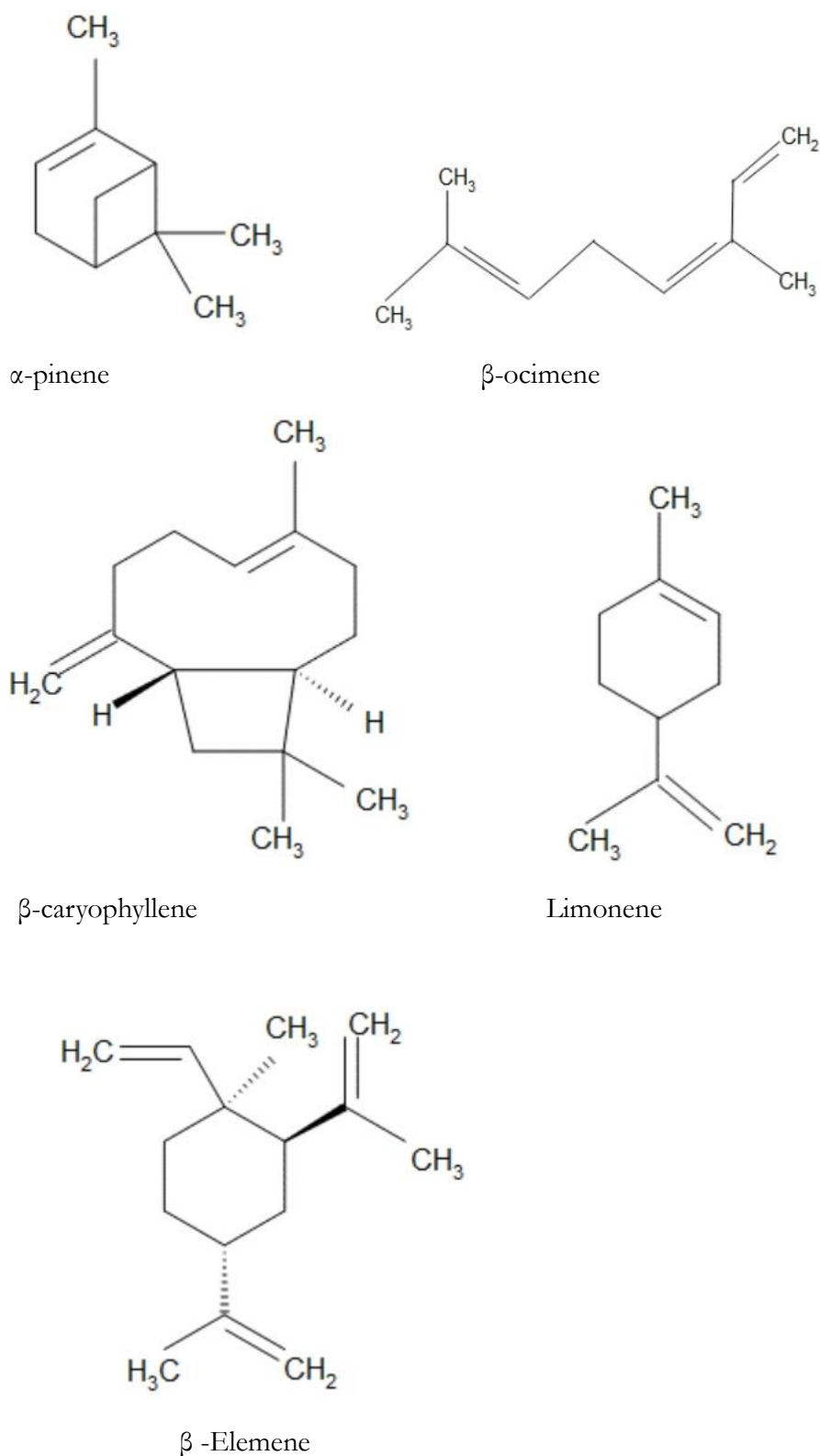


Figure 5.1. Molecular models of the studied terpenoid components of essential oils.

5.4.1. Models

The molecular structure of limonene [1-methyl-4-(1-methylethenyl)-cyclohexen] was taken from the crystal structure of a sulfonate derivative (CCDC 2312929), the molecular structure of β-elementene, [(1S,2S,4R)-1-methyl-2,4-di(prop-1-en-2-yl)-1-vinylcyclohexane], β-

caryophyllene, (1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene, and β -ocimene [(3E)-3,7-dimethylocta-1,3,6-triene], were extracted from PUBCHEM. The molecular structure of α -pinene was extracted from crystal structure determined by X-ray diffraction (ref. CSD-176009) (Bond & Davies, 2001).

The phyllosilicate employed in this study is MNT, whose crystallographic structure was adopted from a previous work (Meruvia-Rojas et al., 2025b). Our MNT model is a dioctahedral (T-O-T) clay mineral, and its chemical composition closely resembles that of Veegum, a pharmaceutical-grade clay commonly used in pharmacological applications (Borrego-Sánchez et al., 2020). The clay exhibits a low degree of Al^{3+} substitution in the tetrahedral sheets and a minor presence of Mg^{2+} in the octahedral sheets, with a representative unit cell composition of $\text{Na}(\text{Al}_{3.17}\text{Mg}_{0.83})(\text{Si}_{7.83}\text{Al}_{0.17})\text{O}_{20}(\text{OH})_4$.

For adsorption modeling, a $3 \times 2 \times 1$ supercell was constructed with the composition $\text{Na}_6(\text{Al}_{19}\text{Mg}_5)(\text{Si}_{47}\text{Al}_1)\text{O}_{120}(\text{OH})_{24}$. Each interlayer Na^+ cation was coordinated by two water molecules. Based on prior studies of cation substitution in MNT (Sainz-Díaz et al., 2005a), Mg^{2+} cations in the octahedral layer and Al^{3+} cations in the tetrahedral (T) layer were distributed to achieve maximal spatial dispersion, following the methodology reported by Escamilla-Roa et al. (2017). For larger adsorption systems a $6 \times 4 \times 1$ supercell was also used. For simulating the external surface of MNT, we included an empty space on the (001) mineral surface applying $c = 30 \text{ \AA}$. In the hydrated models, a box with 3-D periodical boundary conditions of $18 \times 18 \times 18 \text{ \AA}^3$ was filled with water molecules (200 molecules) with Monte Carlo method using INTERFACE at 1 g/cm^3 density (waterbox). One molecule of the organics was placed in this waterbox for modeling the hydration states (water-org).

5.4.2. Methodology

Our calculations were conducted using empirical interatomic potentials incorporated in the INTERFACE (Sainz-Díaz et al., 2005b) and COMPASSIII (Sun, 1998) force fields (FF) under three-dimensional periodic boundary conditions. The INTERFACE has previously demonstrated excellent accuracy in systems similar to the one studied here (N. L. Jorge et al., 2024). Structural optimizations were carried out using the Forcite module within the Materials Studio software package (Biovia, 2024). The convergence was taken at 2×10^{-5} kcal/mol threshold. Van der Waals interactions were evaluated using the Lennard-Jones potential, $V(r) = \epsilon[(\sigma/r)^{12} - 2(\sigma/r)^6]$. Both van der Waals and Coulombic non-bonded interactions were treated using the Ewald summation method. For the hydrated systems, three-dimensional periodic boxes containing water molecules were generated via Monte Carlo method to achieve a density of 1 g/cm^3 . Each molecule is introduced in the centre of these water-boxes. On the other hand, several molecular dynamics simulations have been performed with INTERFACE at NVT and NPT ensembles with 1 fs steps along several simulation times: 5 ps (NVT) and 16.2 ns (NPT). In the last simulation and after the equilibration stage in the first 50,000 steps, we sampled 324 structures from 16,200,000 frames, one each 50,000 frames for further analysis.

For comparison, some quantum mechanical calculations based on DFT were performed using the CASTEP code (Segall et al., 2002) with GGA (Generalized Gradient Approximation) and PBE (Perdew-Burke-Ernzerhof) as correlation and exchange functional

and norm-conserving pseudopotentials generated on the fly (OTFG) with the Koelling-Harmon relativistic treatment with 3-D periodical boundary conditions. For dispersion forces, the Grimme G06 semi-empirical correction was applied (Grimme, 2006). This methodology was successfully used previously in similar systems (Bond & Davies, 2001). After a full optimization, a vibrational frequencies analysis was performed assigning atomic vibration modes by means of Density Functional Perturbational Theory (DFPT) Rafson et al., (2006) by linear response of phonon calculations with CASTEP for the Raman and Infrared (IR) spectra. The IR spectra were smoothed by a Lorentzian function with a full width at half maximum parameter (FWHM) of 30 cm⁻¹. The Raman spectra were simulated with a 514.5 nm incident light (Ar laser) at 298 K applying the same Lorentzian function as above.

5.4.3. Results

5.4.3.1. Crystallographic and spectroscopic properties of α -pinene crystal structure.

Crystal structure of α -pinene was calculated by using both FF, INTERFACE and COMPASS, performing full optimization runs (atomic positions and the lattice cell parameters at a time). Both FF yield similar values of cell parameters being slightly closer to experimental one those of Interface FF. However, we found that COMPASS FF does not reproduce neither distinguish the C=C double bond of α -pinene, $d(\text{C-C}) = 1.545 \text{ \AA}$, $d(\text{C=C}) = 1.543 \text{ \AA}$ (Table 5.1), whereas the INTERFACE FF distinguishes quite well both bonds, $d(\text{C-C}) = 1.520 \text{ \AA}$, $d(\text{C=C}) = 1.341 \text{ \AA}$, being the experimental values: $d(\text{C-C}) = 1.506 \text{ \AA}$, $d(\text{C=C}) = 1.320 \text{ \AA}$ (Bond & Davies, 2001). Computed bond lengths are slightly larger than experimental values, however, they are below the experimental error threshold. Hence, we can consider that the experimental crystal cell parameters have been quite well reproduced by the Interface FF (Figure 5.2). These results validate the use of INTERFACE and it will be used for the rest of this work. For comparison we calculated this crystal structure also at DFT level finding similar results to INTERFACE (Table 5.1). This fact corroborates the selection of this FF for this work.

Table 5.1. Experimental and calculated main geometrical features of the crystal structure of α -pinene (distances in $\text{\AA}$). In all cases, the angles are 90° .

Geometric features	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i> (C-C)	<i>d</i> (C=C)
experimental	7.19	7.59	15.92	1.506	1.320
Calculated INTERFACE FF	7.10	7.59	15.73	1.520	1.341
Calculated COMPASS FF	7.19	7.46	15.34	1.545	1.543
Calculated DFT	7.13	7.57	15.79	1.554	1.345

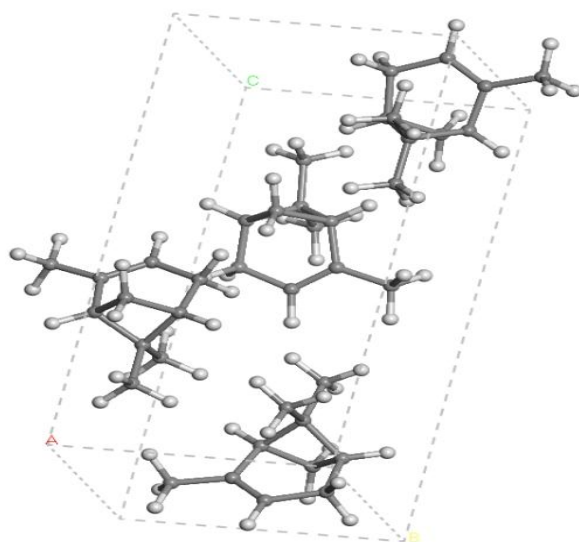


Figure 5.2. Optimized unit cell of the crystal structure of α -pinene. The H, and C atoms are in white and grey colours.

We can calculate the packing energy of this crystal comparing its energy with that of the isolated molecule. Then, the isolated molecule of α -pinene was optimized, including it in a 3-D periodical box of $15 \times 15 \times 15 \text{ \AA}^3$, which is big enough to avoid interactions with vicinal molecules. Taking into account that there are 4 molecules per unit cell, the packing energy will be:

$$E_{\text{pack}} = E_{\text{cryst}} - Z * E_{\text{mol}}$$

where E_{pack} is the packing energy, E_{cryst} is the energy of the unit cell of the optimized crystal structure, Z is the number of molecules per unit cell, and E_{mol} is the energy of the optimized isolated molecule. Our calculations yielded a packing energy of -62.15 kcal/mol per unit cell, being -15.54 kcal/mol per molecule.

On the other hand, the experimental spectroscopic properties are based on the vibration of the atoms of our systems and our calculations are based on atoms. Hence, both approaches, experimental and theoretical, are directly related. Our DFT calculations allow us to study the spectroscopic properties of this crystal. In general, theoretical analysis of IR and Raman spectra are performed with isolated molecules and compared with experimental data (Borrego-Sánchez et al., 2017). However, nowadays the experimental spectra are performed at solid state where the compound is at crystal state not as isolated molecule. In this work we compare the experimental data with frequency values calculated directly in crystal structure of α -pinene as a first time. The spectra of IR and Raman shown in Figure S1 (see Supplementary Material) are calculated for the crystal structure of α -pinene as a first time. The theoretical IR spectrum is close up to the experimental spectrum of pinene ices reported in refs. (De Barros et al., 2025). The Raman spectrum is consistent with the experimental one in frequency values (An et al., 2016).

In general, the calculated frequencies are consistent with the experimental data (Table 5.2). Our calculations can distinguish the vibrations of the C-H bonds of methyl and CH_2 groups

that are in the cycle of 4 C atoms from those of the cycle with 6 C atoms. The stretching $\square$ (C-H) frequencies of those from cycle of 4 C atoms are higher than those of the cycle of 6 C atoms, whereas the opposite is observed in the bending $\square$ (C-H) frequencies. Our calculated alkenyl $\square$ (C-H) frequencies are slightly higher than the experimental, though in both cases appear at higher frequency than this mode of CH₂ or CH₃ groups. Although the zone of the $\square$ (C-H) bands a combination of several vibrations occur, we can observe that the symmetric umbrella $\square$ (C-H) mode of CH₃ appear at lower frequencies than the asymmetric mode.

Table 5.2. Calculated by DFPT and experimental IR frequencies of the main vibration modes (in cm⁻¹) of α -pinene.

