UNIVERSIDADE DE SÃO PAULO
Faculdade de Ciências Farmacêuticas
Programa de Pós-Graduação em Fármacos e Medicamentos
Área de Produção e Controle Farmacêuticos

Preparation and characterization of organogels containing vitamin E for cosmetic application

Preparação e caracterização de organogéis contendo vitamina E para aplicação cosmética

Renata Miliani Martinez

Tese para obtenção do grau de DOUTOR

Orientador: Prof. Dr. André Rolim Baby

São Paulo

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Ficha Catalográfica elaborada eletronicamente pelo autor, utilizando o programa desenvolvido pela Seção Técnica de Informática do ICMC/USP e adaptado para a Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP

Bibliotecária responsável pela orientação de catalogação da publicação:
Marlene Aparecida Vieira - CRB - 8/5562

M115p

Martinez, Renata Miliani
Preparação e caracterização de organogéis contendo vitamina E para aplicação cosmética / Renata Miliani Martinez. - São Paulo, 2022.
54 p.

Tese (doutorado) - Faculdade de Ciências Farmacêuticas da Universidade de São Paulo. Departamento de Farmácia - Programa de Pós-Graduação em Fármaco e Medicamentos.
Orientador: Baby, André Rolim
Coorientador: Lannes, Suzana Caetano da Silva

1. Organogéis. 2. Bigéis. 3. Vitamina E. 4. Ácido 12-hidroxiesteárico. 5. Cera de candelila. I. T. II. Baby, André Rolim, orientador. III. Lannes, Suzana Caetano da Silva , coorientador.

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Comissão Julgadora da Tese para Obtenção do Título de DOUTOR

Prof. Dr. André Rolim Baby

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1° Examinador

2° Examinador

3° Examinador

São Paulo, ___ de_____de 2022.

ACKNOWLEDGEMENT

I would like to thank special persons and institutions that direct or indirect contributed to this work:

To Professor André Rolim Baby for the opportunity and privilege of being his PhD student throughout these years. Thank you for believing in me and my work.

To Professor Cristiano Luis Pinto de Oliveira for the support and contribution with a part of this work.

To Pedro Leonidas Oseliero Filho and Barbara Bianca Gerbelli for all the support and long hours of work to finish our paper.

To David, secretary from the PhD program, for the patience and caring with all students.

To Chemyunion, specially Wagner Vidal Magalhães, for the trust and partnership.

To CAPES for funding this work (This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001).

To my laboratory colleagues, that turned this period more fun and entertaining.

To my family, that supported me to continue this journey, specially my husband.

To my sons – this work was all made for you.

ABSTRACT

MARTINEZ, R.M. **Preparation and characterization of organogels containing vitamin E for cosmetic application.** 2022. 54f. Thesis (PhD) – Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2022.

Human skin is attacked daily by ultraviolet (UV) radiation and pollutants. Such aggressions can promote lipid peroxidation mediated by free radicals in cells, generating DNA damage and inflammatory processes, in addition to endogenous vitamin E depletion. The use of antioxidant cosmetic products has potential for treatment; however, the stability and permeation of these active ingredients may be a limiting factor in their use. Thus, the present work aimed at the development and characterization of organogels containing vitamin E and their dispersions in bigels, focusing on cosmetic application. The results derived from this investigation are ordered in three articles, being: (1) review on organogels in the cosmetic area; (2) application of organogels containing vitamin E in systems of bigels, their physicochemical characterization and evaluation of efficacy *in vitro* and *ex vivo*; and (3) characterization of organogels containing vitamin E regarding their microstructure and rheological profile. The results indicated that despite the potential for permeation of hydrophilic and hydrophobic actives through the skin, increased stability and sensory modification, organogels were still little explored in the cosmetic area. The bigels were characterized as weak viscoelastic oil-in-water gels, with adequate stability determined by thermal and centrifugal stress. The presence of vitamin E generated little influence on the rheological profile and efficacy *in vitro* and *ex vivo* of the bigels, emphasizing the need for further studies in the presence of stress in the biological sample. Organogels were characterized as weak pseudoplastic gels. The type of organogelling agent was relevantly influenced in the presence of vitamin E. 12-Hydroxysesteric acid had a reduction in gel strength, while candelilla wax showed increased strength. All phase transition temperatures were reduced in the presence of vitamin E. The results indicated the potential use of organogels and bigels in the delivery of vitamin E for topical application, enabling the development of formulations with stability and modulation of the rheological profile as needed.

Keywords: organogels, bigels, vitamin E, 12-hydroxystearic acid, candelilla wax.

RESUMO

MARTINEZ, R.M. **Preparação e caracterização de organogéis contendo vitamina E para aplicação cosmética**. 2022. 54f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2022.

A pele humana é agredida diariamente pela radiação ultravioleta (UV) e poluentes. Tais agressões podem promover a peroxidação lipídica mediada por radicais livres nas células, gerando danos ao DNA e processos inflamatórios, ademais da depleção da vitamina E endógena. O uso de produtos cosméticos antioxidantes apresenta potencial para o tratamento, porém, a estabilidade e permeação desses ingredientes ativos pode ser um limitante em sua utilização. Assim, o presente trabalho teve como objetivo o desenvolvimento e caracterização de organogéis contendo vitamina E e suas dispersões em bigéis, visando aplicação cosmética. Os resultados derivados dessa investigação estão ordenados em três artigos, sendo: (1) revisão sobre organogéis na área cosmética; (2) aplicação de organogéis contendo vitamina E em sistemas de bigéis, sua caracterização físico-química e avaliação de eficácia *in vitro* e *ex vivo*; e (3) caracterização de organogéis contendo vitamina E quanto à sua microestrutura e perfil reológico. Os resultados indicaram que apesar do potencial na permeação de ativos hidrofílicos e hidrofóbicos pela pele, aumento de estabilidade e modificação no sensorial, os organogéis ainda foram pouco explorados na área cosmética. Os bigéis foram caracterizados como géis viscoelásticos fracos tipo óleo-em-água, com estabilidade adequada determinada por estresse térmico e centrífugo. A presença de vitamina E gerou pouca influência no perfil reológico e eficácia *in vitro* e *ex vivo* dos bigéis, ressaltando a necessitando de estudos posteriores na presença de estresse na amostra biológica. Os organogéis foram caracterizados como géis pseudoplásticos fracos. O tipo de organogelificante sofreu influência relevante na presença da vitamina E, sendo que o ácido 12-hidroxiesteárico teve redução na força do gel, enquanto a cera de candelila apresentou aumento de força. Todas as temperaturas de transição de fases foram reduzidas na presença de vitamina E. Os resultados indicaram o potencial uso de organogéis e bigéis na veiculação de vitamina E para aplicação tópica, possibilitando o desenvolvimento de formulações com estabilidade e modulação de perfil reológico conforme a necessidade.

Palavras-chave: organogéis, bigéis, vitamina E, ácido 12 hidroxiesteárico, cera de candelilla.

SUMMARY

1. INTRODUCTION

Oxidative stress promotes skin aging, represented by the formation of spots and wrinkles. The target cells in the skin are keratinocytes, fibroblasts and melanocytes (Nakamura et al., 2015; Qiao et al., 2017; Soeur et al., 2017). Extrinsic skin aging, caused by external agents, is closely related to oxidative stress, especially after exposure to ultraviolet (UV) radiation, increasing the expression of genes that translate matrix metalloproteinases enzymes (MMPs) into dermal fibroblasts, degrading collagen and elastin (Burke, 2018). Clinical studies corroborate the evidence of cutaneous aging *in vitro* (Hüls et al., 2016; Vierkötter et al., 2010). Despite the difficulties in isolating a single variable in a clinical study, skin aging has a strong correlation with exposure to solar radiation and pollutants (Krutmann et al., 2017). These "triggers" promote the formation of free radicals, usually in the form of reactive oxygen species, and lipid peroxidation products that follow the deleterious cascade, reducing the concentration of endogenous vitamin E to the cell nucleus, where they will activate transcriptional factors for the formation of enzymes capable of disrupting collagen and inflammatory factors, culminating in signs of premature skin aging and inflammation (Park, 2015). Chronic exposure to pollutants causes cumulative oxidative damage in the *stratum corneum* and a significant reduction in the reserves of endogenous antioxidants such as vitamin C and E. In the mitochondria, damage also occurs, reducing levels of adenosine triphosphate (ATP) and sirtuin-3 (protein involved in neutralizing oxidizing agents) (Mancebo & Wang, 2015). Thus, the replacement of vitamin E to the skin is essential for the maintenance of the protective antioxidant system of cells.