Mode ^a	Exp. ^b	calculated
$\square$ (C-H) (HC=C)	3022, 3023 ^f	3079, 3078
$\square$ (C-H) CH ₂ ^c	2990 ^f	3050 _{as} , 2985 _s
$\square$ (C-H) CH ₃	2968-2938 _{as} ^f , 2874 _s ^f	3025-2964 _{as} , 2925-2922 _s , 2906 _s ^d
$\square$ (C-H) cyclic	2918 ^f	2943
$\square$ (C-H) CH ₂ ^d	2982-2837, 2832 ^f	2888 _s
$\square$ (C=C)	1653, 1659 ^c , 1661 ^f	1651
$\square$ (C-H) CH ₂	1443, 1469-1443 ^c 1472-1442 _s ^f , 1265-1204 ^c , 1470-1465 _s ^d , 1409-1406 _s ^c , 1190 1337-1205 ^f	
$\square$ (C-H) CH ₃	1371, 1333, 1380-1367 _{umb} ^c , 1449 ^f , 1381-1452-1416 _{as} , 1354-1349 _{umb} 1363 _{umb} ^f	
$\square$ (C-H) CH	1261, 1211, 1124 ^c , 1249-1126 ^f	1308-1200, 1171-1029

^a exp. = experimental, $\square$ = stretching mode, $\square$ = bending mode in-plane, s = symmetric, as = antisymmetric, umb = umbrella. ^b Experimental values (Pradhita & Rahman, 2015). ^c Cycle of 4 C atoms. ^d Cycle of 6 C atoms. ^e Experimental data (An et al., 2016). ^f Experimental data at 50 K (De Barros et al., 2025).

5.4.3.2. Intercalation of the organic compounds on clay mineral interlayer space.

Each isolated molecule was included in a 3-D periodical box of 18x18x18 Å³, being big enough to avoid interactions with vicinal molecules and optimized as isolated molecule inside this box (Figure 3). Hence, these models represent a gas phase state of these molecules or in an inert media. In the α -pinene molecule the geminal methyl groups are oriented in a perpendicular plane with respect to the bicycle moiety (Figure 5.3a). The $\square$ -ocimene molecule maintains the extended linear orientation where one methyl H atom is oriented to the π electron cloud of the terminal C=C double bond (Figure 5.3b). In the $\square$ -caryophyllene molecule the terminal double bond and the methyl groups are in a perpendicular orientation with respect to the bicycle (Figure 5.3c). The large cycle of 9 C atoms has a C=C double bond in *trans* configuration and the vinyl and methyl substituents are in the same site of the cycle (*syn* configuration), whereas the cycle of 4 C atoms are in *trans* configuration with respect to the big cycle. The isopropenyl group of limonene is close to coplanar orientation with the ring plane (Figure 5.3d). In the $\square$ -elemene molecule the cyclohexyl group is in chair conformation, and two isopropenyl groups are in an approximately perpendicular orientation with respect to the ring plane, the same as the terminal C=C group, whereas the other

isopropenyl group is close to a coplanar orientation with respect to the cyclohexenyl ring plane. This ring is in an envelope configuration (Figure 5.3e).

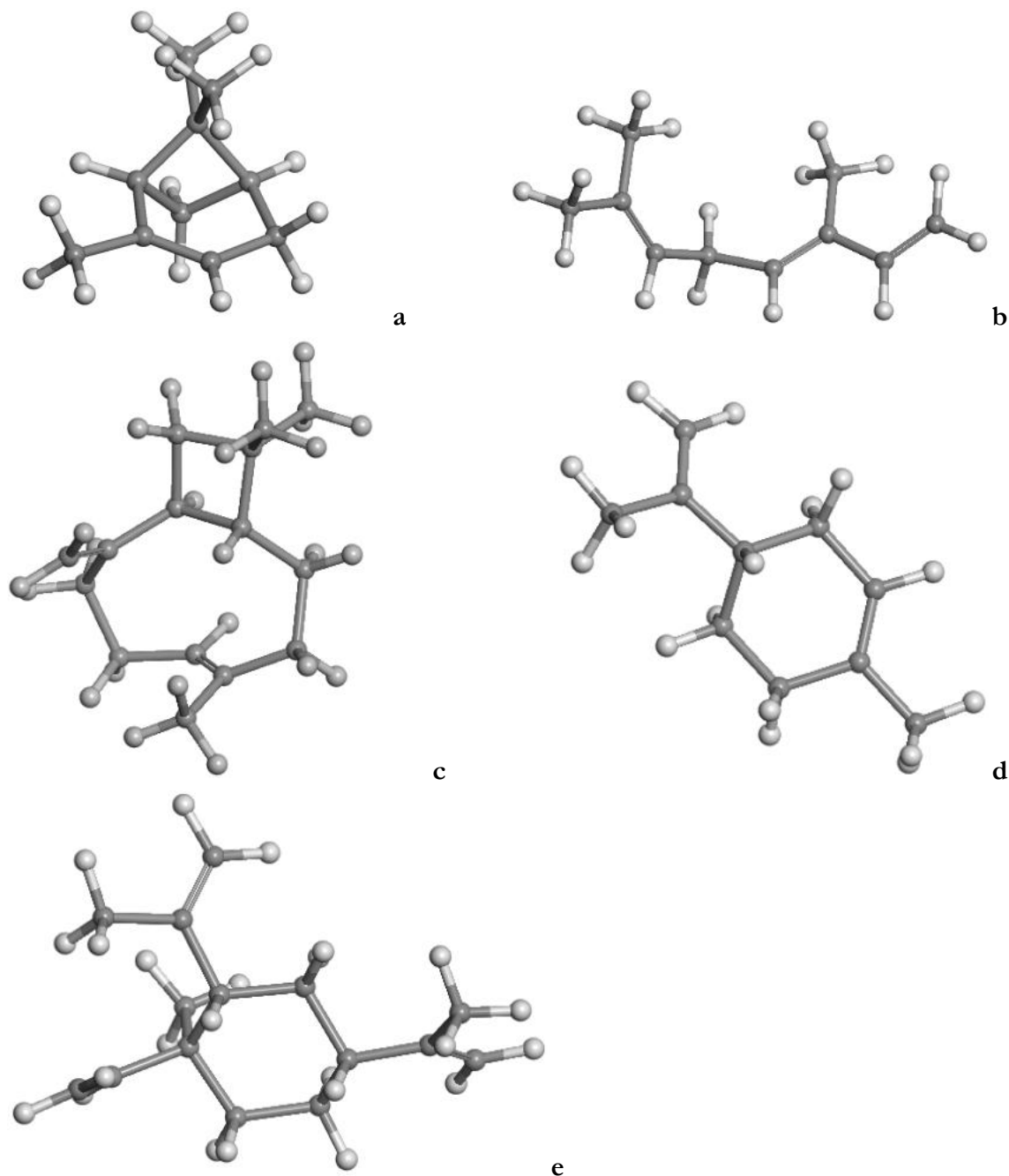


Figure 5.3. Optimized molecular structures of (a) α -pinene, (b) β -ocimene, (c) β -caryophyllene, (d) limonene, and (e) β -clemene.

The MNT structure was fully optimized considering the atomic positions and lattice cell parameters. Each molecule was placed in the center of the interlayer space and optimized. The crystal structure of MNT was not disturbed significantly with the interlayer adsorption of essential oils components changing only the interlayer spacing $d(001)$ that increases with the intercalation (Table 5.3). In all cases some changes in the molecular structure of adsorbates are observed with respect to those as isolated molecules. The increase of the interlayer space is greater for α -pinene and β -caryophyllene due to

their bicyclic structure and higher volume. Indeed, the conformation of these bicycles and the disposition of the substituents yields the largest cycle perpendicular to the interlayer surface opening the interlayer space. So, α -pinene-MNT complex increases 2 Å the c axis with respect to MNT (Table 5.3, Figure 5.4a) Therefore, this molecule is not placed in a parallel orientation with respect to the mineral surface adopting a globular form and some C-H bonds are oriented to the mineral surface O atoms, especially those of the terminal alkenyl group. For similar reasons, β -caryophyllene-MNT complex increases 1.6 Å the c axis with respect to MNT, being the second one in c -axis increasing order of our molecules (Table 5.3, Figure 5.4c). On the contrary, the increase of interlayer space in the adsorption of β -ocimene is very low due to the linearly extended disposition of the molecule adopting a parallel orientation along the mineral surface, where some H atoms are oriented towards the basal O atoms of the interlayer mineral surface $d(\text{CH}\dots\text{OSi}) = 2.34\text{--}2.84$ Å (Figure 5.4b). A similar behaviour is observed in limonene where the adsorbate adopts a parallel orientation with respect to mineral surface producing the smallest increase of interlayer spacing, where the H atoms are interacting with the surface O atoms $d(\text{CH}\dots\text{OSi}) = 2.28\text{--}2.72$ Å. Similarly, the intercalation of α -elemene the cycle and the terminal C=C double bond are parallel to the mineral surface, where methyl and other C-H bonds are oriented towards the mineral surface O atoms. In general, in the interlayer space some Na^+ cations are coordinated by water molecules and mineral surface O atoms.

One of the aims of this work is not focused only to the innovative academic research, but also to a more applied research especially to the possible therapeutic and clinical application for skin treatment and thermal baths. Then, the essential oils come from extracts of natural plants and really these extracts are complex and composed by many natural components. So, we do not use only one chemical compound in the preparation of a clay-essential-oil composite, but we introduce also several compounds to the clay mineral phase. Hence, we generate a model of MNT with the simultaneous intercalation of all molecules studied in this work. For this purpose, a $6\times 4\times 1$ supercell of MNT was generated with an interlayer space of $c = 20$ Å and one molecule of α -pinene, β -ocimene, β -caryophyllene, α -elemene, and limonene were intercalated randomly and simultaneously (2568 atoms) in the interlayer space of MNT (Figure 5.4). This combined system was fully optimized yielding a higher interlayer space (Table 5.3). Between all these molecules there are different interactions with water molecules and interlayer cations. We can see that some Na^+ have been pushed aside to the mineral surface on the tetrahedral cavities (Figure 5.4).

A molecular dynamics simulation was performed with this combined structure using 1 fs steps at a NVT ensemble at 298 K (5 ps, 100 samples from 5000 frames) as an equilibration phase. After this first range of dynamics for equilibrium, the two lowest energy configurations were extracted and fully optimized. The most stable structure was used for adsorption energy calculations. This dynamics continued later at a NPT ensemble at 298 K during 16.2 ns (324 structures sampled from 16,200,000 configurations) for production. In both cases the adsorbates tend to be close together (Figure 5.5) due to the hydrophobicity of all these molecules. During the molecular dynamics simulations the adsorbate molecules are close to the center of the interlayer space

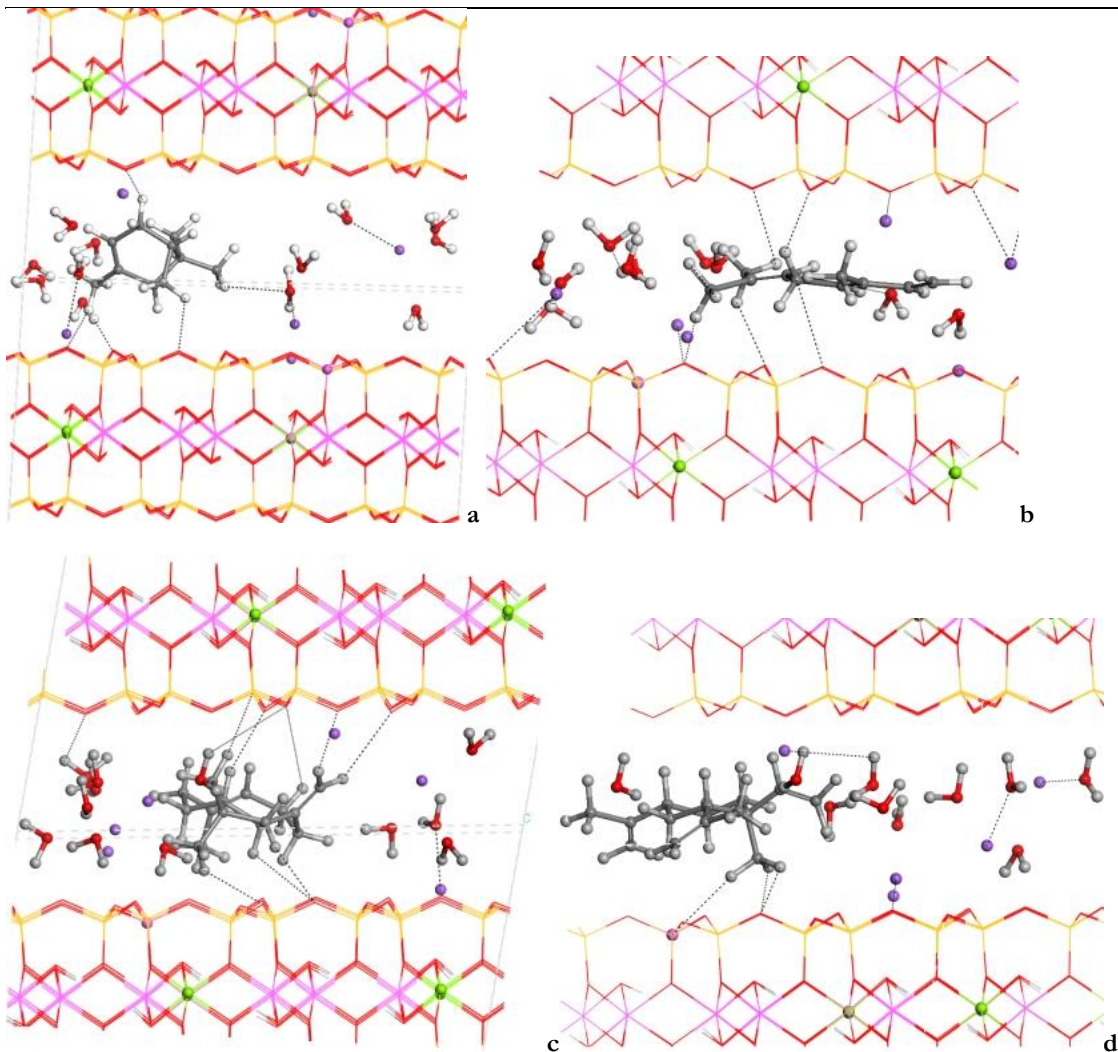
From these simulations the mean square displacement (MSD) was calculated and the slope of MSD with respect to the simulation time after the equilibrium time yielded the diffusion coefficient, being 0.0116 ($R^2 = 0.9982$) and 0.10 ($R^2 = 0.9949$) Å²/ps for C, and O atoms, respectively (Figure S1). This indicates that the diffusion of the water molecules is much higher (almost one order of magnitude) than the adsorbates along the confined interlayer space of MNT. We performed a similar molecular dynamics simulation of a waterbox of 200 water molecules with the mixture of the five organic molecules. We calculated the MSD and the diffusion coefficient was 1.24 ($R^2 = 0.9893$) and 4.6 ($R^2 = 0.9945$) Å²/ps for C, and O atoms, respectively. These values are drastically higher than in the interlayer of MNT. This confirms that the diffusion of these organic molecules is much slower in

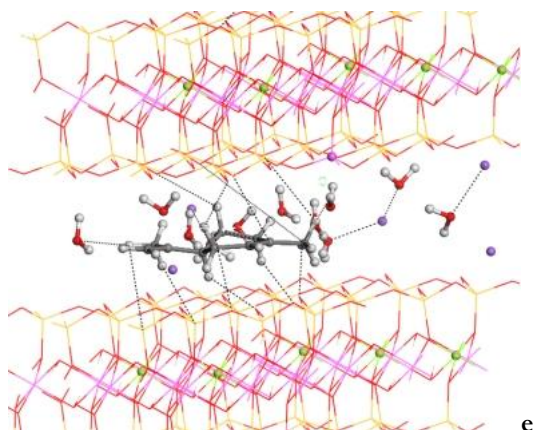
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the confined interlayer space of MNT than in aqueous media. This indicates than the delivery to the external water is not drastic but is a control release way.