Vitamin E is a classic anti-aging agent used in the cosmetic and dermatological field. When applied topically, vitamin E can act against photoaging, reducing lipid peroxidation and photocarcinogenic markers, such as transcription factors of matrix metalloproteinases (MMP-1) and thymine dimers (Chen et al., 2012). It is a lipophilic compound formed by several molecules, and alpha-tocopherol is the most important to humans, as it is available in both the *stratum corneum*, epidermis and dermis. Unlike other antioxidant agents, vitamin E is not produced by the human body and should, therefore, be obtained through diet or topical application. Vitamin E is recognized for its antioxidant property, acting in the reduction of erythema caused by exposure to UVB radiation (Pegoraro et al., 2017) and attenuation of signs of skin aging (Rinnerthaler et al., 2015). In the presence of UV radiation, the alpha-tocopherol available in the *stratum corneum* decays at, approximately, 50% of its initial concentration. This process may be associated with a direct mechanism of absorption of UVB radiation and/or an indirect mechanism of interaction of the molecule with reactive oxygen

species formed by UVA radiation photosensitive compounds. In this sense, vitamin E aids photoprotection when combined with oral or topical formulations, the latter being of great cosmetic interest. Topical formulations containing 0.1 to 1.0% (w/w) of alpha-tocopherol have effective potential to increase the antioxidant skin barrier protection (Thiele & Ekanayake-Mudiyanselage, 2007). However, its instability in the face of UV stress and heat lead to pharmacotechnical difficulties. Thus, the use of encapsulated or nanostructured vitamin E can be used (Pegoraro et al., 2017). In some cases, the high production cost and technical difficulties of preparing these systems may discourage formulators, and new ways of stabilizing vitamin systems are needed, such as organogels (Shi et al., 2014).

Organogels are thermoreversible organogelled matrices capable of functioning as asset delivery systems, convenient preparation and low cost. This technology comes from the food area, however, already has several pharmaceutical applications (Esposito et al., 2018). In the cosmetic field, organogels can be *used per se, as used* for the delivery of anticellulite agents (Morales-Rueda et al., 2009), or through the development of organogelled nanoparticles for dermocosmetics (Kirilov et al., 2014). There is also the possibility of incorporating organogels in emulsifiable systems, known as bigels (Lupi et al., 2016).

Among the various applications of organogels, its use as a delivery system differentiates this technology from other carrier systems for its convenience in preparation and low cost. Despite the possibilities described, there are still no reports in the literature for the development of organogelled cosmetic formulations containing vitamin E. Thus, the present study aimed at the development of stable dispersions of organogels and bigels containing vitamin E for cosmetic application.

2. OBJECTIVES

This research work aimed to produce and evaluate organogels and bigels loaded with vitamin E for skin cosmetic application.

2.1 SPECIFIC OBJECTIVES

- Review the literature regarding organogels in cosmetics.
- Produce and characterize organogels with candelilla wax or 12-hydroxystearic loaded with vitamin using rheology, polarized microscopy and small angle X-ray scattering (SAXS).
- Incorporate organogels into bigels and characterize them by rheology, microscopy, laser diffraction, antioxidant assay (DPPH) and *in vitro* and *ex vivo* efficacy tests.

3. PUBLICATIONS

The Thesis was organized in accordance with three published papers, derived from this investigation work, ordered by date of publication:

- Article 1 (Main features and applications of organogels in cosmetics): a review paper about the organogel's state-of-art into the cosmetic field, published at **International Journal of Cosmetic Science**.
- Article 2 (Vitamin E-loaded bigels and emulsions: Physicochemical characterization and potential biological application): a full-length article about the application of vitamin E-loaded organogels into bigels, their characterization and biological effects, published at **Colloids and Surfaces B: Biointerfaces.**
- Article 3 (Influence of the Mixtures of Vegetable Oil and Vitamin E over the Microstructure and Rheology of Organogels): a full-length article about the characterization of vitamin E-loaded organogels, published at **Gels**.

Article 1

Main features and applications of organogels in cosmetics

International Journal of Cosmetic Science

International Journal of
Cosmetic Science

International Journal of Cosmetic Science, 2019, 41, 109–117 doi: 10.1111/ics.12519

Review Article

Main features and applications of organogels in cosmetics

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Received 20 November 2018, Revised 25 January 2019, Accepted 30 January 2019

Keywords: cosmetics, delivery/vectorization/penetration, emulsions, formulation/stability, organogel

Abstract

Cosmetic treatments aim at improving skin appearance through vehicles of good sensory properties. Those vehicles are mainly emulsions and gels designed to deliver safe and effective compounds to skin. Creams and serums are widely used to achieve these goals, but recently a new type of formulation known as organogels triggered scientific attention, particularly in the design of both topical and cosmetic formulations. It has been established that the lipophilic nature of organogels makes it an excellent candidate for the delivery of cosmetic molecules through skin. In this review, we discuss the properties and characteristics of organogels and present the advantages of the application of these systems in cosmetics.

Résumé

Les traitements cosmétiques visent à améliorer l'apparence de la peau grâce à des véhicules dotés de bonnes propriétés sensorielles. Ces véhicules sont principalement des émulsions et gels conçus pour livrer des composants sûrs et efficaces à la peau. Crèmes et sérums sont largement utilisés pour atteindre ces objectifs mais un nouveau type de formulation appelé organogels a récemment attiré l'attention des scientifiques, en particulier en ce qui concerne la conception de formulations à la fois topiques et cosmétiques. Il a été établi que la nature lipophile des organogels en fait d'excellents candidats pour la libération de molécules cosmétiques à travers la peau. Dans cette analyse, nous discutons des propriétés et des caractéristiques des organogels, et présentons les avantages de l'utilisation de ces systèmes dans la cosmétique.

Introduction

Organogel technology was extensively reviewed for human health applications in food (specially as a trans-fat texture mimetic) and pharmaceutical purposes (mainly as drug delivery system) [1–4]. However, cosmetic applications of organogels are still scarce in the literature. Organogel or oleogel is a semi-solid material composed of gelling molecules organized in the presence of an appropriate organic solvent, via physical or chemical interactions, into a continuous network, preventing solvent flow as a result of surface tension. The organogel successful formation depends on the organic phase, temperature, process time and characteristics of the gelling molecules. The latter can be divided into two main categories: polymeric or low molecular weight organogelators (LMWO). Polymers can either immobilize the organic phase by forming a network of chemical crosslinked chains or entangled physical chains that are

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Table I Organogelators used in cosmetics

Organogelator	Uses	Reference
Fatty esters of polyols, fatty ethers of polyols, mixtures of fatty esters of polyols and mixtures of fatty ethers of polyols	Organogel in the form of an oil/glycerol emulsion to deliver an oxidation-sensitive hydrophilic molecule	[7]
Pluronic–lecithin	Deliver *Centella asiatica* to cellulite treatment	[8]
Amine and amide molecule derivatives	Structuring silicone to make-up formulations	[9, 10]
Lecithin	Delivery of anti-ageing molecules	[11]
	Delivery of caffeine to cellulite treatment	[12]
12-hydroxystearic acid	Stabilize UVB blockers to sunscreens Improve rheological properties like spreadability over the skin to antiperspirant gel formulations	[13,14]
Monoglycerides of fatty acids	Template to hydrophilic	[15]
Mixture of glyceryl stearate and policosanol	and lipophilic cosmetic molecules delivery	[16]
Dialkyl-Nacylglutamides, polyamides and their mixtures	Composition with long-lasting brilliance and pleasant (not sticky) when applied on skin or lips	[17]

UVB = ultraviolet B.

stabilized by weak interchain interactions (e.g. hydrogen bonding, van der Waals forces and p-p stacking bonds). Likewise, the LMWO can undergo self-assembly by physical interactions for the formation of aggregates that are sufficiently long to overlap and induce solvent gelation [5]. Those aggregates are seen microscopically as tubular rods, fibrils, fibres or crystal networks [6]. Organogelators' orientation may lead to solid-matrix gels that are more rigid owing to their (pseudo)crystalline permanent solid-like networks; or to fluid-matrix gels that have transient junction points formed by simple chain entanglements. The difference between the types of gels is important to deliver the active cosmetic ingredient to skin and to the formulation shelf life.

There are several molecules that show organogelator capacity. Some of the main examples in cosmetics are lecithin, glyceryl fatty acid esters, sorbitan-derived esters, fatty acids, fatty alcohol and vegetable waxes. Their uses are discussed in Table I.