Table 5.3. Crystallographic parameters of the crystal structures of MNT with the adsorbates optimized with INTERFACE (distance are in Å and angles in degrees).

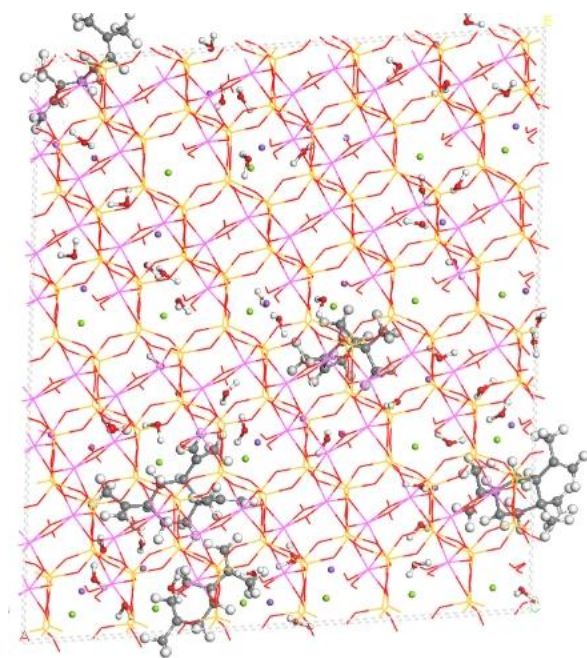
cell parameters	<i>a</i>	<i>b</i>	<i>c</i>	α	β	γ	<i>d</i> (001)
MNT	15.46	17.87	12.0	97.4	105.4	90.1	11.6
α -pinene-MNT	15.48	17.88	14.26	87.9	103.0	90.1	13.90
β -ocimene-MNT	15.48	17.88	13.26	106.3	103.3	90.0	12.90
β -caryophyllene-MNT	15.48	17.89	13.87	100.7	98.9	90.1	13.87
Limonene-MNT	15.48	17.89	13.19	91.1	110.7	90.1	12.34
β -elemene-MNT	15.48	17.88	13.60	93.7	100.6	90.1	13.37
Combined-MNT	31.46	36.90	15.69	108.7	103.4	90.7	15.26





e

*Figure 5.4. Optimized structures of MNT with organics intercalated: α -pinene (a), *b*-ocimene (b), *b*-caryophyllene (c), *b*-elemene (d), and limonene (e). Some non-bonding interactions are shown with dashed lines. The H, C, Si, Al, Mg, Na and O atoms are in white, grey, yellow, pink, green, purple, and red colours.*



a

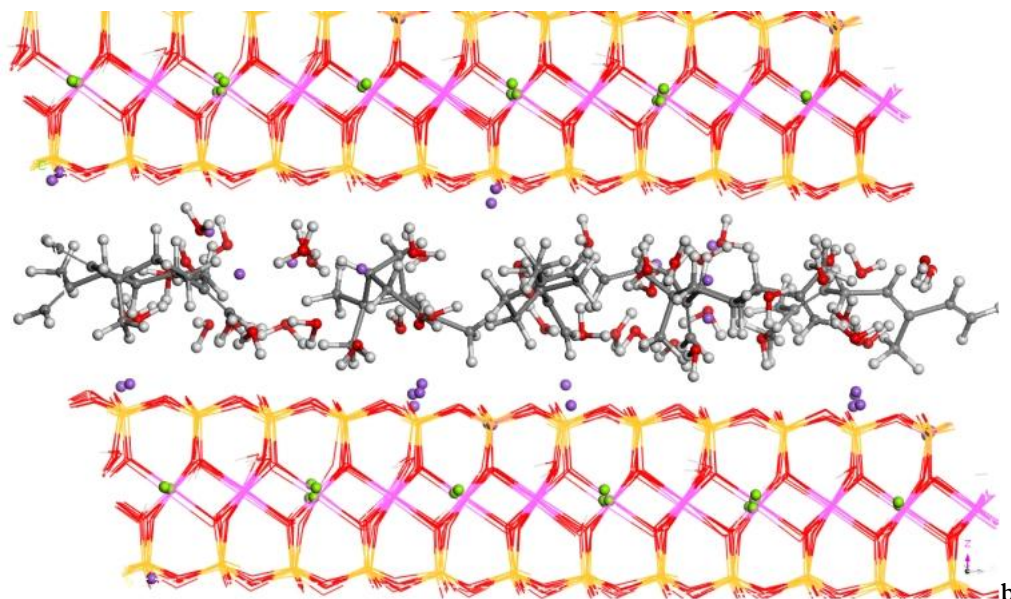
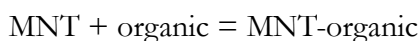


Figure 5.5. A combined hybrid composite of MNT with all adsorbates studied in this work intercalated simultaneously extracted from molecular dynamics simulations and fully optimized with INTERFACE. Views from 001 (a), and 010 (b) planes.

The adsorption energy was calculated following the reaction:



Where MNT-organic is the optimized structure of MNT with the organic molecule.

The adsorption energy is:

$$E_{\text{ads}} = E_{\text{MNT-organic}} - E_{\text{org}} - E_{\text{MNT}}$$

Where E_{ads} is the adsorption energy, $E_{\text{MNT-organic}}$ is the energy of the optimized structure of complex of MNT with the organic molecule, E_{org} is the energy of the optimized molecular structure of the essential oil component, and E_{MNT} is the energy of MNT without the organic molecule.

The calculations are expressed in Table 4 for interlayer, and external surface, respectively. Overall, the direct adsorption energies associated with the intercalation of these organic molecules into the interlayer space of MNT are comparable and positive. This indicates that the direct intercalation process is not energetically favorable, primarily due to the hydrophilic nature of MNT and the hydrophobic character of the organic molecules (Table 5.4).

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Table 5.4. Adsorption energy (in kcal/mol) of the organic molecules on interlayer, and external surface of MNT.

Adsorbate	Interlayer E_{ads}	external surface E_{ads}
α -pinene	38.55	-10.14
$\square$ -ocimene	23.04	-7.42
$\square$ -caryophyllene	42.49	-10.4
Limonene	34.13	-14.72
$\square$ -elemene	53.54	-9.6
All combined	112.7	

The computed adsorption energies for interlayer incorporation were uniformly positive, ranging from 23.04 kcal/mol for $\square$ -ocimene to 53.54 kcal/mol for $\square$ -elemene. These positive values clearly indicate that direct intercalation of the organic molecules into the interlayer space is energetically unfavorable. This can be attributed to the hydrophilic nature of the MNT interlayer, which is typically occupied by polar or charged species such as water and cations. The intrinsic hydrophobic character of the organic molecules studied here leads to unfavorable energy contributions upon confinement within the polar interlayer environment. In addition the intercalation produces a drastic change of the conformation of some components. So, the adsorption of linear $\square$ -ocimene and the quasi-planar limonene is less unfavourable because their intercalation does not force any conformational change. This trend is consistent with previous findings (Meruvia-Rojas et al., 2025b) on hydrophobic molecule–clay interactions and suggests limited spontaneous intercalation in the absence of mediating solvents or modifications.

On the other hand, a simultaneous intercalation of all components is energetically more favourable with adsorption energy of 112.7 kcal/mol being significantly lower than the sum of the adsorption energies of all components separately (191.8 kcal/mol). Indeed, this better intercalation energy for all organics at a time should be able to be explained by a certain stabilizing interaction in between the organics, in spite of the large distances between the different organics in the 6x4x1 cell model (Figure 5.5a). The presence of some components increases the hydrophobicity of the interlayer space acting as an organo-clay for the rest of members in the series.

5.4.3.3. Adsorption on external clay surface

Another alternative for adsorption is on the external surface of the clay mineral. Initially we can create an ideal clean external surface without water molecules and Na^+ cations. However, this surface does not exist in nature and some water molecules and Na^+ cations can exist close to the adsorption sites of the external surface of MNT. Then, a model representing the external surface of MNT was constructed by introducing a vacuum space of 20 Å above the (001) basal plane ($c = 30$ Å). The organic molecule was initially positioned 2 Å above the surface, oriented parallel to the mineral surface (001) plane. Following the geometry

optimization of these adsorption complexes maintaining the external empty space (Figure 5.6), the organic molecules retained a parallel orientation relative to the MNT surface. In contrast to interlayer, adsorption on the external surface of MNT was found to be energetically favorable for all molecules, as evidenced by negative adsorption energies (Table 5.4). All adsorbates showed similar moderately negative adsorption energies, being the highest the limonene adsorption. These values suggest that while adsorption is generally favorable, the extent of interactions vary based on molecular structure, conformation, and surface affinity of each component.

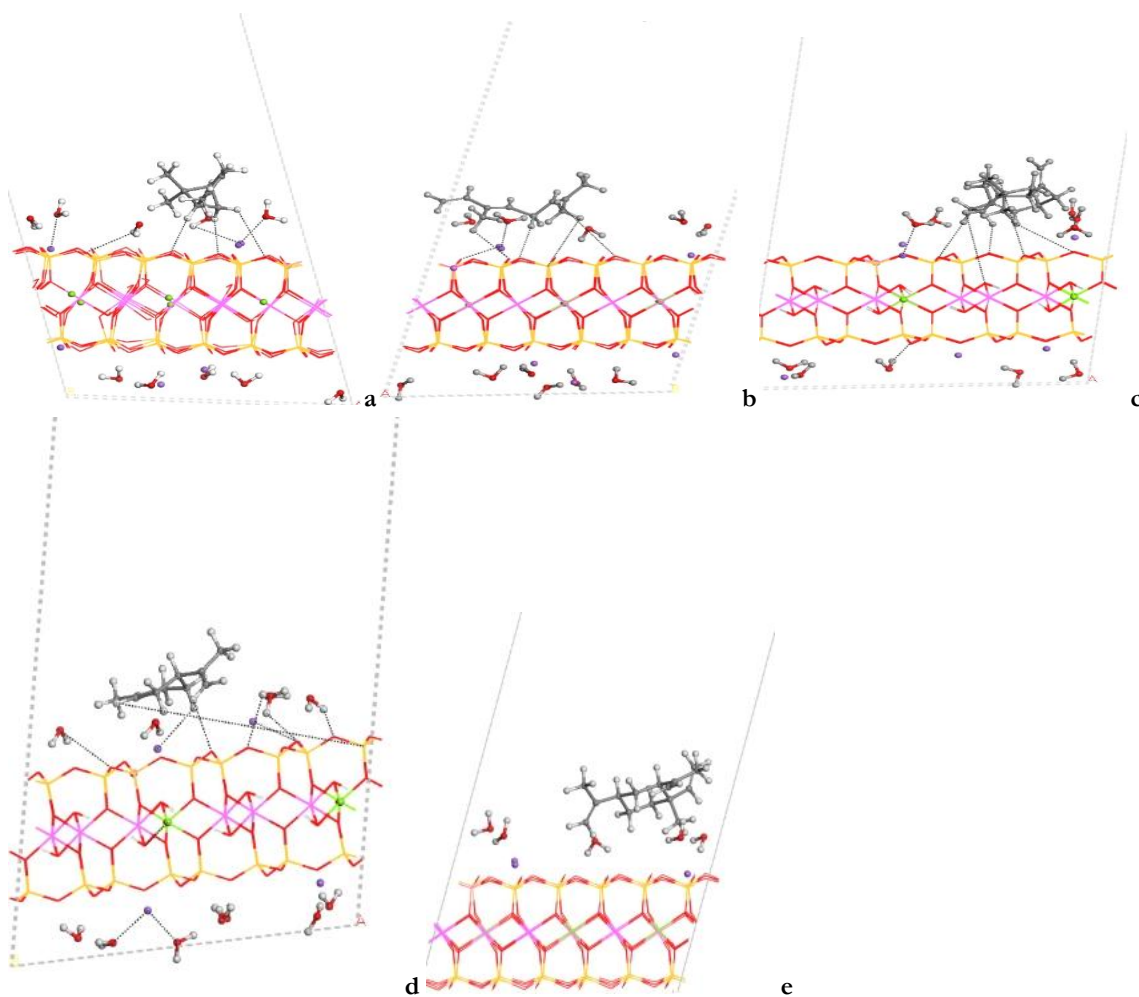


Figure 5.6. Optimized structures of the adsorption complexes of α -pinene (a), β -ocimene (b), β -caryophyllene (c), limonene (d), and β -elemene (e) on the external surface of MNT.

Overall, the results reveal that external surface adsorption is energetically more favorable than intercalation for all studied molecules. MNT provides both types of surfaces at the same time, the interlayer space and the external surfaces existing in the interstitial borders of grains in pores. The relative accessibility to these surfaces will depend on the experimental conditions. These findings highlight the importance of considering both the physicochemical

properties of the adsorbate and the structural environment of the clay substrate when designing clay-organic hybrid materials.

5.4.3.4. Desorption process and delivery of essential oils components to external media.

To complement the adsorption analysis, desorption energies were calculated for the same set of organic molecules from the interlayer, and the external surface of MNT clay, in the presence of an explicit water box ($18 \times 18 \times 18 \text{ \AA}^3$, with 200 water molecules). The desorption energies provide insights into the potential use as delivery system under aqueous conditions. The desorption process was calculated following the next equation (Figure 5.7):

$\text{MNT-organic} + \text{water} = \text{MNT} + \text{water-organic}$, where MNT-organic is the optimized structure of MNT with the adsorbate, water is the waterbox optimized, MNT is the optimized structure of the pristine MNT, and water-organic is the adsorbate molecule embedded into the waterbox system. Then, the energy was calculated with the expression:

$$E_{\text{desor}} = E_{\text{MNT}} + E_{\text{wboxorg}} - E_{\text{wbox}} - E_{\text{MNT_org}}$$

, where E_{desor} is the desorption energy, E_{MNT} is the energy of the optimized pristine MNT, E_{wboxorg} is the energy of the optimized waterbox with the organic molecule included inside, E_{wbox} is the energy of the optimized waterbox system, and $E_{\text{MNT_org}}$ is the energy of MNT with adsorbate.