The gelation capacity is also closely related to the properties of the organic solvent. Mineral and vegetable oils are mainly used as organic phases in cosmetic organogels. Mineral oil is a transparent, thermo-stable and secure material [18]. Its properties and low cost turn it into one of the major solvents used in cosmetics. Lately, some brands are replacing mineral by vegetable oils to achieve 'green' claims. Despite the health benefits brought by medium and small triglyceride chains present in vegetable oils, these ingredients have limited thermal and oxidative stability owing to their unsaturated carbon chains [19]. Nevertheless, the use of antioxidants and low temperature processes are effective strategies to stabilize short shelf-life products. Another interesting feature of using vegetable oils is the impact on gelation properties. Since vegetable oils contain unsaturated fatty acids and triacylglycerols, the entanglement between organogelators and the organic phase is affected by p-p stacking bonds and the organogel may present different properties, mainly by softening its structure. It should be noted that unsaturated oils are far more polar than mineral oils, limiting the organogelator solubility [20]. Other solvent characteristics, like polarity, may influence gelation by the intensity of intermolecular interactions [21].

Preparation of organogels in cosmetics

There are mainly three methods used for the organogel preparation. The first is the fluid-filled fibre mechanism, characterized by the formation of micellar aggregates by the addition of water into the solution of apolar solvent and surfactant (e.g. lecithin). In this case, linear networks are constructed by the linkage of surfactant molecules induced by water, entrapping the organic phase in the spaces among the entangled micelles [11]. The second is the solid-fibre mechanism, characterized by heating–cooling process of the solution containing the organic phase and the organogelator. In this case, organogelator molecules precipitate out as fibrils, forming a three-dimensional fibrillar network structure that holds the organic phase [22]. The third is the mechanical homogenization and microirradiation method, characterized by a high-speed homogenization followed by a microwave heating of the solution of organic phase and organogelators. In this occasion, the final organogel is like the one obtained by the solid-fibre mechanism but with a reduction in time preparation and energy consumption [23]. The methods used to obtain topic application organogels are mostly through solid-fibre mechanism [24] or fluid-filled fibre mechanism (when lecithin is applied) [25–28].

The process involved in organogel formation is also a critical parameter to obtain successful gelation. First, it is very important to guarantee total melting or dispersion of organogelators in the organic phase for the posterior network formation. High temperatures, above the melting point of organogelators, are needed to fully dissociate the initial ordering. When fatty acids and esters are used as organogelators, it is important to make sure that the crystalline and liquid memories are totally destroyed during melting and recooling, respectively, to obtain a new, different and more stable structure that will entrap the organic phase [29]. Second, the cooling rate of the mixture, when solid-fibre mechanism is applied, is also crucial to organogel formation. Changes in the cooling rate affect the rate of nucleation, crystal growth and the degree of branching of organogelators. These processes are governed either by mass transfer or thermodynamics. At rapid cooling rates (e.g. above 5°C min^{-1}), time-dependent chemical potential difference between the molten solid in solution and the crystallized solid is the driving force for both nucleation and crystal growth. On the other hand, slow cooling rates are governed only by time [30]. For solid-fibre mechanism organogels, the temperature is an important factor because of its influence on all phase transition parameters: gel–sol or sol–gel phase transition domains (PTDs), Tmelt (organogel melting temperature), Tgel (gelation transition temperature), Tsol (organogel liquefaction temperature) and Tform (organogel formation temperature). The gel–sol PTD (organogel liquefaction) or sol–gel PTD (organogel formation) are regions observed by rheology thermic studies that present both the gel and sol phases. When the material is heated, the Tmelt is observed as an abrupt decrease of the viscoelastic modulus (G^0) and it indicates the beginning of the organogel melting process whereas Tsol indicates the temperature above which the organogel is completely liquefied. On the other hand, when cooling, Tgel is observed as an abrupt increase of the viscoelastic modulus (G^0) and it indicates the beginning of the gelation process whereas Tform indicates the organogel formation temperature, above which the material shows constant viscoelastic properties with the temperature decrease, as shown in Fig. 1 [31].

In the case of the fluid-filled fibre mechanism, the content of phospholipids drives the organogel formation. Poorly purified lecithin, which presents residues of saturated fatty acids, carotenoids or synthetic hydrogenated soybean lecithin, does not possess gel-forming properties [32]. To achieve the desired gelation, lecithin must contain more than 95% phosphatidylcholine and must be free from fat, as well as moisture [11]. For that reason, lecithin-organogels display a high cost. An alternative is using lower cost surfactants (e.g. sorbitan monostearate) in hot solvent dispersions without water addition. In this process, the cooling rate deeply influences gel characteristics, but it can provide an opaque, thermoreversible and stable gel with potential topical applications [33, 34].

And finally, the amount of organogelator and its homogeneous dispersion on the organic phase are also important to ensure organogel formation. For LMWO, concentrations of <2.0% are enough to form a stable and rigid organogel. Candelilla wax (*Euphorbia cerifera* and *Euphorbia antisyphilitica*, from the Euphorbiaceae family), a natural and food grade ingredient, has a highly promising LMWO capacity requiring only 0.5% (w/w) concentration in safflower oil [35].

Characterization of organogels in cosmetics

The characterization of organogels is required to assure the structure, stability and security of the formulation. The methods can be

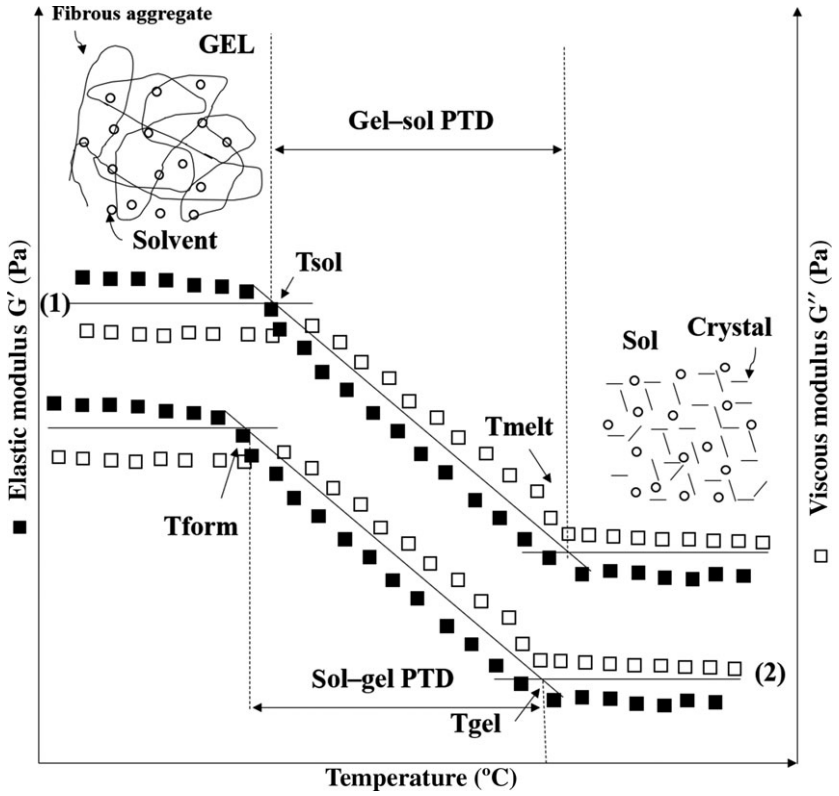

Figure 1 Schematic representation of phase transition parameters of solid-fibre mechanism organogels. (1) Heating curve; (2) cooling curve. Adapted from Kirilov and collaborators, 2017 [25].

divided into categories depending on the feature characterized and they are discussed in Table II.

Rheological properties

Rheology studies describe relevant information about the structure, thermal and shear behaviour of organogels. Stress ramp and steady flow drag analysis are used to show how organogels flow. The results indicate the viscosity and viscoelasticity of the organogel. This method is valuable to determine non-Newtonian flow characteristics, like when a decrease in the viscosity is observed with increasing shear rates, known as pseudoplasticity [42]. That phenomenon is named as shear-thinning behaviour and it is considered very beneficial to topical application of formulations, since this ensures the formation of a thin layer of the gel spread over skin surface and a consequential efficient delivery of bioactive agents. Also, high viscosity at low shear rates is related to long-term stability during storage [43]. Shear stress rheological measurements may also be used to assess sensory properties, which are very useful to the development of cosmetic products, where pleasant organoleptic properties are critical. Measuring the viscosity as a function of shear stress may be used to quantify primary and secondary skin feel. Primary skin feeling is related to the moment when the product is first applied over the skin, whereas secondary skin feeling is determined at high shear rates, which are related to the shear applied during product application [44]. In order to select the best formulations, a panel of subjects that can attribute real sensory aspects to the products is required. With the subjected information acquired from the panel, the viscosity at fixed shear stress is set as a goal to new developments. Since the use of sensory panels is cost and time consuming, the use of rheology measurements are promising alternatives to R&D laboratories. Despite the benefits, information on organogel formulations tested by this methodology is still scarce.

Oscillatory shear (stress and frequency sweeps) evaluates the values of elastic and viscous moduli (G^0 and G″, respectively) and the critical gelling concentration (Cg, e.g. minimal organogelator concentration required to obtain an organogel). It is often preferable to obtain low Cg values, indicating good organogelator properties. The material is characterized as gels when $G^0 > G''$, and 'strong gels' are defined when the relation between G″ and G^0 (G''/G^0) ($\omega \leq 0.1$). Solid matrix gels are more robust, as demonstrated in rheology studies by smaller ω values [6, 45]. Strong gels may have longer shelf lives but the delivery of active ingredients to the skin may be impaired, since diffusion through the formulation will be hindered. The determination of G^0 and G″ moduli is also useful to estimate the minimum gel concentration, corresponding to the minimum concentration of the organogelator needed to obtain an organogel [13].

Rheology also generates useful information about the temperature behaviour of organogels. Temperature ramps are valuable to understand how temperature affects organogelators' entanglement into organogels [45]. Since a great number of organogels are made by LMWO heating and cooling processes, it is important to understand what happens to the structure in each temperature.

Table II Organogel characterization methods in cosmetics

Category	Feature	Technique	Reference
Structure of the organogel	Isotropic and optical clarity Molecular network packing architecture Determination of chemical bonds	Fourier transform infrared (FTIR)/small angle neutron scattering (SANS)/nuclear magnetic resonance (NMR)/scanning electronic microscopy (SEM)/transmission electronic microscopy (TEM)	[21, 36–39]
Thermo-associated properties	Thermo-reversibility Gelation kinetics Phase transition temperature	Differential scanning calorimetry (DSC)/Hot stage microscopy/Rheological methods	[28, 40]
Viscoelastic behaviour	Elastic (G^0) and viscous moduli (G'')	Rheological methods	[31]
Safety issues	Histopathological studies Skin irritation Biocompatibility	*In vitro*/*ex vivo* methods	[41]
Efficacy issues	Drug release profile	Skin permeation tests (e.g. Franz diffusion cells)	
Drug content and stability	Analytical methods	High-performance liquid chromatography (HPLC)/ultraviolet spectrophotometry (UV)/physical–chemical analysis	

This phenomenon is observed by the gelation temperature (Tg). The difference between slow and high temperature ramps can show how the system is affected by cooling rates, indicating optimization possibilities of processes. At high cooling rates, the organogelator molecules have less time to organize when compared to low cooling rates, requiring a lower temperature to achieve molecular packing for organogel formation and consequential lower Tg [35]. Likewise, the concentration of organogelators also affects the Tg. The higher the organogelator concentration, more fibrils are formed to trap the solvent and a lower Tg is observed [46, 47]. That information is important to modulate the production of a high temperature resistant organogel or an organogel that will melt when applied over the skin (37°C). Shelf-life stress temperature cycle ramps may be used to quality control final formulation [48].

Skin permeation properties

The effect of viscosity on drug release from gel formulations is well established, and most authors have found inverse relationships between the viscosity of preparations and matrices and drug diffusion coefficient [49, 50]. Different types of penetration enhancers can, thus, be added to the formulation to promote transcutaneous delivery, including surfactants, bile salts, chelators, fatty acid salts, phospholipids, glycyrrhetinic acid derivatives, cyclodextrins and unsaturated fatty acids (such as oleic acid (18:1), eicosapentaenoic acid (20:5) or docosahexaenoic acid (22:6)) and glycols [51].

However, the organogel itself can also promote the permeation of active molecules into the skin, such as in the case of lecithin-organogels [12, 52–54]. These systems have demonstrated to significantly enhance the permeation and absorption of both lipophilic and hydrophilic molecules owing to the organized microstructural matrix, amphiphilicity, super-solubilizing capacity and the interaction of the biolipids with skin tissue [55]. Figure 2 shows the formation and interaction between lecithin-organogels and skin. Lecithin-organogels can also hydrate the upper skin layer (*stratum corneum*) and present an occlusive effect, which not only promotes permeation of hydrophilic compounds, but also provides pleasant sensory properties after application [11]. When pluronic–lecithin is used, the permeation is even greater owing to a synergic action of pluronic (surfactant) and lecithin.

Transdermal penetration is the major benefit that organogels have over hydrogels. When using the same ingredients, organogels promote skin permeation to a greater degree. For that reason, organogels developed to cellulite treatment are very promising, since the ingredients must reach the deepest layer of the skin (subcutaneous layer) to act against adipose tissue cells [8, 12]. Although this phenomenon has been widely studied in drug delivery systems [51], there is still lack of information on the possibilities available for cosmetic formulations.

In some types of cosmetics, the permeation of actives is desirable, like in anti-ageing or anti-cellulite formulations, but in other cases safety requirements demand that penetration is limited to the *stratum corneum*, such as in sunscreens. Thus, depending on the application of the organogel, the structure and rigidity can be modulated to achieve optimal results [56]. This modulation capability is why the lipophilic nature of organogels makes it an excellent candidate for the delivery of cosmetic molecules through the skin. Deeper penetration formulations often include the fluid-filled fibre mechanism organogels (e.g. lecithin [11, 12] or pluronic–lecithin organogels [3]) whereas superficial delivery formulations often include solid-fibre mechanism organogels (e.g. 12-hydroxyestearic acid or fatty acid glycerides [13–16]).

Stability properties

Organogels present good physical and chemical stability properties. Their inherent thermostability is a very desired characteristic for longer shelf-life requirements (e.g. deliver bioactive agents and cosmetic applications) [57]. They also have shown the ability to improve formulation stability by different mechanisms. In a sunscreen formulation, organogels obtained with 12-hydroxystearic acid (HSA) and almond oil showed an improvement in sun protection factor (SPF) value attributed to the organogelator. The presence of HSA increased the ultraviolet B (UVB) absorption ability of the immobilized organic filter 2-ethylhexyl-*p*-dimethylaminobenzoate and its water resistance property. The organogelation turned the formulation more diffusing (transfused aspect) and increased its

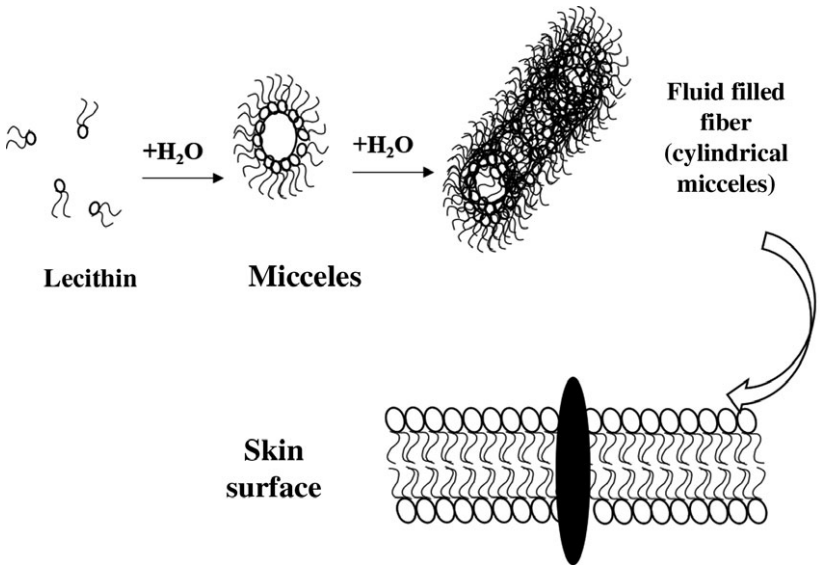

Figure 2 Schematic representation of fluid-filled fibre mechanism organogel formation by adding water into a lecithin system. Notice the interaction between the cylindrical micelle and skin and their structured phospholipid resemblance.

capacity to absorb the UVB radiation. The improvement in SPF value shows that a better photostabilization of the organic ultraviolet (UV) filter was attained in the formulation with organogels [13].

Another interesting finding was achieved by Shi and collaborators by using tea polyphenols in organogel systems to protect peanut oil from oxidation. Since polyphenols are mainly hydrophilic, they prepared a pre-organogelator mixture by freeze-drying tea polyphenols and stearic acid. The product was used as organogelator on peanut oil. They achieved a 2.5-fold increase in the protective performance when compared with other classical antioxidant molecules such as butylated hydroxytoluene and propyl gallate [58]. Similar results were obtained by Lupi and collaborators protecting ferulic acid in a system of olive oil and policosanol organogels. Ferulic acid was submitted to a drastic acidic environment, simulating gastric digestion, but the organogels guaranteed homogenous and suitable rheological properties and melting temperature to this molecule in nutraceutical applications [59]. These studies were performed in edible oil applications, but similar effects could be expected on cosmetics.

Organogelation can also improve the physical stability of emulsions. Water-in-gelled-oil emulsions with HSA showed no coalescence, indicating that the emulsion gels were stable, despite the large size of the water droplets in the internal phase [60]. Likewise, coalescence was prevented in organogel-in-water emulsions when strong gels were formed, since more fibrils are interacting to form the gel and a better stabilization of the emulsion was, thus, achieved [61].