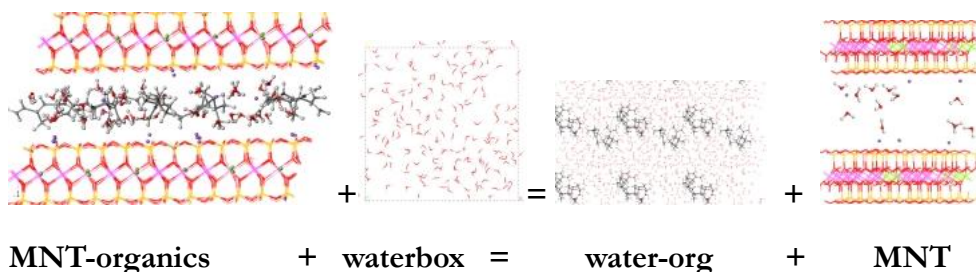


Figure 5.7. Optimized models for desorption process. This example is for the combined case, but is similar to all organics.

The desorption energies of organic molecules from interlayer, and external surface of MNT are presented in Table 5.5. Desorption energies from the MNT interlayer were consistently large and more negative for all molecules-MNT systems than from the MNT external surface, indicating an energetically favourable process for a spontaneous release of these essential oil molecules to external aqueous environments like the skin and therapeutic treatments. β -caryophyllene (-90.64 kcal/mol) and $\square$ -elemene (-90.58 kcal/mol) showed the strongest release from the interlayer space, followed by α -pinene (-63.49 kcal/mol).

Desorption energies from the external surface were lower, ranging from -3.47 kcal/mol (limonene) to -27.44 kcal/mol ($\square$ -elemene). All desorption energies are negative indicating that the delivery from the external surface of MNT is also energetically favourable.

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Table 5.5. Desorption energy, E_{des} , (in kcal/mol) of the organic molecules from interlayer, and external surface of MNT.

Organic molecule	Interlayer E_{des}	Surface E_{des}
α -pinene	-63.49	-14.8
$\square$ -ocimene	-55.33	-7.33
$\square$ -caryophyllene	-90.64	-21.28
limonene	-52.32	-3.47
$\square$ -elemene	-90.58	-27.44
Combined one	-231.51	

5.5. Discussion

The methodology, based in INTERFACE FF, chosen in this work has been validated reproducing the crystallographic parameters of α -pinene and its spectroscopic properties. In addition, our calculations have let us know the assignments of the IR and Raman spectra bands of the crystalline solid of α -pinene.

These validated methodology calculations show that the intercalation of these essential oils components into the confined interlayer space of MNT is not energetically favourable. These terpenoids are highly hydrophobic non-polar molecules with no friendly tendency to enter into the hydrophilic interlayer space of our clay mineral. This is consistent with previous experimental works of adsorption of *Eucalyptus globulus* essential oil, containing α -pinene, and $\square$ -caryophyllene, with phyllosilicates, where the adsorbed amount of these components were much lower than the rest of components of the plant extract (Saucedo-Zuñiga et al., 2021b). These results predict the necessity of using no solvent or non-polar solvents for the intercalation of these molecules into the interlayer space of MNT. Previous experimental works reported the adsorption of essential oils on clay minerals by using an organic solvent (Ghrab et al., 2018; Ngumtchouin et al., 2015), or without solvent vapor essential oils on clay mineral solids (Essifi et al., 2022a; Molina-Montes et al., 2008), or mixing directly oil-clay (Giannakas et al., 2017) according with our results. The diffusion of these organic components is one order of magnitude slower than water in the interlayer space of MNT, as found with our molecular dynamics simulations. This fact corroborates their difficulty and slowness intercalation process. Nevertheless, these calculations describe only the initial and final steps of the adsorption process without considering the temperature effect. Therefore, this adsorption will be possible at experimental conditions of time and temperature where the Brownian movement of atoms and molecules could increase the probability of the adsorbate-mineral surface intercalation. This probability will be higher at the external surfaces in the interstitial spaces between pores, being an initial step for a possible further intercalation process. Previous experimental reports required long reaction time even 10 days (8.64×10^{14} ns). Obviously this reaction time is out of our theoretical calculations. Our models do not include real temperature and physiological conditions, or complex solvent

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environments typical in practical drug delivery scenarios. The study of thermodynamics, kinetic parameters, and the influence of buffers or skin mimetics at atomic scale is out of the scope of this work.

On the other hand, more representative models of plant extracts where most of the compounds studied here are present can be also calculated. The adsorption of the components of this mixture in the same interlayer space is more probable than the adsorption of each component separately or in different interlayers of MNT. The presence of some apolar molecules in the interlayer increases the hydrophobicity of this space facilitating the adsorption of more apolar components. This is consistent with previous experimental works where the modification of MNT with additives forming organo-clays enhances the adsorption capacity of these systems. However, we explore the most simple system without using additives and avoiding possible toxicologic problems.

Nevertheless, our calculations predict the possible increase of the $d(001)$ spacing produced by the intercalation of some of these compounds. This fact can be an useful information for future experimental works of powder X-ray diffraction (XRD) analysis in adsorption of essential oils components. Besides, our calculations indicate that adsorption of these compounds on the external surfaces of MNT is more favourable than intercalation. The hydrophobicity of the tetrahedral silanol layers of the external surfaces of this clay mineral can be more friendly for these adsorbates. This result predict also the possibility of adsorption of these organic compounds in the external surfaces of the interstitial pores of clay minerals without observing variation in the $d(001)$ spacing by XRD experimentally. This phenomenon was observed in previous adsorption experiments of essential oils and MNT, where the adsorption was on the external surface (Essifi et al., 2022a).

These essential oil components are stable into the interlayer space of MNT. This means that these hybrid organics-MNT composites can be handled during their applications in skin treatment. Besides, our calculations predict that these bioactive compounds with therapeutic properties can be delivered to external aqueous media with an energetically favourable process. This fact can facilitate the release of these compounds to the skin surface in treatments with possible application in warm thermal baths. Our calculations enhance to experimentalist groups to perform release research of these systems with kinetics of delivery, and bioactivity of post-release media.

5.6. Conclusions

The combination of the theoretical methodology used in this work has let to calculate the crystallographic parameters and IR and Raman spectra of the crystals of α -pinene as a first time and reproduce the known experimental data. Adsorption of some of the main components of the most used essential oils onto the surfaces of montmorillonite has been studied in this work by means of molecular modelling methods. We selected the terpenoid components of essential oils without hydroxy groups belonging to a hydrophobic compounds series. In general, the intercalation of these molecules into the confined interlayer space of montmorillonite was not energetically favourable due to the hydrophilia of the interlayer space. However, the adsorption of these compounds on the external surfaces is more favourable owing to the hydrophobicity of the silanol layers of the external

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surface of montmorillonite. We have to consider that our calculations are without temperature and taking into account only the initial and final steps of the adsorption process. Therefore, this adsorption will be possible at experimental conditions of time and temperature where will increase the probability of the adsorbate-mineral surface interaction. Besides, the adsorption of a real mixture of these components within an essential oil is more energetically favourable than adsorbing each pure component separately. Hence, our calculations predict that these adsorbates can be adsorbed in the phyllosilicate clay mineral by using non-polar solvents or without solvents. Molecular dynamics simulations indicate that the diffusion of organics is one order of magnitude slower than water in the interlayer space of MNT, predicting the high stability of these guest compounds into the confined interlayer space of MNT. On the other hand, our calculations predict that these components can be easily delivered to the aqueous media, indicating these composites as good candidates for drug-delivery bioactive compounds for therapeutic skin-treatment, especially for specialized thermal baths.

5.7. Clinical additives- anti-inflammatory essential oils.

Essential oils and their bioactive constituents have attracted increasing attention for their anti-inflammatory potential and their ability to serve as functional excipients or *clinical additives* in topical and transdermal drug delivery systems. Among these, thymol, carvacrol, eugenol, and menthol have demonstrated significant pharmacological activity relevant to inflammation modulation and tissue repair.

5.7.1. Thymol

Thymol, a monoterpenoid phenol found primarily in *Thymus vulgaris* and *Origanum* species, exhibits potent anti-inflammatory activity through multiple pathways. It inhibits the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), thereby reducing the synthesis of prostaglandins and nitric oxide, two key mediators of inflammation (Gago et al., 2025). Thymol also downregulates NF- κ B activation, a central transcription factor in the inflammatory cascade, and enhances antioxidant enzyme activities, including superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Tavares et al., 2022; Yildiz et al., 2021). In clinical and preclinical contexts, thymol-containing formulations have shown efficacy in reducing dermal inflammation, erythema, and edema (Wang et al., 2020).

5.7.2. Carvacrol

Carvacrol, a structural isomer of thymol also present in oregano and thyme oils, exerts anti-inflammatory and analgesic effects through suppression of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 (Han & Parker, 2017). It modulates the TRPV3 and TRPA1 ion channels, influencing sensory neuron signaling and contributing to its analgesic profile (de Cássia da Silveira e Sá et al., 2017). Additionally, carvacrol exhibits membrane-stabilizing and antioxidant effects that reduce leukocyte infiltration and oxidative tissue damage during inflammation (Avola et al., 2020). Its dual role as a bioactive compound and permeation enhancer makes it a valuable additive in clinical topical formulations.

5.7.3. Eugenol

Eugenol, the main constituent of *Syzygium aromaticum* (clove oil), has been extensively studied for its anti-inflammatory, local anesthetic, and antioxidant properties. Mechanistically, it inhibits COX and lipoxygenase (LOX) pathways, leading to decreased prostaglandin and leukotriene production. Eugenol also interferes with NF- κ B signaling, suppressing the expression of inflammatory mediators such as TNF- α and IL-8 (Pires Costa et al., 2025). Clinically, eugenol has been used as a therapeutic additive in dental, dermatological, and wound-healing applications, owing to its anti-inflammatory and antimicrobial actions (Barboza et al., 2018; Maurya et al., 2020). Its lipophilic structure also enhances dermal penetration of co-administered drugs, supporting its dual function as a bioactive and functional additive.

5.7.4. Menthol

Menthol, a cyclic monoterpene alcohol derived from *Mentha* species, is widely recognized for its cooling, analgesic, and anti-inflammatory effects. It acts primarily through transient

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receptor potential (TRP) channels, especially TRPM8, inducing calcium influx and modulating neuronal excitability. Menthol has been shown to inhibit arachidonic acid metabolism and reduce neutrophil recruitment, contributing to its anti-inflammatory activity (Cheng & An, 2022; Rozza et al., 2021). Topically, menthol-containing formulations are clinically used to alleviate pain, pruritus, and inflammatory skin conditions. Moreover, its penetration-enhancing property aids the delivery of co-formulated active agents, making it an important clinical additive in transdermal systems (L. Chen et al., 2019; Lan et al., 2016).

5.8. Adsorption of volatile anti-inflammatory essential oils on phyllosilicate surfaces. A natural drug delivery system. (Article 5)

Essential oils (EOs) are concentrated mixtures of volatile organic compounds extracted from various parts of plants, including flowers, leaves, bark, and roots. These natural substances have long been valued in traditional medicine for their broad spectrum of biological activities, particularly their anti-inflammatory, antimicrobial, and antioxidant properties (Bakkali et al., 2008; Burt, 2004). In dermatology, essential oils have shown promising effects as natural therapeutic agents for treating a variety of skin disorders, such as acne, atopic dermatitis, psoriasis, and eczema, owing to their ability to modulate inflammatory responses, regulate microbial balance, and promote skin regeneration (Ali et al., 2015; Orchard & Van Vuuren, 2017).

Despite these well-documented properties, the practical application of essential oils in topical therapies remains limited due to several physicochemical challenges. Their high volatility, low water solubility, and sensitivity to environmental factors (light, temperature, and oxygen) contribute to rapid degradation and loss of activity, compromising their stability, bioavailability, and therapeutic efficacy when directly applied to the skin. These limitations have driven growing interest in natural carrier systems capable of protecting EO molecules from volatilization and degradation, while providing controlled release and enhanced skin interaction.

One promising strategy involves integrating essential oils into natural mineral-based systems used in thermal and balneotherapy treatments. Thermal waters, rich in minerals such as sulfur, magnesium, calcium, and bicarbonates, have been used for centuries in the management of chronic skin disorders (Antonelli & Donelli, 2018; Nasermoaddeli & Kagamimori, 2005). In balneotherapy, these waters are often combined with mineral-rich clays or muds to form peloids, which improve skin contact, heat retention, and penetration of bioactive components (Fioravanti et al., 2011). Incorporating essential oils into these clay–thermal water matrices could further enhance their therapeutic efficacy by merging the mineral, thermal, and bioactive benefits within a single formulation.

The clay component in these systems plays a pivotal role as a carrier and stabilizer of volatile compounds. Among the different mineral classes, phyllosilicates—including kaolinite, illite, and smectites such as montmorillonite—exhibit layered crystalline structures, high surface areas, and interlayer spaces capable of adsorbing and retaining organic molecules (Aguzzi et al., 2007b). These structural characteristics make phyllosilicates ideal candidates for the encapsulation and stabilization of essential oils, improving their thermal and oxidative stability and facilitating sustained release during topical application.

Recent studies have explored this approach and demonstrated the potential of EO–clay hybrid systems in enhancing the stability and biological activity of essential oils. For example, Agougui et al. (2022) reported that Caraway essential oil adsorbed onto sodium montmorillonite (MNT) exhibited improved antibacterial and antifungal efficacy, with adsorption capacity influenced by the polarity and molecular composition of EO

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components. Similarly, Essifi et al. (2022b) developed montmorillonite–EO nanostructured hybrids through environmentally friendly synthesis methods, achieving controlled release and enhanced bioactivity of volatile compounds. Other encapsulation approaches, such as those by Pinto et al. (2024), demonstrated that immobilizing EOs within silica-based matrices significantly improved their stability, extended their release, and enhanced antifungal and analgesic effects. Collectively, these studies underscore the importance of mineral carriers in preserving EO functionality and enabling long-term bioavailability in therapeutic formulations.

The integration of essential oils with phyllosilicate-rich clays, particularly within the context of thermal therapy, thus represents an innovative and eco-sustainable strategy for developing natural dermocosmetic and therapeutic formulations. These systems combine the bioactivity of essential oils, the adsorptive and rheological properties of clays, and the mineral composition of thermal waters, offering synergistic benefits for skin health. Such hybrid formulations align with current trends in clean beauty, natural therapeutics, and biocompatible product development, potentially providing enhanced efficacy in managing inflammatory skin conditions, barrier dysfunction, and oxidative stress.

To advance this emerging field, it is necessary to develop a fundamental understanding of the molecular mechanisms governing EO–clay interactions. In this study, molecular modeling and simulation techniques are employed to investigate the adsorption and intercalation behavior of selected volatile essential oils—particularly thymol, menthol, carvacrol, and eugenol—on phyllosilicate surfaces, with emphasis on montmorillonite (MNT) as a representative clay mineral. Through our calculations at molecular and atomic scale, this study explores adsorption energetics, molecular orientation, hydrogen bonding, and van der Waals interactions that determine the stability and loading capacity of EO–clay hybrids.

Complementing the theoretical work, experimental studies are conducted to validate the computational predictions. Synthesized EO–clay hybrids are characterized using XRD, FTIR, to confirm intercalation, identify functional interactions. By correlating computational and experimental data, this research aims to establish structure–property relationships that can guide the rational design of EO–clay systems for dermocosmetic applications.