Safety properties

Safety guidelines to assess cosmetics are still a controversial subject and tests are not harmonized throughout the different markets. It should also be noted that the legal definition of cosmetics is not standardized globally, and some countries consider some ingredients as cosmetics whereas in others they are classified as quasi-drugs or even over-the-counter drugs . However, one rule is common to all regulations: cosmetics must be safe under conditions of normal use [62]. The safety and the skin compatibility of organogels are entirely dependent on their composition. In cosmetic applications, organogels are formulated using mainly food grade ingredients as organogelators and solvents, like monoglycerides of fatty acids and vegetable oils. Food ingredients have more significant systemic effect on humans through digestion and oral absorption of molecules, suggesting that applying those same ingredients on the skin will be safe, except when there is history of individual allergies. It is also important to notice that the most common organogelators and organic solvents are already used as cosmetic ingredients in regular formulations and certified as safe by international offices (e.g. FDA, Cosmetics Europe). Nevertheless, as in any other cosmetic, tests must be submitted to regulatory agencies to certify the safety of organogel formulations. Unfortunately, there is still scarce toxicology information available on organogels specifically to cosmetics applications, despite their potential safety tests for drug delivery [3, 63], and the recent ban on animal testing has increased this problem.

The available studies address the toxicology issues of pluronic–lecithin organogels for drug delivery, because they have shown promising permeation effects. Safety data were obtained by a study on transdermal delivery of fluoxetine in cats, as well as in the use of diclofenac in patients suffering from knee osteoarthritis [64].

Organogel applications: bigels and nanoparticles

Hedonic and descriptive sensory analysis showed good acceptance on cholesterol and paraffin oleogels. The trained panel declared that

the skinfeel was closer to a water-in-oil (W/O) cream and highlighted the good spreadability, which was very positive considering that there was a petrolatum ointment between samples [65]. However, depending on its composition, organogels can exhibit an intense oily texture that is often detrimental to the sensory expectations of customers [8]. For that reason, formulations containing organogels were developed to beneficiate from its positive properties and minimize its bad organoleptic properties. Emulsions are good candidates to deliver organogels, because they carry surfactant molecules that can induce crystal migration and adsorption on the water/oil (W/O) interface stabilizing W/O emulsions [56–67]. Surfactants can either increase the affinity between the crystals and the aqueous phase or change the intercrystal interaction, or even alter the crystal lattice [67–69]. However, traditional methods to obtain emulsions comprise a heating stage, when all the oily phase is melted in one compartment and all the aqueous phase is heated to the same temperature, after which both phases are mixed at high speed before cooling. Since most of organogelators are also molecules used as consistency agents in traditional emulsions (e.g. fatty acids and fatty alcohols), when melted with other ingredients, they may become fibrils out of the oily globules in the emulsion and the organogel formation may be compromised [70]. Thus, to guarantee that organogelators are exclusively engaged in the organogelation process, the viscosity of emulsions should be ensured using polymeric swallow when water is applied – in other words, the crystals must be recruited to form the organogel and not to stabilize the aqueous phase of emulsion. Polymers may add emulsion stability sterically and through globule charge repulsion [71]. Synthetic or natural polymers can be used, like polysaccharides and proteins.

The emulsions containing organogels and hydrogels in the same formulation are known as bigels. This type of organogel application was first described in cosmetics by Almeida and collaborators [72] as an organogel in hydrogel system (O/W emulsion-like). The authors studied the organogelation process of several oily phases and polyacrylic acid hydrogel in order to determine the moisturizing effect of bigels in clinical trials. The results indicated that bigels showed simultaneously an enhanced moisturizing effect when compared to organogels and hydrogels alone. Lupi and collaborators [15] studied olive oil and a mixture of monoglycerides of fatty acids to obtain the organogel and modified potato starch as the polymer to form the hydrogel. Their goal was the development of a cosmetic matrice that could deliver hydrophilic or lipophilic active molecules. Other possibilities are hydrogel in organogel system (W/O emulsion-like) and bi-continuous system [16]. The benefits of bigels in cosmetics are still under discussion since little information is available. However, stability and rheological properties may represent major features of this technology.

Kirilov and collaborators [13] also developed an application of organogels when nanodispersions of organogelated oil with HSA were used to stabilize UVB blockers. In order to stabilize the colloidal dispersions, surfactant molecules and some polymers (e.g. sodium hyaluronate and polyvinyl alcohol) were added. The results indicated a better photostabilization of UVB blockers in organogel systems, but some stability issues were described since the dispersions were polydisperse. The authors suggested that an increase in the organogelator concentration could solve this problem. However, despite the presence of an internal phase of organogel particles and of hydrophilic polymers in the external phase, this system is not a bigel (organogel in hydrogel). The presence of surfactant molecules to obtain the dispersion and the choice of weak hydrogel polymers in the aqueous phase are not considered compatible strategies used to obtain bigels [3, 15, 73]. More recently, this type of dispersion is called as gelled-oil nanoparticles and gelled sun protection nanoparticles and it is an interesting application for organogels because of its better stability when compared with solid lipid nanoparticles (SLN) [31].

Limitations and challenges of organogels in cosmetics

It is important to notice that despite the potential uses of organogels, there are some drawbacks listed above [74, 75]:

- Toxicity of the non-polar solvents and skin enhancers when deep permeation is required.
- Difficulty to predict the success of gelation process between the gelator and the solvent required.
- Swelling (increase of liquid volume) and syneresis (liquid pressed out) may occur.
- Dependency on the quality of ingredients (contaminants may influence gelation).
- Fine control of process variables (pH, temperature etc.).

Understanding how organogels are formed, the critical points and limitations, is crucial to obtain a good quality product and achieve consumers' expectations.

Future perspectives

In the last years, the potential of organogels in cosmetics is starting to emerge in scientific and industrial literature. There are still issues in the preparation processes that need to be addressed, mainly in the crystallization patterns of LMWO in organic phases. Studies on toxicology, efficacy and long-term skin compatibility are required to further guarantee the safety of organogel formulations. Some cosmetic industries are already using the benefits of this technology forming organogels by cold or hot emulsification processes [17, 76], achieving promising perspectives and better sensory properties.

Conclusions

The cosmetic applications of organogels are still not fully explored. Some of the advantages of using organogels are low-cost composition, improvements in chemical stability of active molecules and physical stability of formulations, better rheological properties and enhanced delivery profiles. It is a versatile form to present hydrophilic and lipophilic molecules to skin and it can be modulated to achieve the expectations. Its simple and low-cost preparation processes seem to be particularly beneficial to large-scale uses, but their potential in complex systems like emulsions is also starting to emerge. There is still lack of information on safety and toxicological effects. However, a great amount of robust knowledge can be transferred from food and drug delivery applications, helping to turn organogels into a promising delivery system for cosmetics.

Acknowledgements

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Article 2

Vitamin E-loaded bigels and emulsions: Physicochemical characterization and potential biological application

Colloids and Surfaces B: Biointerfaces

Colloids and Surfaces B: Biointerfaces 201 (2021) 111651

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Vitamin E-loaded bigels and emulsions: Physicochemical characterization and potential biological application

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ARTICLEINFO

Keywords:
Bigel
Organogel
12-hydroxystearic
Candelilla wax
Rheology
Stability

ABSTRACT

Bigels have been studied as topical formulations for its benefits over sensory and drug delivery parameters. However, there is still few evidences about the properties of the combination of organogelators, oily phases and bioactive molecules into rheological and stability behavior. We investigated the use of classical organogelators (candelilla wax and 12-hydroxystearic acid) and oily phases (sunflower and mineral oil) in 5/95 organogel/polymeric hydrogel ratio to compare vitamin E bigels with its corresponding emulsions. The rheological measurements, microstructure, physical and oxidative stability properties and biological behavior were evaluated. The obtained oil-in-water bigels and emulsions showed crystallization pattern at the interface with high thermal and centrifuge-stress stability. Viscoelastic weak gels were obtained with higher thixotropy and consistency of 12-hydroxystearic bigels. The diameter of the inner phase was increased by vitamin E, despite its little influence over physical and oxidative stability of bigels and emulsions. Those findings indicated that sensory attributes may be regulated by the organogel composition.

1. Introduction

Bigels are biphasic systems formed by two gelled phases: (1) an organogel dispersed into hydrogel (O/W); (2) a hydrogel dispersed into organogel (W/O) or (3) a bi-continuous system [1]. The combination of both phases in a colloidal system could bring advantages due the synergetic effect between the organogel and hydrogel, depending on the application. For pharmaceutical and cosmetic bigels, the moisturize and emollient effects, good spreadability, water washability [2] and the ability to deliver both hydrophilic and lipophilic agents are mainly focused [3]. Nevertheless, understanding how the structure and the distribution of each phase can be manipulated plays an important role on the development of the system.