Overall, this work bridges traditional thermal therapy practices with modern molecular-level insight, contributing to the scientific foundation for natural, clay-based drug delivery systems. The combined modeling–experimental approach not only enhances understanding of the physicochemical behavior of EO–clay hybrids but also supports the development of stable, sustainable, and bioactive formulations for skin health and therapeutic care.

With these premises, the aims of this paper was to theoretically and experimentally investigate the adsorption behavior of volatile anti-inflammatory essential oils (thymol, menthol, carvacrol, eugenol) Figure 5.8 on phyllosilicate surfaces through molecular modelling and simulation techniques. By simulating the interactions between essential oil molecules and phyllosilicate surfaces, this research provides at atomic scale a fundamental understanding of the physicochemical mechanisms governing adsorption, stability, and

potential release behavior. The adsorption of these molecules (thymol, menthol, eugenol, carvacrol) on MNT surfaces (VF, VHS) has also been studied experimentally.

5.8.1. Theoretical Methodology

The phyllosilicate used in this work was MNT, whose structure was obtained from previous works (Meruvia-Rojas et al., 2025c). This clay mineral belongs to dioctahedral phyllosilicates. Its chemical composition is close to one used often for pharmacological applications, Veegum. This clay mineral has a low proportion of tetrahedral Al^{3+} cations and a small amount of octahedral Mg^{2+} cations with a unit cell composition $\text{Na}(\text{Si}_{7.83}\text{Al}_{0.17})(\text{Al}_{3.17}\text{Mg}_{0.83})\text{O}_{20}(\text{OH})_4$. For the adsorption studies a 3x2x1 supercell was generated with the composition $\text{Na}_6(\text{Al}_{19}\text{Mg}_5)(\text{Si}_{47}\text{Al}_1)\text{O}_{120}(\text{OH})_{24}$. The interlayer Na^+ cations were coordinated with two water molecules each one. Considering previous studies of Mg substitutions in this clay mineral (Sainz-Díaz et al., 2005c), the Mg^{2+} cations and the tetrahedral Al^{3+} cations were placed with a maximal dispersion distribution in tetrahedral and octahedral sheets of MNT (Escamilla-Roa et al., 2017).

The molecules selected for this work are the main constituents of some essential oils (EO) with anti-inflammatory effect. The thymol molecule, 5-methyl-2-(propan-2-yl)phenol, was extracted from the crystal structure provided from the CSD database (ref. 2069355) from crystallographic X-ray diffraction (XRD) studies (Alhadid et al., 2021). The (-)-menthol, (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanol, molecule was extracted from the crystal structure (CSD-ref.112473) determined from XRD (Bombicz et al., 1999). The carvacrol, 5-isopropyl-2-methyl-phenol, was extracted from a crystal structure obtained along with secnidazole (CSD-ref 2330750) and determined by XRD (Oliveira Pontes et al., 2024). The Eugenol, 2-methoxy-4-(2-propenyl)phenol, molecular structure was extracted from a crystal structure determined by XRD (CSD-ref 121082).

Main calculations have been performed by using empirical interatomic potentials condensed in the INTERFACE force field (FF) (Heinz et al., 2013) with periodical 3-D boundary conditions. This FF was used previously with excellent results in similar systems to this work (M. Jorge, 2024). The optimization of these structures were performed using the Forcite code within the Materials Studio package (Biovia, 2024). The Lennard–Jones potential, $V(r) = \epsilon[(\sigma/r)^{12} - 2(\sigma/r)^6]$, was used for the calculations of the van der Waals interactions. The Ewald summation method was used for the van der Waals and coulomb non-bonding interactions. In the hydrated systems 3-D periodic boxes with water molecules were generated with a Monte Carlo method for a density of 1 g/cm³.

The Molecular Dynamics (MD) simulations were performed using the same of above methodology within a NPT ensemble with steps of 1 fs during 5 ns (5 millions of structures). We sampled 1000 structures, one each 5000 steps. After the simulations we selected the configurations with the lowest energy. These selected samples were fully optimized with variable lattice cell.

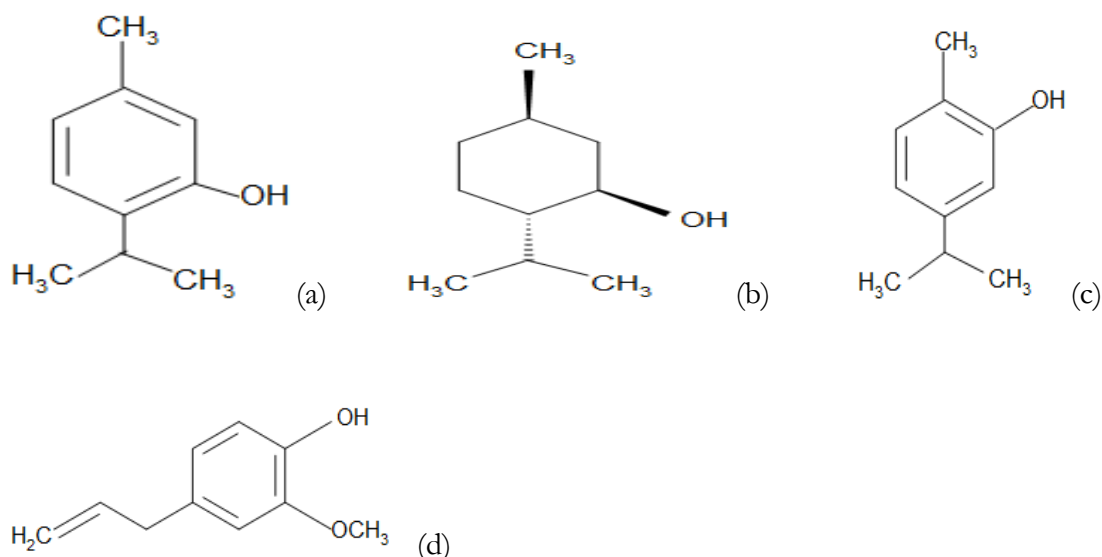


Figure 5.8. Molecular models of the studied volatile components of essential oils: thymol (a), menthol (b), carvacrol (c), and eugenol (d).

5.8.2. Experimental techniques

Montmorillonite samples of bentonite VF, VHS are used in experiments that are pharmaceutical-grade magnesium aluminium silicate clays (Vanderbilt Ltd., USA) commercially used as an excipient. The materials complies with the specifications of the European Pharmacopoeia (8.0) and United States Pharmacopoeia (USP 32) monographs.

Essential oil of menthol samples were purchased to Adrian & Klein (Castellon, Spain) and the thyme essential oil sample was from Ventós S.A. (Barcelona, Spain). Both samples were analysed by gas chromatography coupled with mass spectrometry by using a AGILET 7890A model gas chromatographer coupled with a WATERS XEVO TQ-S model mass spectrometer triple-quadrupole analyzer.

5.8.2.1. Essential oil characterization

The chemical composition of the essential oil was determined by gas chromatography–mass spectrometry (GC–MS). The sample was diluted 1:100 (v/v) in hexane and 1.0 μL was injected in split mode (1:50). Analyses were performed on a GC–MS system equipped with an HP-5MS capillary column (30 m $\times$ 0.25 mm, 0.25 μm film thickness).

Helium was used as the carrier gas at 1.0 $\text{mL}\cdot\text{min}^{-1}$. The oven program was: 60 $^{\circ}\text{C}$ (2 min) ramp to 180 $^{\circ}\text{C}$ at 3 $^{\circ}\text{C}\cdot\text{min}^{-1}$ ramp to 260 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}\cdot\text{min}^{-1}$, hold 5 min. Injector temperature: 250 $^{\circ}\text{C}$.

Mass spectra were acquired in electron ionization (70 eV), scanning m/z 40–450. Compounds were identified by comparison with the NIST mass spectral library, and relative abundances were calculated from normalized peak areas.

5.8.2.2. Intercalation of menthol and thymol in VF and VHS

To prepare the samples, we began by weighing 0.5 g of the previously obtained extract, which was diluted in 30 ml of acetone. This preparation was carried out in a 30 mL amber topaz

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glass bottle, and the resulting solution was stirred on a hot plate for 30 minutes to ensure adequate homogenization. Next, 0.5 g of the corresponding clay was weighed and added to the bottles. The mixture was left stirring continuously for 24 hours at 220 rpm to promote interaction between the components. The solutions were then distributed into glass containers, and an additional 10 mL of acetone was added to the bottles to rinse and recover any residue. The containers were covered with perforated parafilm and placed in an active extraction hood. Finally, the samples were left to stand for 24 hours in the hood, until the solvent had completely evaporated.

5.8.2.3. FTIR analysis.

The raw and treated samples of montmorillonite was analysed by Fourier transformed infrared (FTIR) spectroscopy by using a JASCO 6200 spectrophotometer, equipped with an ATR accessory, in the range of 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1} . A minimum of 50 spectra were collected from each experiment. The data were processed using Spectra Manager v2 software, including baseline correction, CO_2 removal, and smoothing, as needed for each spectrum. The results are represented in transmittance (T) units.

5.8.3. Theoretical Results and Discussion

5.8.3.1. Crystal structures of essential oils. Validation of force field

The crystal structures of studied essential oils components have been calculated in order to validate the FF used in this work. We have fully optimized these structures (atom positions and cell parameters). Our theoretical results reproduce the experimental crystal lattice cell parameters with relative differences lower than 5 % (Table 5.6). This result validates the use of our FF for the adsorption studies of these molecules on phyllosilicates surfaces.

Table 5.6. Comparison of calculated and experimental crystal cell parameters of some essential oils (Fig. 5.9). Distances are in Å and angles are in degrees.

Parameters ^a	<i>a</i>	<i>b</i>	<i>c</i>	α	β	γ
thymol exp	14.45	14.45	23.03	90	90	90
thymol optimized	14.59	14.59	21.97	90	90	90
Relative dif.	-0.009	-0.009	0.048	0	0	0
menthol exp	21.26	21.26	5.98	90	90	120
Optimized	21.35	21.35	6.02	90	90	120
Relative dif.	-0.004	-0.004	-0.006	0	0	0

^a exp: experimental values; optimized: calculated by using INTERFACE FF; relative dif: relative standard deviation.

In both compounds the hydrogen bonds of the hydroxyl groups are the main interactions stabilizing the crystal structures, being $d(\text{OH}\dots\text{O}) = 1.85$ and 1.77 Å in thymol and menthol, respectively. Some π - π interactions can be observed between the aromatic rings in the thymol crystal lattice which are in parallel planes. In both crystals the alkyl residues are grouped forming hydrophobic zones and the OH groups are forming the polar zones (Figure 5.9).

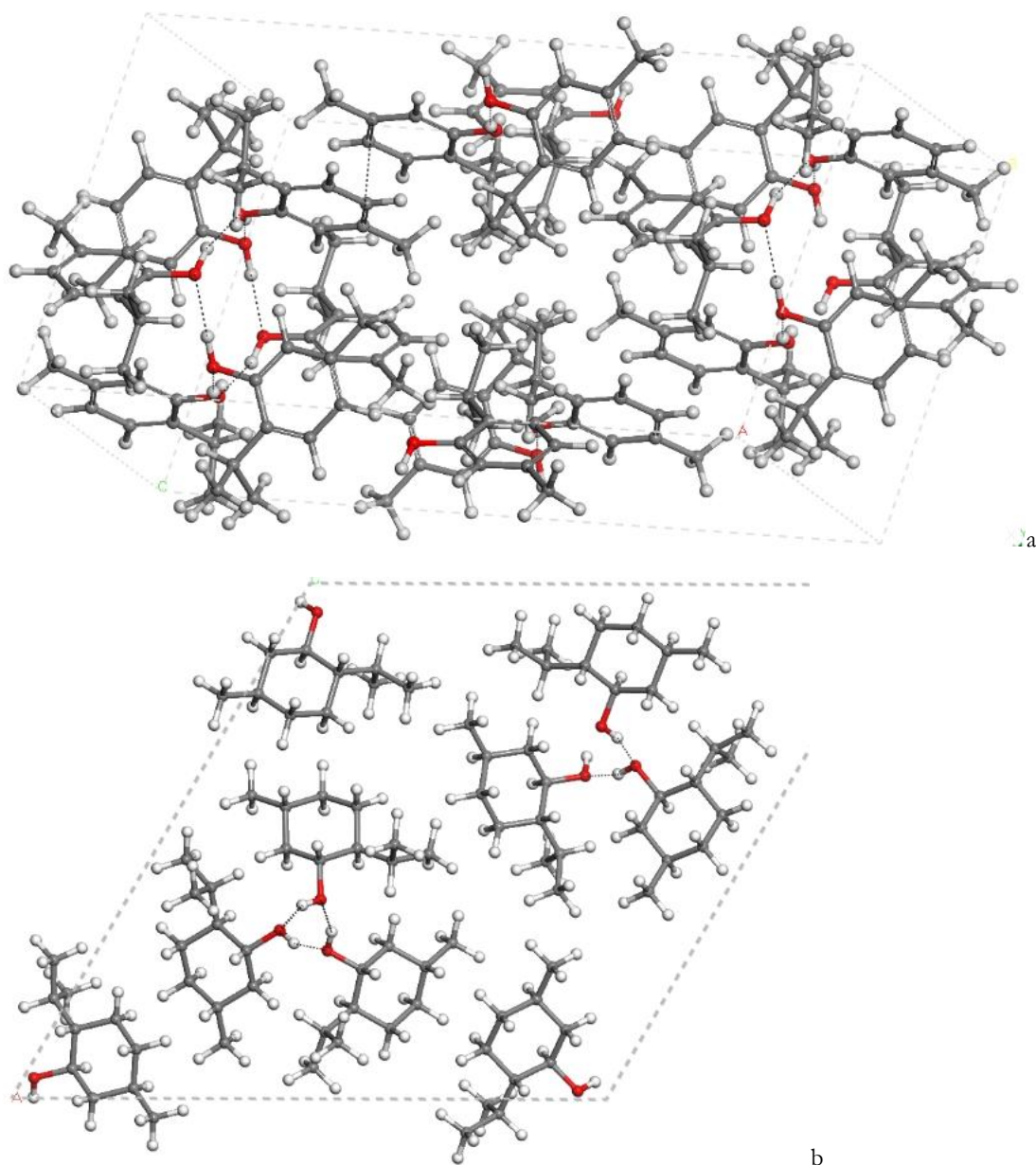


Figure 5.9. Optimized crystal structures of thymol (a) and menthol (b). Some non-bonding interactions are shown with dashed lines. The H, C, and O atoms are in white, grey and red colours.

The isolated molecule was extracted from the crystal structures and included in 3-D periodical boxes being big enough to avoid interactions with vicinal molecules. The optimized molecules maintain the same conformation as in crystal (Figure 5.10). The isopropyl group adopts a perpendicular orientation with respect to the aromatic ring in thymol and carvacrol, and the cyclohexyl ring of menthol. The O-H bonds are co-planar to the aromatic ring in most molecules. This conformation is maintained by an electrostatic

interaction between the hydroxyl O atoms and the isopropyl C-H bond $d(\text{HO}\dots\text{HC}) = 2.46$ Å.

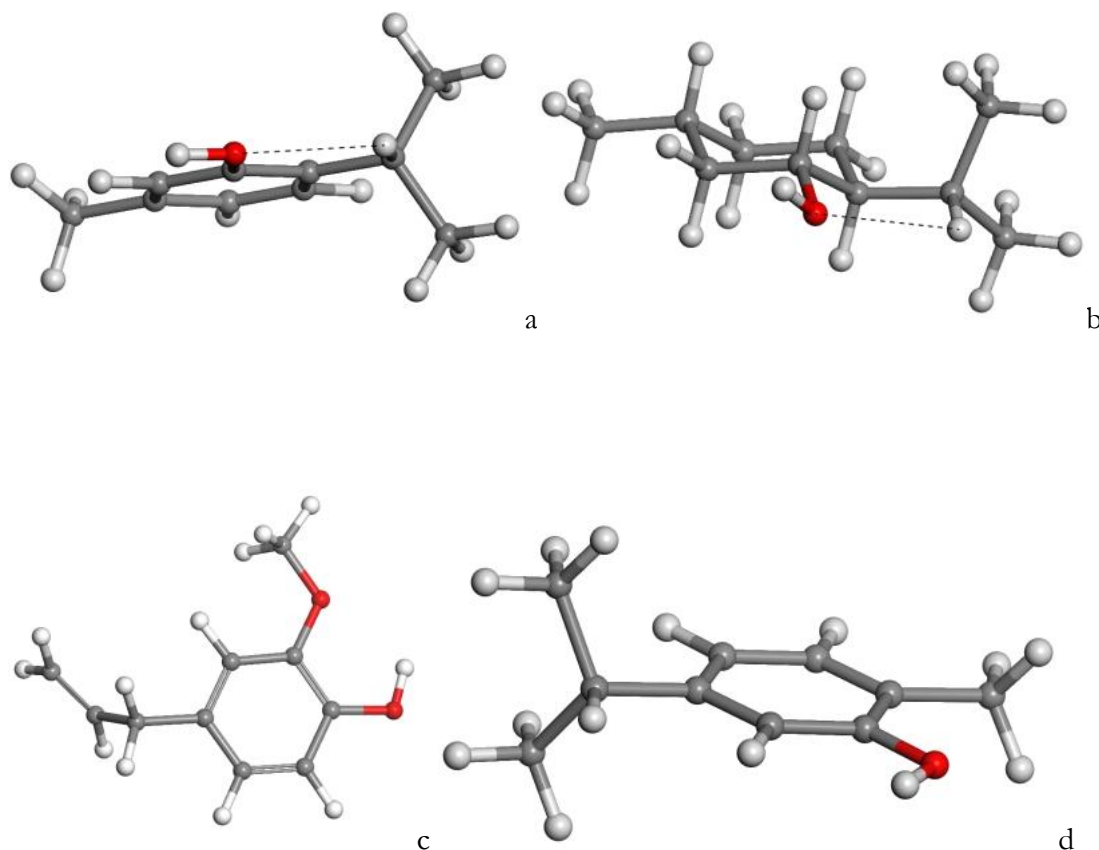


Figure 5.10. Optimized crystal structure of thymol (a), menthol (b), eugenol (c), and carvacrol (d). The main non-bonding interactions are shown with dashed lines.

5.8.3.2. Adsorption on mineral surfaces.

The MNT structure was fully optimized reproducing the experimental structure (Meruvia-Rojas et al., 2025c). Each molecule was placed in the center of the interlayer space of MNT and optimized. After the optimization the organic molecule remains in the center of the interlayer space where the aromatic and acyclic rings are coplanar with the mineral interlayer surface (Figure 5.11). The alkylic H atoms of the adsorbed molecules are interacting with the O atoms of the mineral surface in all samples, $d(\text{CH}\dots\text{OSi}) = 2.20\text{-}2.88$ Å. The OH group of the sorbates are oriented to the mineral surface, forming strong hydrogen bonds between the hydroxy H atoms and the surface O atoms, $d(\text{OH}\dots\text{OSi}) = 1.60\text{-}1.89$ Å, in all molecules except thymol. In thymol the OH group remains coplanar to the aromatic ring of the molecule. Some water H atoms interact with the mineral surface O atoms $d(\text{HOH}\dots\text{OSi}) = 1.60\text{-}1.90$ Å. Besides, these water molecules are coordinating some Na^+ $d(\text{Na}\dots\text{OH}) =$

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2.43-2.58. Some Na^+ cations have electrostatic interactions with the mineral surface O atoms $d(\text{Na}\cdots\text{OSi}) = 2.32\text{-}2.43 \text{ \AA}$.

These interatomic interactions have provoked some changes in the conformations of the adsorbate molecules. In thymol, the OH group has rotated 180° and the isopropyl moiety is not perpendicular to the aromatic ring. The central CH isopropyl H atom has rotated 90° and is more oriented towards the mineral surface O atoms than to the OH group of the molecule. The same is observed in carvacrol.

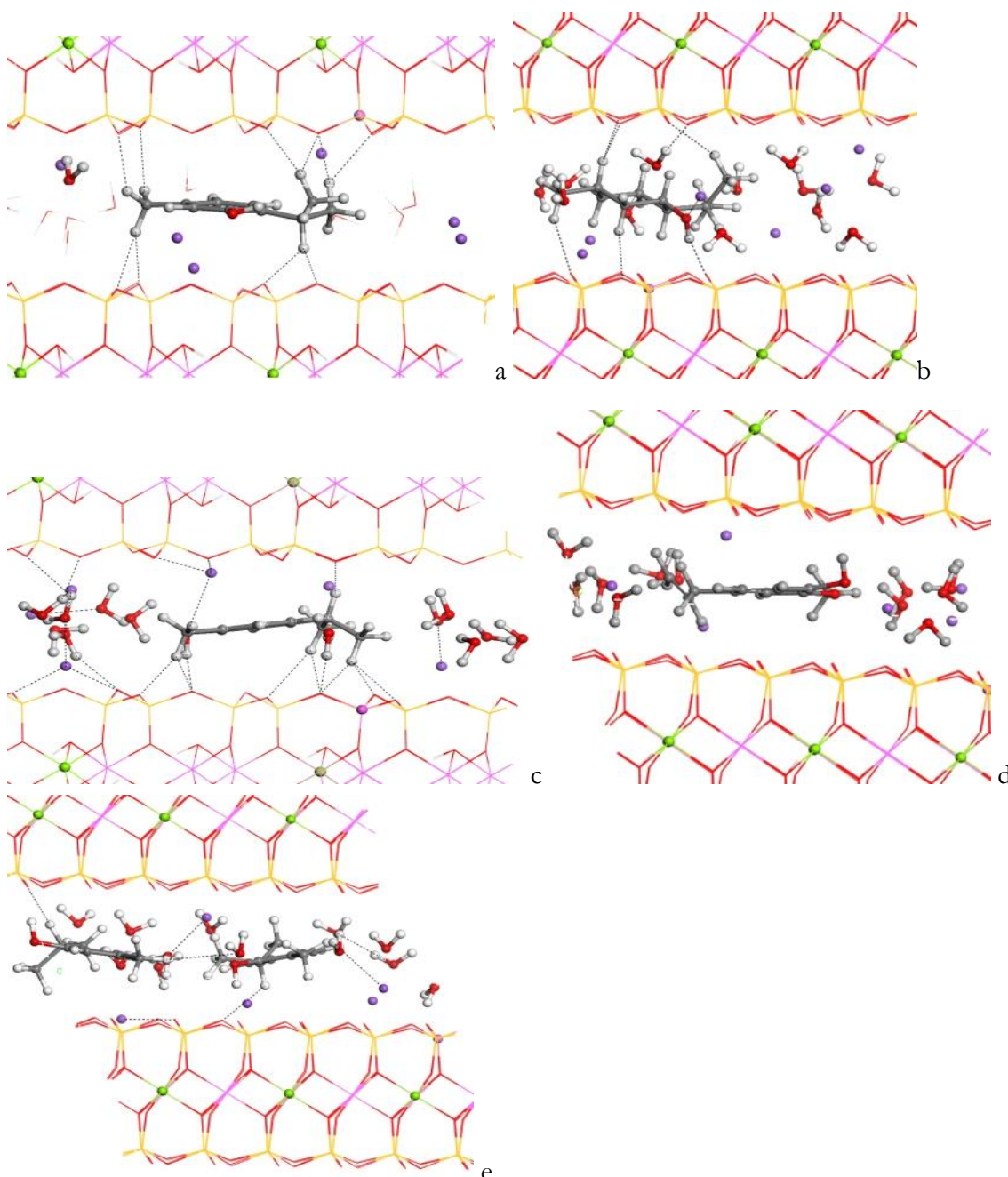


Figure 5.11. Optimized structures of MNT with organics intercalated: thymol (a), menthol (b), carvacrol (c), Eugenol (d), and a mixture of thymol and carvacrol (e). Some non-bonding interactions are shown with dashed lines. The H, C, Si, Al, Mg, Na and O atoms are in white, grey, yellow, pink, green, purple, and red colours.

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On the other hand, the crystal structures of the mineral do not alter significantly in the intercalation process and the cell parameters do not change properly. Only an increase of the interlayer spacing is observed due to the intercalation of the molecules (Table 5.7).

Taking into account these optimized structures, we can compare their total energy values and calculate the adsorption energies. The adsorption energy was calculated following the expression:

$$\text{MNT} + \text{organic} = \text{MNT-organic}$$

Where MNT-organic is the optimized structure of MNT with the organic molecule.

The adsorption energy will be:

$$E_{\text{ads}} = E_{\text{MNT-org}} - E_{\text{org}} - E_{\text{MNT}}$$

Where E_{ads} is the adsorption energy, $E_{\text{MNT-org}}$ is the energy of the optimized structure of complex of MNT with the organic molecule, E_{org} is the energy of the optimized molecular structure of the essential oil component, and E_{MNT} is the energy of MNT without the organic molecule.

In general, the direct adsorption energies in the intercalation of these organic molecules into the interlayer space of MNT are similar and positive. This means that the direct intercalation process is not energetically favourable due to the hydrophylia of MNT and the hydrophobicity of these molecules (Table 5.8). However, the analysis of the thyme essential oil used in our experiments indicated that the main components were thymol and carvacrol (see later below). Then, we designed a model of montmorillonite with the interlayer cations hydrated placing a mixture of one molecule of thymol with another of carvacrol in the center of the interlayer space (MNT-THYM-CARV). This structure was optimized and both adsorbates remaining close with the center of the interlayer space. Taking into account that can exist multiple minimum points in the configurations of these adsorbates. We performed a MD simulations in a NPT ensemble in order to explore multiple structures with minimal energy. The selected samples were optimized again with variable cell and compared the adsorption energies obtaining a more energetically favourable intercalation, being together much better than with thymol and carvacrol separately (Table 5.8). This indicates that a molecular association stabilized the intercalation process (Figure 5.11e).

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Table 5.7. Lattice cell parameters (distances in Å and angles in degrees) of the organic molecules in interlayer, on surface and on clean surface of MNT.

<i>Structure</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>α</i>	$\square$	$\square$	<i>d(001)</i>
MNT	15.47	17.88	12.0	97.4	105.4	90.1	11.57-11.76
MNT-thymol	15.47	17.88	12.92	97.5	106.9	90.1	12.36
MNT-menthol	15.46	17.88	13.28	94.3	104.0	90.1	12.89
MNT-carvacrol	15.47	17.88	12.72	99.1	102.0	90.1	12.44
MNT-eugenol	15.48	17.88	12.60	97.6	103.1	90.1	12.27
MNT-THYM-CARV	15.48	17.88	13.72	90.8	112.9	90.1	12.64

Table 5.8. Adsorption energy (in kcal/mol) of the essential oil components on clay mineral surface.

<i>structure</i>	<i>E_{ads} Interlayer</i>	<i>E_{ads} Surface</i>
MNT-thymol	31.42	-20.24
MNT-menthol	32.87	-9.74
MNT-carvacrol	34.92	-18.02
MNT-eugenol	27.08	-6.14
MNT-THYM-CARV	-26.64	-43.23

In addition, a model of an external surface of MNT was created with an empty space of 20 Å over the 001 clay surface ($c = 30$ Å), and the organic molecule was placed at 2 Å from the surface in a parallel disposition with respect to the mineral surface. After the optimization of these adsorption complexes (Figure 5.12), we observe that the organic molecules remain in a parallel disposition with respect to the MNT surface with a slightly twisting. The adsorption energies indicate that thymol and carvacrol adsorption is energetically favorable much better than the intercalation process. However, the adsorption energies of menthol and eugenol were low on the external surface of MNT.

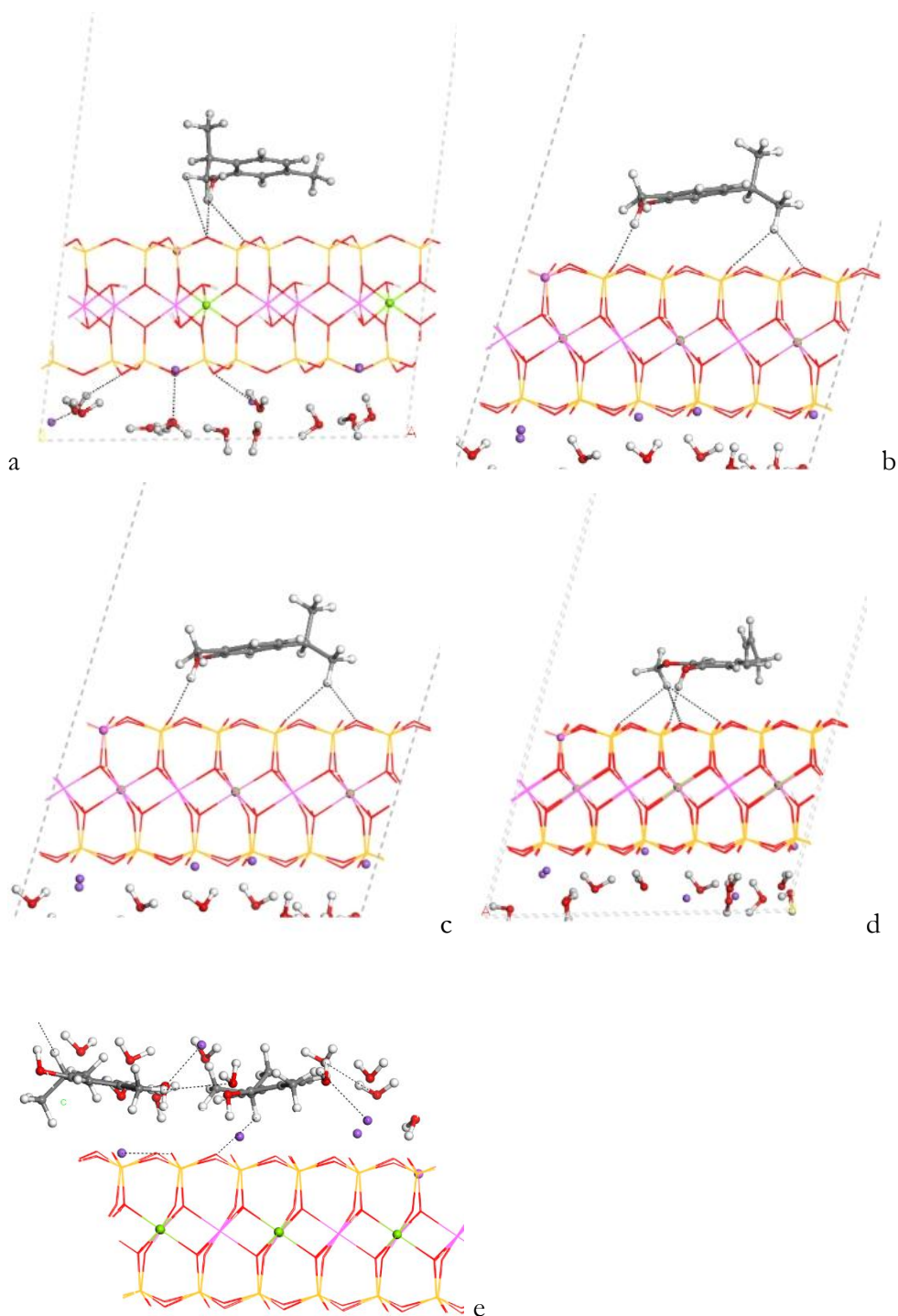


Figure 5.12 Adsorption complexes with the organic molecules on the external surface of clay mineral fully optimized. Thymol (a), menthol (b), carvacrol (c), eugenol (d) and thymol-carvacrol (e).