Rheology, microscopy and the average droplet size of dispersed phase are important parameters to determine the flow and thermal behavior, stability prediction and the microarchitecture of bigels [4]. Recently, a review article has been published on the characteristics that influence the mechanical, structural, thermal, physical, rheological and electrical properties of bigels. The organogel/hydrogel ratio, hydrogel polymer composition and concentration, the source of oily phases into organogel and organogelator properties were highlighted along with the process parameters for the preparation of bigels, such as the mixing temperature, mixing speed and the storage of bigels [5]. The outcomes showed that small changes into composition of bigels may have a major impact in the final product characteristics and applications.

Focusing on topical bigels, studies conducted to stablish the ideal organogel/hydrogel ratio showed that smaller ratios (e.g. 10/90) had better lipophilic drug delivery properties with good stability, since higher organogel ratios acted as a depot for drugs and led to controlled release profiles [3,6,7]. Most of hydrogels were obtained by natural

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https://doi.org/10.1016/j.colsurfb.2021.111651
Received 31 October 2020; Received in revised form 12 January 2021; Accepted 16 February 2021
Available online 21 February 2021 0927-7765/

polymers, like guar gum, gelatin, pectin and alginate [2,3,7,8], however, the use of synthetic polymers such as carbopol, poly(vinyl alcohol) (PVA) and poly (vinyl pyrrolidone) (PVP) were not detrimental to achieve good sensory, delivery and biocompatibility properties [9–11]. For the design of organogels, the natural sources of oils coming from fish, sunflower, olive and almond [2,9–11] were preferred instead of mineral sources for their constitutions of fatty acids. The organogelator molecules at high concentrations (10.0–15.0 %) were mainly based in solid-fiber mechanism of organogelation (when organogel is formed upon melting and cooling processes) from beeswax, rice wax and stearate derivates [2,6,9,10]. Surfactants were sometimes needed to improve the release profile [8] and drugs were tested mainly in small concentrations (0.3 – 0.5 %) [3,12]. Those studies compared bigels in several organogel/hydrogel ratios with organogels and hydrogels alone and there was a brief discussion about the influence of the drug into the system.

Bigels were designed as a solution for mechanical and stability problems in emulsion gels/emulgels. Nevertheless, as an emerging class of materials, further analysis of these systems are still needed for commercial applications. Despite rheological models available for bigels are not enough accurate to predict their properties, it can bring extensive information about stability and sensory aspects. Literature showed that bigels possessing superior mechanical properties exhibited lower drug release rate when compared to the commercial formulations [5]. However, better sensory attributes can be beneficial for adherence and compliance to therapies for pharmaceutical and cosmetic purposes.

This investigation was designed to understand the structure, rheology and stability of bigels and their corresponding emulsions in the presence of vitamin E as a model of lipophilic drug. We fixed the organic/aqueous phase ratio (5/95) in order to stabilize the oily phase with small concentration (2.0 %) of organogelators. We explored the comparison between vegetable and mineral oil since they are typically used for topical applications. Despite several works reported the use of candelilla wax and 12-hydroxystearic acid in organogels [13,14], there are still lack of information about those organogelators in bigels. The interaction between organic and aqueous phases was studied concerning their microstructure, rheological, thermal and oxidative stability properties in the presence of vitamin E. This is the first study comparing bigels and their emulsions.

Our research group has been studying the influence of antioxidants into colloidal systems for the past decade [15] and has recently started exploring organogels and bigels for cosmetics [16]. In the present research work vitamin E suits as a model of lipophilic antioxidant to understand its effects over the system structure. Vitamin E is a classic dermatological compound not produced by human body and, therefore, must be topically or orally delivered. In skin, this antioxidant plays an important role in anti-aging mechanisms and acts as coadjutant in *atopic dermatitis* and melanoma treatments [17–19]. Therefore, our work shows an important contribution for the development of topical applications.

1. Material and methods

Bigels were produced by preparing, separately, the hydrogel and the organogel, and mixing them with a mechanical (rotor-stator) homogenizer at 25 °C. After 15 min, the preservatives were introduced. Control emulsions were prepared the same way as bigels, but the oily phase was the oil itself and not the organogel.

Organogels were prepared with two types of oily phases: sunflower (*Helianthus annuus*) oil with high oleic content (Agri Pure 80, Cargil Agrícola S/A, Brasil) (VO) or mineral oil USP 70 (Emcaplus 070 L F, Oxiteno, Brasil) (MO). The organogelators candelilla wax (Double Refined Candelilla Wax 102 P, Koster Keunen Inc, USA) (CW) and 12-hydroxstearic acid (A. Azevedo, Brasil) (12HSA) were used at 2.0 % (w/w). Vitamin E (dl-α-Tocopherol, DSM, USA) (VE) was tested at 0.0 and 20.0 % (w/w). Sodium polyacrylate (SNF, France) was used to obtain hydrogels. Phenoxyethanol and caprylyl glycol were used as preservatives at 0.55 % (w/w) and 0.45 % (w/w), respectively.

1.1. Organogels and hydrogels

The oily phase of organogels was heated to 85 °C and organogelators were added upon continuously stirring (RW 20, IKA-Werke, Germany) at 200 rpm. After complete melting of organogelator, vitamin E was added, and mixing was continued for 5 min. Finally, it was cooled naturally. The batch sizes were standardized at 50 g to insure the same thermal heating transfer characteristics. Organogels remained still at room temperature (25 °C) for 24 h before their characterization. We fixed the amount of organogelator in 2.0 % (w/w), the amount of vitamin E in 0.0 or 20.0 % (w/w) and the rest of the formulation to achieve 100.0 % of oil (vegetable or mineral). We used the code of “CW” for candelilla wax, “12HSA” for 12-hydroxstearic acid, “VO” for vegetable oil (sunflower oil), “MO” for mineral oil and “VE” for vitamin E. We prepared six types of organogels, described as follows: 1) 2.0 % CW: 78.0 % VO: 20.0 % VE; 2) 2.0 % CW: 98.0 % VO; 3) 2.0 % 12HSA: 78.0 % VO: 20.0 % VE; 4) 2.0 % 12HSA: 78.0 % MO: 20.0 % VE; 5) 2.0 % 12HSA: 98.0 % VO; and 6) 2.0 % 12HSA: 98.0 % MO.

The hydrogel was prepared by mixing the acrylate polymer with purified water at 300 rpm using a laboratory stirrer (RW 20, IKA-Werke, Germany) at 25 °C for 15 min. The final amount of polymer in the hydrogel was fixed to 1.0 % (w/w).

1.2. Bigels

All bigels (batches of 200 g) were prepared by slowly incorporation of the organogel into the hydrogel at 25 °C while stirring at 600 rpm for 15 min with a stirrer (RW 20, IKA-Werke, Germany). The preservatives were introduced while still stirring. Bigels were stored at room temperature (approximately 25 °C) for 24 h, and afterward the tests were carried out. We used 5% (w/w) of oily phase, 94 % (w/w) of hydrogel and 1% (w/w) of preservatives. The composition of the oily phases was used to code the formulations. We prepared 10 formulations in total. Besides the six organogels described in 2.1, we prepared four formulations using only liquid oily phases: 1) 100.0 % MO; 2) 80.0 % MO: 20.0 % VE; 3) 100.0 % VO; and 4) 80.0 % VO: 20.0 % VE.

1.3. Rheological characterization

The rheology of bigels and emulsions were investigated with a controlled strain rheometer (TA Instruments, DHR-2, USA) and a Peltier system for temperature control. The geometry was the parallel plates of 40 mm (gap 500.0 μm), typically used to evaluate bigels [2,20]. Approximately 0.5 g of samples was transferred to the geometry and left still for 3 min to temperature equilibrium before every test. Dynamic temperature ramp tests were performed at 1 Hz in the linear viscoelastic regime, ranging from 10 to 70 °C and from 70 – 10 °C in five cooling-heating cycles, with intervals of 60 s between curves in a ramp rate of 5 °C/min. Frequency sweep tests at 25 °C were carried out in the range 0.1–100 rad/s. Finally, three flow curve tests (up–down–up) at 25 °C were performed between 0 and 100 s^{-1} shear rate sweeps in order to eliminate thixotropy and to fit steady state rheological models [21]. Intervals of 60 s were adopted between every curve. Rheological investigation profile was carried out using GraphPadPrism® Software and TRIOS® Software (TA Instruments, USA).

1.4. Microscopy tests

The inner microstructure of the bigels and emulsions was investigated with optical and polarized microscopy (DM2700, Leica Microsystems, Germany), at 40x magnification (Leica HI Plan 40x/0.65 POL). The images were captured with a digital camera Leica® MC120 HD (Leica Microsystems, Germany) and analyzed with the Leica®

Application Suite Software (Leica, Wetzlar, Germany). All samples were loaded on a glass slide and covered with a cover slip without dilution.