We have to consider that this model is an ideal one, however the external surface will have some Na^+ cations with some water molecules in a model closer to experiment. Then, we generate more realistic models in the same way that above but maintaining some Na^+ cations and water molecules on the external surfaces. The optimization of these models showed that

the adsorption process on the external surface of MNT was energetically favorable in all cases (Table 5.8). The adsorption of carvacrol and thymol are more favorable than the rest on the external surface of MNT. In MNT-THYM-CARV the adsorption on external surface of MNT is also more favourable than both adsorbates separately. In all cases the adsorbate adopts a twisted configuration with respect to the clay mineral surface.

5.8.3.3. Desorption and delivery process

The desorption process was calculated following the next equation:

And the energy was calculated with the expression:

$$E_{\text{desor}} = E_{\text{MNT}} + E_{\text{wboxorg}} - E_{\text{wbox}} - E_{\text{MNT_org}}$$

Where E_{desor} is the desorption energy, E_{wboxorg} is the energy of the optimized structure of complex of waterbox with the organic molecule, E_{wbox} is the energy of optimized water-box. E_{MNT} is the energy of the optimized structure of the clay, and $E_{\text{MNT_org}}$ is the energy of MNT with the organic molecule.

The desorption energies of organic molecules are presented in Table 5.9 describing the release of the adsorbate from the interlayer, surface and clean surface of MNT to the external media. In all cases the desorption is favorable, especially from the intercalation complexes. The desorption of menthol and eugenol on the clean surface of MNT are much more favorable than the rest due to the hydrophobic property of the clean siloxane and the non-polar character of these molecules.

Table 5.9. Desorption energy (in kcal/mol) of the organic molecules from interlayer, surface and clean surface of MNT.

Organic molecule	Interlayer $E_{\text{desorption}}$	Surface $E_{\text{desorption}}$
Thymol	-68.04	-16.43
Menthol	-59.54	-16.93
Carvacrol	-51.65	-8.4
Eugenol	-61.03	-27.81

These results predict that the adsorption of these components of essential oils should be in a non-aqueous media. Besides, we can predict that the adsorption will be mainly in the external surface of the interstitial spaces of clay minerals with some amount of intercalate complexes. This last amount will depend on the experimental conditions, temperature, maturation time, etc.

Our calculations predict these essential oil components can be delivered to the external media in presence of aqueous solutions. This means that these bioactive molecules can be delivered from clay mineral to the skin.

5.8.4. Experimental results and discussion.

5.8.4.1. Essential oil Characterization.

The essential oils used were analysed by Gas chromatography/mass spectrometer (GC/MS) indicating that the thyme essential oil from *Thymus zygis* was mainly thymol (59 %) with an important amount of carvacrol (24 %) along with other components [orto-cimene (12 %), linalool (3.9 %) and eucalyptol (1.7 %)]. This composition is within previous analysis of similar samples (Rota et al., 2008), although with a higher amount of carvacrol due to possible oxidation process during storage. The mentha essential oil was mainly menthol (64 %) along with other components [menthol acetate (14 %), menthone (13 %), and eucalyptol (9 %)].

5.8.4.2. FTIR Analysis

The raw clay mineral samples and the adsorption composites were analysed by FTIR. The main bands observed and their possible assignment are described below:

VHS and VF

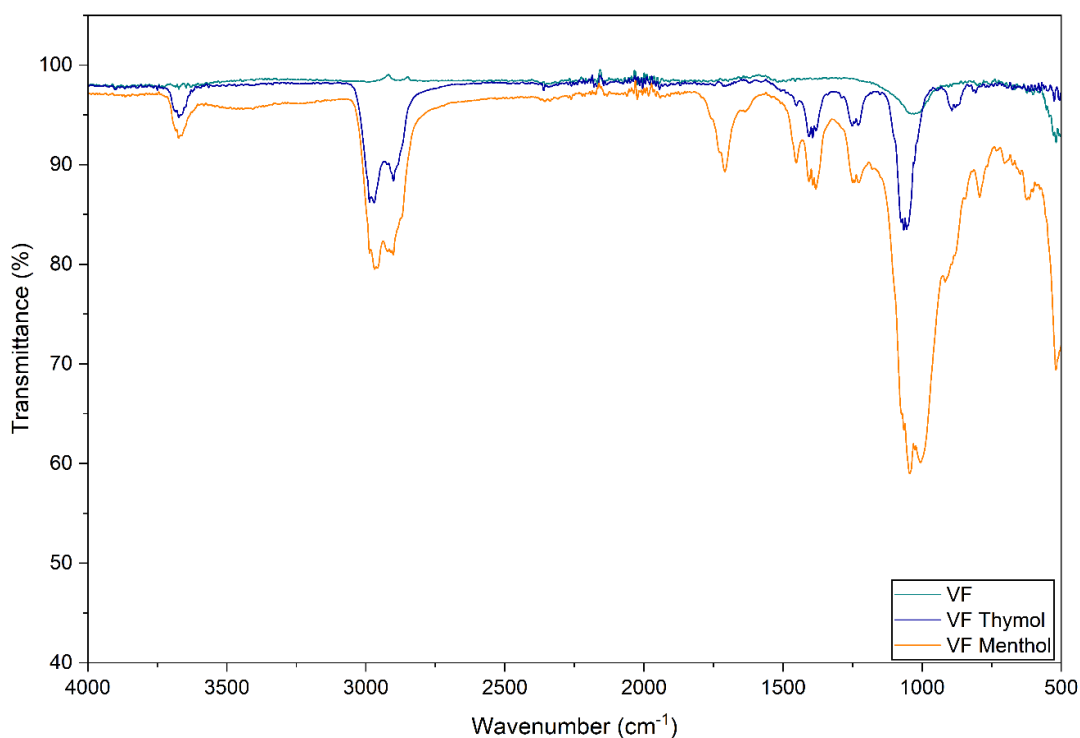
Due to the similarity in the composition of VHS and VF, the main bands appear very close. The bands at $3650\text{--}3580\text{ cm}^{-1}$ correspond to the ν (OH) stretching vibrations of the O-H bonds of the hydroxy groups in the octahedral layer of the clay mineral (Al–OH–Al, Al–OH–Mg, and Mg–OH–Mg vibrations) and also the ν (OH) bands of water molecules. The band at 1634.86 cm^{-1} corresponds to the δ (OH) bending vibrations of the H_2O molecules coordinated to the exchangeable cations in the interlayer space. The characteristic wide band at $1200\text{--}900\text{ cm}^{-1}$ is assigned to the Si–O–Si and Si–O–Al bonds vibrations being characteristic of layered phyllosilicates (García-Villén et al., 2019).

VF_menthol and VHS-menthol

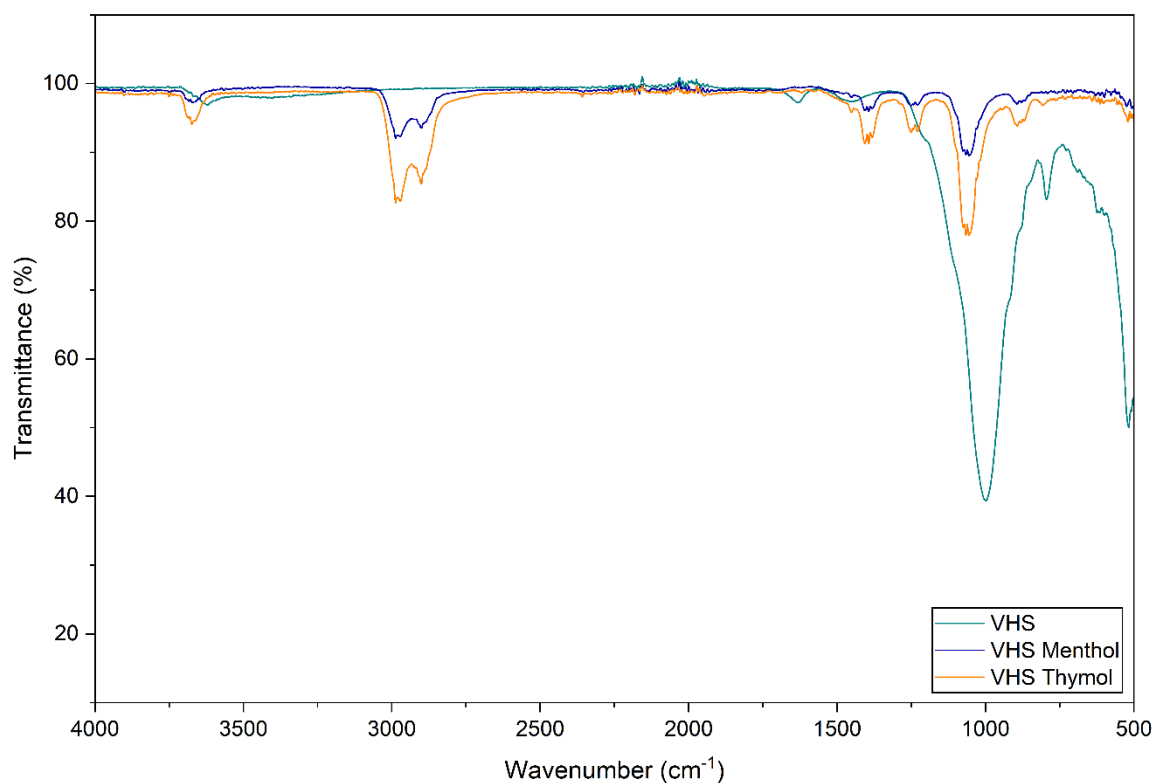
Regarding the spectra corresponding to VF_menthol and VHS-menthol adsorption composites, the characteristic bands of clay are observed in both materials. We can also confirm the presence of menthol detecting the essential oil bands in both solids. In the $3650\text{--}3580\text{ cm}^{-1}$ complex band overlap the ν (OH) band of menthol, water and clay mineral. Nevertheless, a certain asymmetry is observed at high frequency (3673 cm^{-1}) corresponding to the menthol hydroxyl group. The complex band at $3000\text{--}2800\text{ cm}^{-1}$ correspond to the ν (CH) stretching vibrations of the CH_3 and CH_2 groups of menthol. Comparing with these bands in isolated menthol molecule (Al-Bayati, 2009), a slightly shift to lower frequencies is observed due to the interactions of these bonds with the mineral surface. At 1393 and 1250 cm^{-1} mainly two multiplets band appear corresponding to bending δ (CH) vibrations of the CH_3 and CH_2 groups. These multiples is not observed in an isolated menthol molecule spectrum. Then, a drastic change of these bands is produced during the intercalation due to the interactions between the adsorbate and the mineral surface in the confined interlayer space of clay mineral (Al-Bayati, 2009). In the complex bands at $1100\text{--}1150\text{ cm}^{-1}$ the vibrations of clay minerals and menthol are overlapped observing bands at high frequency shoulder corresponding to the menthol presence. The band observed at 1711 cm^{-1} in VF-menthol correspond of the ν (C=O) of the rest components of the menthol essential oil sample (menthone 13%, menthol acetate 14%) (Figure 5.13).

VF_thymol and VHS-thymol

Regarding the spectra corresponding to VF_thymol and VHS-thymol, the characteristic bands of clay are observed in both adsorption products. Furthermore, the presence of thymol (59%) and carvacrol (24%) in our thyme essential oil sample can be confirmed in both solids by the band at 3673 cm^{-1} , corresponding to the stretching vibration of the O–H bond, associated with the phenolic groups of both compounds. A multiple form is observed in this band due to the frequency differences between thymol and carvacrol components and the conformational effect of the OH group observed clearly at low temperature in previous works (Lopes Jesus et al., 2022). No frequency shift is observed in this band in the intercalation of this essential oil into clay mineral probably due to the steric effect of the vicinal isopropyl (thymol) and methyl (carvacrol) groups in the molecular structure. In the $3000\text{--}2800\text{ cm}^{-1}$, two group of bands appear: one at $3000\text{--}2900\text{ cm}^{-1}$ assigned to the sp^2 (C-H) stretching vibrations of the aromatic C-H bonds and another one at $2900\text{--}2800\text{ cm}^{-1}$ corresponding to the aliphatic C-H bonds of methyl and isopropyl groups. On the other hand, the band at 1452 cm^{-1} can be attributed to the sp^2 (CC) stretching of the aromatic C=C bonds of thymol and carvacrol that appears at $1583\text{--}1459\text{ cm}^{-1}$ (Luna-Martínez et al., 2024). Hence, a low frequency shift is observed in these bands due to the interactions of these aromatic rings with the mineral surface. Finally, the bands at 1394 cm^{-1} and 1382 cm^{-1} are related to the symmetric and asymmetric bending in-plane sp^2 (C-H) vibrations of the isopropyl and methyl groups, respectively (Luna et al., 2024). The bands at $1066\text{--}1028\text{ cm}^{-1}$ range corresponding to sp^2 (C-H) and the out-of-plane bending sp^2 (C-H) vibrations are overlap with the characteristics bands of both clay minerals (Figure 5.13).



(a)



(b)

Figure 5.13: FTIR spectra of VHS, VF loaded with menthol and thymol. VF-thymol-Menthol (a) VHS-thymol-Menthol (b).

5.8.4.3. XRD Analysis

The powder XRD patterns of the adsorption complexes showed a clear shift of the (001) reflection from 12 Å to 14.7 Å after the treatment with EO. These shifts in the 001 reflections were observed in both clay minerals. This result confirmed the intercalation of both EO into the interlayer space of both clay minerals (Figure 5.14). Our calculations reproduced the interlayer space of the clay minerals observed experimentally (12 Å) and predicted also a shift of the $d(001)$ spacing from 11.7 Å in the pristine clay minerals to 12.64 Å in the intercalated materials. However, our experiments showed a higher shift in $d(001)$ (14.7 Å) (Figure 5.14). This can be explained by an adsorption of a higher amount of adsorbates because we used high EO concentrations. We explored the intercalation of a higher amount of menthol and thymol molecules. We obtained a $d(001)$ spacing of 14.9 and 14.85 Å for 4 thymol and 4 menthol molecules per 3x2x1 supercell of MNT, respectively. This value is similar to our experiments (14.7 Å) easily observed with the horizontal red line drawn in Figure 6, so confirming our hypothesis. The rest of organic molecules will be adsorbed on the external surfaces of the clay minerals used. Hence, our calculations can also predict the relative amount of EO intercalated into MNT.

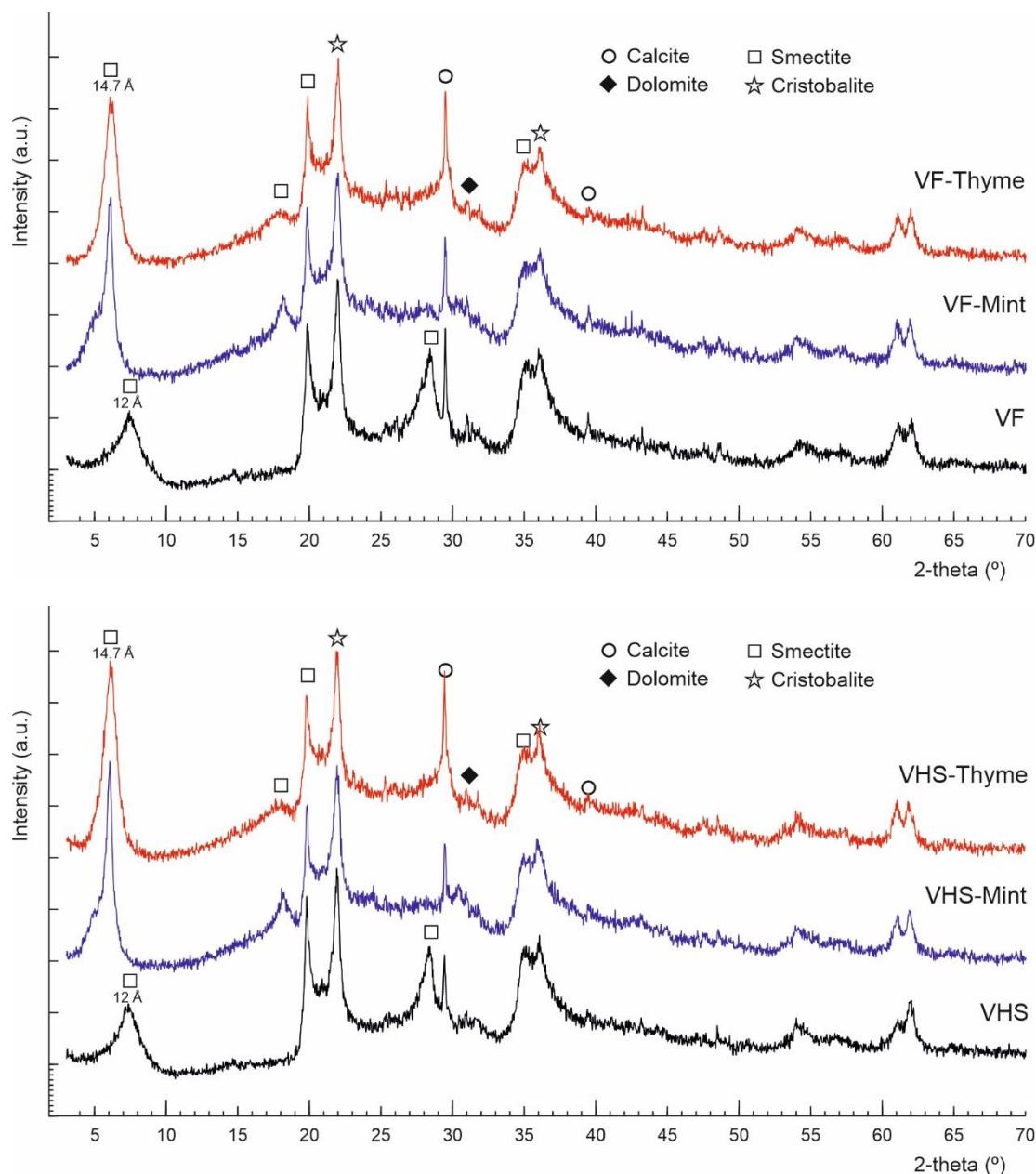


Figure 5.14. Powder X-ray diffraction patterns of the clay minerals (VHS and VF) (in black) and the adsorption composites with the EO of mentha (Mint) (blue) and thyme (red).

5.8.5. Conclusions

Our work is a successfully collaborative research between experimental and theoretical studies. The calculation methodology was validated reproducing the crystal structures of thymol and menthol. This study employed molecular modeling to investigate the adsorption behavior of essential oil components at three distinct sites within a clay mineral system: the interlayer region, the external surface, and the clean external surface. The computed adsorption energies indicate a strong preference for adsorption on the surface and clean surface of the clay, as evidenced by negative adsorption energy values. In contrast, adsorption within the interlayer region resulted in positive energies, suggesting energetically

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unfavourable interactions in that site. This result proposes the best experimental process for preparing a hybrid composite essential oil-clay mineral. We propose to use non-aqueous solvent or no solvent for preparing these materials.

Desorption energy calculations for all adsorption sites yielded negative values, indicating that desorption is energetically feasible in each case. This suggests relatively weak or reversible binding of essential oil components, particularly within the interlayer region.

Overall, the surface characteristics of the clay mineral play a critical role in facilitating favourable adsorption. These modeling results provide mechanistic insight into the interaction behaviour of essential oil molecules with clay, and will serve as a valuable foundation for guiding and interpreting the upcoming experimental work. This integrated approach can support the development of optimized clay-based systems for the encapsulation and controlled release of essential oil components.

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CHAPTER 6

CONCLUSION

Thesis Conclusion:

This thesis presents a systematic and multiscale investigation of clay-based suspensions and peloids, integrating experimental rheology, spreadability, physicochemical characterization, and molecular modeling to establish mechanistic relationships between formulation, processing, and functional performance. The studies demonstrate that clay type, water composition, and processing parameters—including agitator type and mixing energy—critically determine the rheological and textural properties of suspensions. Smectite content, cation exchange capacity, and accessory minerals govern the dominant thickening mechanism: swelling dominates in low-ionic-strength purified water, whereas ion-bridging prevails in mineral-rich Saturnia thermal water. Axial agitators generally yield higher viscosities, radial agitators produce lower viscosities, and medium-energy mixing achieves balanced rheology and extensibility suitable for practical applications. Strong correlations between viscosity, extensibility, adhesiveness, and theoretical bonding strength coefficients provide a quantitative framework for predicting and tailoring suspension performance.

Physicochemical characterization of clays, water, and suspensions—including XRD, FTIR, XRF, cation exchange capacity, pH, and cooling kinetics—clarifies the mineralogical and chemical basis for these behaviors. Comparison of purified water versus thermal water peloids underscores the sustainability aspect: optimized formulations with identical properties of natural peloids can be prepared using purified water and pharmaceutical grade clays, conserving limited thermal water resources without compromising rheological or functional properties. These findings provide practical guidance for eco-friendly design of peloids and topical suspensions.

Complementary molecular modeling studies reveal the adsorption, intercalation, and desorption behavior of essential oils and penetration enhancers in clays. Adsorption is energetically favored on external clay surfaces, while interlayer intercalation is achievable under controlled conditions, supporting the design of clay–bioactive composites for controlled delivery in pharmaceutical and cosmeceutical applications. Molecular dynamics simulations indicate that bioactive molecules are stably retained within clay interlayers while remaining deliverable to aqueous media, highlighting their potential for therapeutic skin treatments, specialized thermal baths, and cosmeceutical formulations.

By integrating macroscopic experimental findings with molecular-level insights, this work establishes a mechanistic and predictive framework for sustainable clay-based material design. It enables the rational optimization of clay type, water composition, and processing conditions to achieve desired rheological, textural, and bioactive delivery properties. These results not only advance the development of eco-friendly, high-performance therapeutic and cosmeceutical clay formulations but also demonstrate the broader value of combining experimental and computational approaches to design resource-efficient, functionally optimized materials for pharmaceutical and dermatological applications.

SUPPORTING INFORMATION

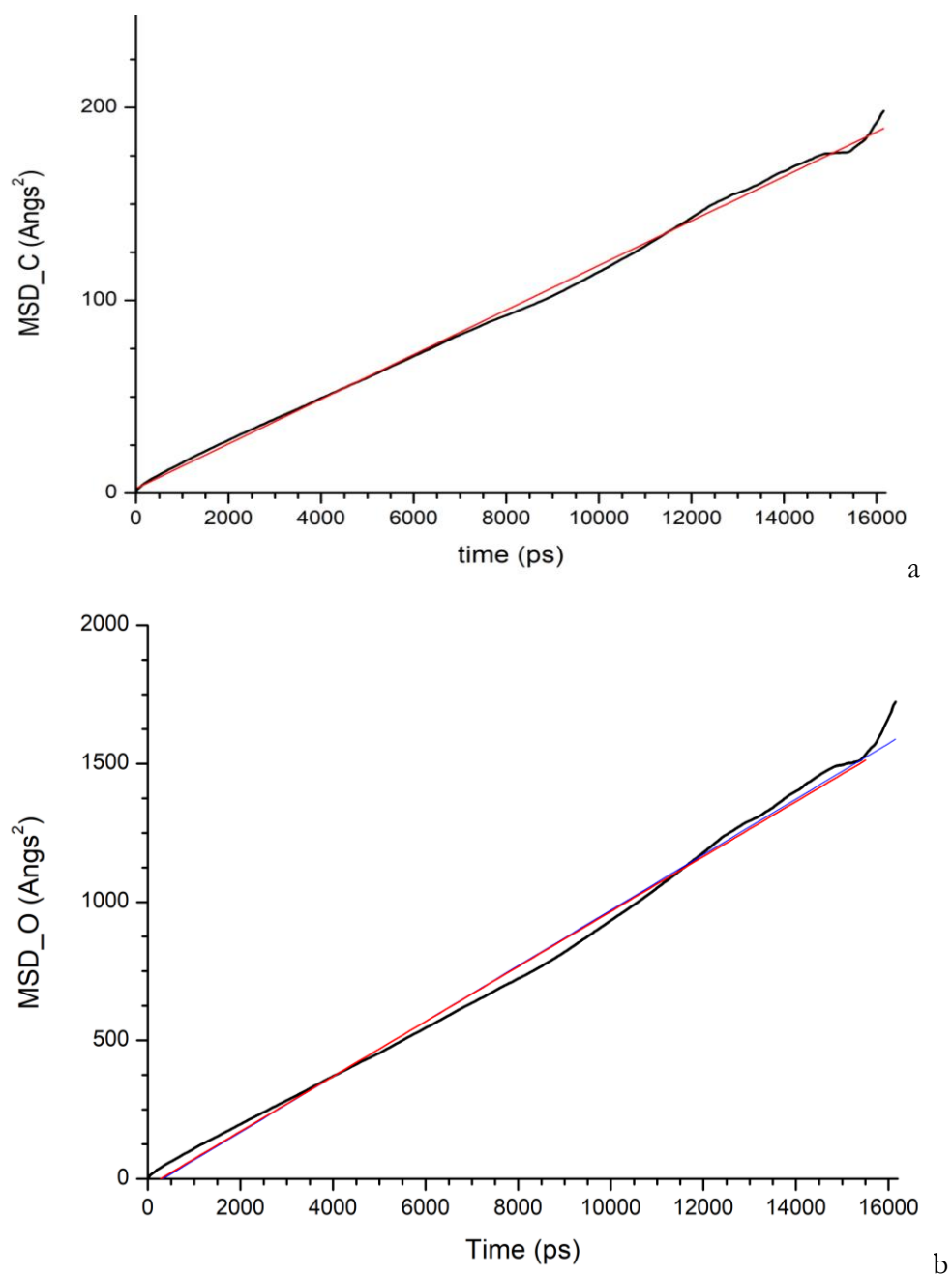


Figure S1. Mean Square Displacement of the C (a) and O (b) atoms of the molecular dynamics simulation of the combined mixture of essential oil components intercalated into MNT interlayer (16.2 ns at NPT). The red line is the linear fitting for calculating the slope of the curve and the Diffusion coefficient.

ANNEX

ANNEX A

Comprehensive Review of Dermatological and Cosmeceutical Manifestations of Thermal Water and Future Insights

Article Type: Review Paper

Authors: *Shamsa Kanwal*, Enass Y. Osman & Imen Khiari

Journal: *International Journal of Biometerology*

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Statement of Inclusion in Thesis:

This article forms part of the manuscript-based thesis submitted by the candidate. The article is included in its published form for reference and verification purposes. An adapted version of this work is presented in the main body of the thesis.

ANNEX B

Molecular Modelling of the Adsorption and Delivery of α -Pinene and Similar Terpenes of Essential Oils on Montmorillonite Surfaces

Article Type: Research Article

Authors

Shamsa Kanwal, Alfonso Hernández-Laguna, and C. Ignacio Sainz-Díaz

Journal: *Nanomaterials*

Publication Details: Volume 15, Issue 20, Article 1573

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Statement of Inclusion in Thesis

This article is included in the annex of this manuscript-based thesis for reference and verification purposes. An adapted and reformatted version of this work is presented and discussed in the main body of the thesis.

ANNEX C

Adsorption of volatile anti-inflammatory essential oils on phyllosilicate surfaces. Toward montmorillonite-based natural topical drug delivery

Article Type: Research Article

Authors

Shamsa-Kanwal, Nathaly Valarezo, Alfonso Hernández-Laguna, Alberto López-Galindo, Fátima García-Villen, Cesar Viseras-Iborra, C. Ignacio Sainz-Díaz*

Journal: *Applied Surface Science*

Publication Status: Submitted (Under Review)

ANNEX D

Characterization of the thermal bath Saturnia muds and comparison with other bentonites. A multiscale study

Article Type: Research Article

Authors

Shamsa-Kanwal, Alberto López-Galindo, Cesar Viseras-Iborra, C. Ignacio Sainz-Díaz

Journal: *Applied Clay Science*

Publication Status: Submitted (Under Review)