1.1. LUMiSizer®

The physical stability of bigels and emulsions was evaluated by LUMiSizer® (L.U.M. GmbH, Germany). The parameters used for the measurement were set to 4000 rpm, 25 °C, with 5 scanning profiles with time interval of 10 s, followed by 995 scanning profiles with 20 s.

1.2. Particle size measurement

The particle size distributions of bigels and emulsions were measured using light scattering via a Mastersizer Hydro EV wet sample dispersion unit (Mastersizer® 3000, Malvern Instruments, UK). Average droplet size was monitored via the Sauter mean diameter, corresponding to surface area (D3,2) and volume mean diameter (D4,3). Span parameter was calculated as: span=(D90-D10)/D50. Samples (0.1 – 0.5 g) were dispersed in 150 mL of purified water at 25 °C (refractive index of 1.33) to provide a constant detector obscuration between 3–12 % at 2000 rpm and no ultrasounds. After 5 min with constant obscuration, we assumed that the matrix was properly dispersed and started the analysis. Fraunhofer model for opaque particles was used to evaluate the results.

1.3. pH

The value of pH was determined in triplicate directly in bigels and emulsion samples at 25 °C (Seven Easy Inlab 413 – Xerolyt® Polymer and Argenthal™, Swiss).

1.4. Antioxidant activity assay

Antioxidant activity was determined by the spectrophotometric DPPH (2,2-diphenyl-1-picrylhydrazyl) analysis using the procedure described by de Oliveira and collaborators [22] in order to determine de free radical scavenging (FRS) potential (Eq. 1).

$$\%FRS = \frac{(Abs_{control} - Abs_{sample}) - 100}{Abs_{control}} \quad (1)$$

where %FRS is the percentage of free radical scavenging, $Abs_{control}$ is the absorbance of the negative control sample and Abs_{sample} is the absorbance of samples. We used a positive (vitamin E at 1.0 % (w/w) in 99.0 % (w/w) mineral oil or sunflower oil) and a negative control (formulation MO and VO without the vitamin E). The DPPH was determined 24 h (T_0), 30 (T_{30}), 60 (T_{60}) and 90 (T_{90}) days after the preparation of emulsions. Each formulation (50 g) was stored in a transparent glass flask and divided into three storage conditions: 5.0 ± 0.5, 24.0 ± 2.0 and 40.0 ± 0.5 °C. At the pre-determined times, samples were removed from the storage conditions and allowed to warm to room temperature (24.0 ± 2.0 °C) prior to the evaluation of the DPPH. All determinations were performed in triplicate.

1.5. Biological tests

Biological tests were conducted as described by Katekawa and collaborators (2019) [23]. *Ex vivo* fragments of human tissue were acquired from elective surgeries (blepharoplasty) from healthy patients aged between 35–70 years old, at the Surgical Center of the Eye Bank of the Sorocaba Ophthalmological Hospital (BOS-HOS), located in Sorocaba City – São Paulo State. The collection and use of these human skin explants derived from voluntary donation with the accepted and the approval of the Ethics Committee of the Hospital (HOS-BOS), and it is under the consubstantiate report number 3.065.484. After the surgical procedure performed by the clinical staff of ophthalmic plastic surgery, eyelid explants were placed in vials containing 0.9 % saline buffer until the time of the experiment. Biological samples were removed from the buffer, immersed in 70 % of ethanol for 60 s and rinsed twice in new saline buffer. Then, they were cut into fragments of approximately 0.5 cm^2. Immediately after, the fragments were transferred to a cell culture dish containing culture medium for up to 72 h for treatment with the bigel or emulsion samples. The product was applied to the skin at a concentration of 12 mg/cm^2.

2.9.1. Quantification of IL-6

The quantification of IL-6 was adapted from the work of Lopes and collaborators (2017) [24]. The IL-6 was quantified in the supernatant of the skin fragment culture, using an immunoenzymatic assay kit (ELISA sandwich) commercially available (OptEIA, BD Bioscience, USA). The anti-cytokine capture monoclonal antibody was added to the 96-well plate and then incubated for 12 h at room temperature. The samples and standards were added to the plate then incubated for 2 h. Thus, the anti-cytokine detection antibody was added and incubated for additional 2 h. After this period, the streptavidin/peroxidase conjugated solution was added to the plate and incubated for 1 h. Finally, the substrate solution (H_2O_2 and TMB – tetramethylbenzidine) was added to each well and incubated for about 30 min in the dark, and a blue color developed in this period. The color reaction was interrupted by the addiction of H_2SO_4 2 N (sulphuric acid) to each well. The absorbance was read at 450 nm in a microplate reader within 30 min after stopping the reaction. The levels of cytokines were expressed in pg/mL and calculated from the reference values obtained with a standard curve constructed with known concentrations of recombinant cytokine.

2.9.2. Evaluation by Fontana-Masson

After incubation with the samples, separately, the *ex vivo* skin fragments were fixed in 4.0 % paraformaldehyde (pH 7.4) for 24 h. After fixation, the material was transferred to 30.0 % saccharose solution for cryopreservation and then included in tissue freezing medium, followed by serial sections about 10 µm thickness made by cryostat (CN1850, Leica Biosystems) and directly collected onto silanized glass slides. Then, the skin cuts were stained by Fontana-Masson technique using a commercially available specific kit (Fontana Masson Kit 4X, FM07223SO, Scientific Exodus). After assembly of the slides containing the skin cuts, the slides were washed with 0.1 M phosphate buffer. The cuts were hydrated with immersion in distilled water. Then, the slides were immersed in ammoniacal silver solution. After this period, the slides were washed with distilled water and immersed in golden chloride solution. Immediately thereafter, the washing procedure was performed again. Slides were immersed in thiosulfate solution, followed by washing. Finally, the slides were dipped in safranin solution and the cuts were dehydrated in ethanol solutions with different gradations. After performed all steps, the slides were left at room temperature for drying, and thus, assembled with specific mounting media and cover slip. Subsequently, the slides were analyzed by an optical microscope (Leica – DM 6000 B) accoupled with a camera of 2.8 M P (Leica, DFC7000 T). The images registered from the skin histological slides were captured using the software LAS (Leica Application Suite v.4.12) linked with the microscope and camera. The evaluated parameter was the pixel emitted by the melanin pigment formation.

1.6. Statistical analysis

Statistical analysis was performed using Minitab® (Version 18) software, adopting a significance level of 5.0 % (α= 0.05). Data were treated using one-way ANOVA followed by the Tukey test for multiple comparisons. For biological tests, data was treated using one-way ANOVA followed by the Dunnett test for comparison with the control group.

1. Results and discussion

1.1. Microscopy characterization

The optical microscopy technique was used to verify the distribution and to identify the type of dispersion obtained. Regardless of the oily phase or the organogelator type, oil-in-water (O/W) structure was observed. The irregular shape of the oil globules was characteristic of the gelled systems, such as bigels. For 12HSA bigels, some oily phase agglomerates appeared (Fig. 1A) and a more dispersed inner phase was observed for CW bigels (Fig. 1C). The stiffer gel structure of 12HSA organogels led to a more difficult dispersion of the oily phase during bigel preparation, when compared macroscopically with CW bigels. Emulsions showed more regular oil phase dispersion and smaller droplets when vitamin E was absent (Fig. 1G and H). In the presence of vitamin E, the dispersion showed some aggregates (Fig. 1E). Depending on the viscoelastic properties, the system can answer to applied deformation (upper glass) in complex ways leading to artifacts in microscopy and rheology [25]. Aggregates may deform under the pressure of the upper glass and show higher particle size and light scattering measurements were used for further investigation. Rheology evaluation was also used to evaluate the system behavior under applied deformation. By subjecting the samples to polarized light, a tendency of the crystals to adhere to the emulsion interface was noticed for all bigel samples (Fig. 1B and D), which may contribute to the stability of the system [26]. Small crystals may adsorb to the interface and lead to full coverage of the oil droplet, acting as a shield that intensifies stability [26]. The presence of vitamin E alone, without organogelators seems to intensify the crystal signal at the interface of the oil globules and small crystals were observed (Fig. 1F) even for control not gellified emulsions (MO:VE and VO:VE). These results suggested that the vitamin E presence may have reduced the size of the crystals formed in emulsions, in addition to affect their polymorphism [27]. This phenomenon suggested that vitamin E, at the concentration of 1.0 % (w/w) in the dispersions, was able to crystallize, adhering around the oil globules and affect the visual crystallization pattern. However, with the techniques used here, it is not possible to ensure that this phenomenon occurred significantly.

1.2. Rheological characterization

Oscillatory measurements are used to determine the properties of viscoelastic materials such as flow and deformation in order to classify samples into strong, weak gels or viscous sols [28]. According to Shakeel and collaborators, frequency sweep tests within linear viscoelastic regime (nondestructive regime) of bigels with higher storage modulus (G') than the loss modulus (G") and an independency of moduli as a function of frequency are classified as strong gels [5]. Our results indicated that G' dominates G" (G' > G") in all range of applied shear evaluated. This behavior marks a solid-like behavior, characterizing our materials as gels [28]. Complex modulus (G*), a combination of G', G" and phase angle, represents both modulus in a slightly frequency of oscillation dependency. Loss tangent was lower than unity in the whole range of frequency investigated (Fig. 2), indicating a solid-like behavior [21] and no sign of structure breakdown at high rates of deformation [28]. Values higher than 0.1 (tan $\delta = G''/G' > 0.1$) are typical of the so-called weak gels [29]. Same behavior was observed for bigels and control emulsions, indicating that the hydrogel properties are dominating the sample frequency sweep rheology. That led us to investigate if the components of the formulations could be hiding organogel influence over bigels. We further analyzed whether the 5/95 organogel/hydrogel ratio was enough to show the different organogels contribution or if the type of polymer used in the hydrogel prevailed over the rheological characteristics.

In order to understand the relationship between the inner and outer phases of our samples, we conducted experiments to calculate the network strength and properties. First, we used the Eq. 2 to classify the nature of the prepared samples so we would be able to use the correct mathematical modelling.

$$\log G' = n \log \omega + k \quad (2)$$

where k and n are constants. The slope of the log–log plot of G' versus ω was denoted by the constant n. Physical gels show $n > 0$, whereas chemical gels $n=0$. All samples showed $n>1$ (1.16–1.84) and therefore classified as physical or reversible gels [5]. In this case, networks are formed by molecular entanglements or secondary forces (e.g. hydrogen bonding and ionic or hydrophobic forces). Physical gel classification and tan $\delta > 0.1$ values encouraged our further investigation using the weak gel model to confirm the bigel network contribution over control emulsions. We used the correlation between the complex modulus as a function of frequency to calculate the parameters "A" and "z", shown in the Eq. 3:

$$G^*(\omega) = \sqrt{(G')^2 + (G'')^2} = A.\omega^{1/z} \quad (3)$$

where, ω is the frequency, "A" is the strength of these interactions and "z" is the coordination number related to the number of interacting rheological units within the three-dimensional network of system [5]. We tested this model and the coefficient of determination R^2 for all fittings ranged between 0.90 and 0.99 for all samples. The "A" parameter indicated that bigels were slightly stronger networks than the control emulsions and this behavior was more pronounced when 12HSA organogelator was used. The hydrogen-bonding interactions among the hydroxyl groups at the 12 position of 12HSA and the hydrogel may explain this phenomenon [30]. Considering that the amount of polymer in the hydrogel was standardized for all samples and that we observed different contributions of organogels over the strength of the network,

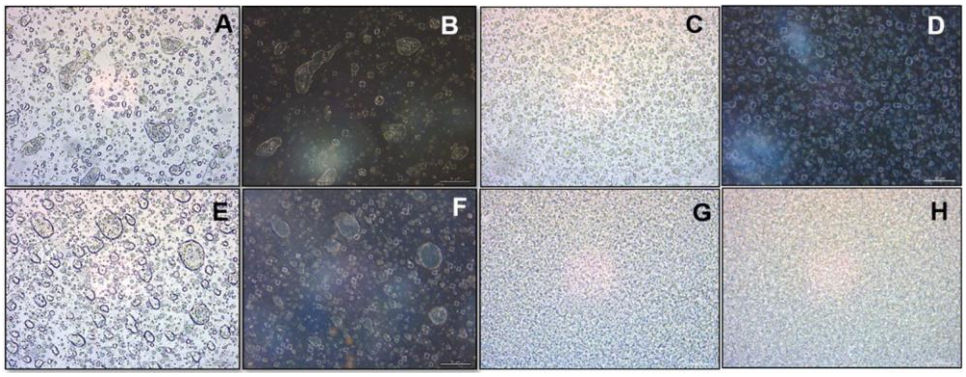

Fig. 1. Optical and polarized microscopy from formulations 12HSA:VO:VE (A and B), CW:VO:VE (C and D) and MO:VE (E and F), respectively. Optical microscopy from formulations MO (G) and VO (H). Bar =50 μm.

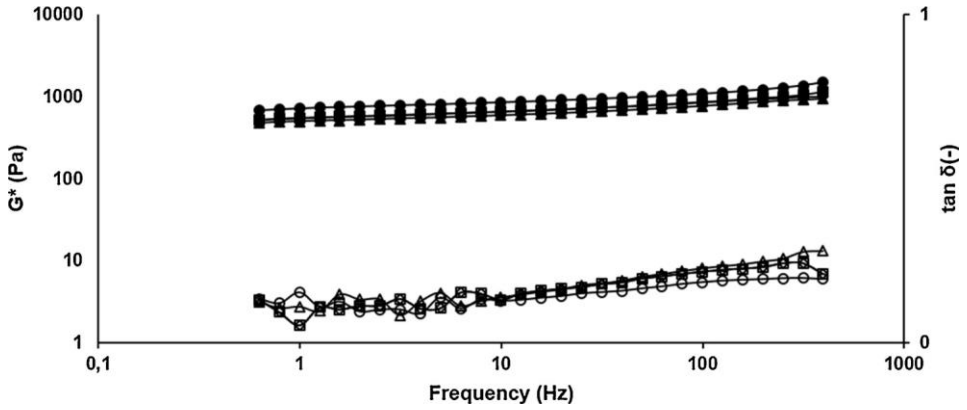

Fig. 2. Frequency sweep test for formulations 12HSA:VO:VE (●), CW:VO:VE (■), MO:VE (◆) and MO (▲). Closed symbols are related to complex modulus (G*) and open symbols are related to tan. δ.

we excluded the hypothesis that the 5/95 organogel/hydrogel ratio was not enough to show the different organogels contribution. However, the type of polymer chosen in our work seemed to show significant impact over rheology outcomes.

The presence of vitamin E showed no effect over "A" parameter when CW organogelator and VO were used, however, the strength of the structure was increased when vitamin E and 12HSA were used in combination (Fig. 3A). The "z" parameter showed similar values for all samples, indicating that they all had approximately the same number of interacting units within the sample network (Fig. 3B). This result also confirmed our hypothesis of the greater contribution of the hydrogel polymer over the frequency sweep test, since it highlighted that hydrogel and organogel interactions occurred at the same extend for all samples.

Rheological measurements can also inform about sensory attributes, which are very important for topical products. Flow curves are tools to estimate viscosity and other flow properties. We estimated thixotropy from the difference between the areas below the flow curves (hysteresis) during increase (S1 — transient) and decrease (S2 — steady state) [21]. In this case, bigels showed higher hysteresis and, therefore, higher thixotropy when compared to control emulsions (Table 1). The flow curve test may represent the shear of a cream being spread over the skin [31]. For topical emulsions, it is interesting that the fluid disorganizes, losing viscosity, and this will be perceived as a sensory attribute of "spreadability". The reorganization of the system after the shear is also required, since when the product is stored, it must remain viscous to avoid instabilities in the formulation [31]. Those properties influence the efficacy of active compounds that require a homogeneous film formed over skin, such as antibiotics and UV filters [32].

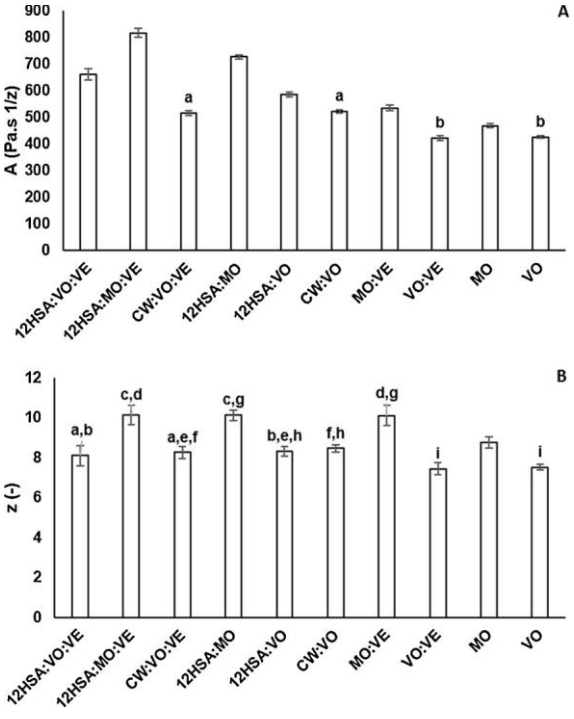

Fig. 3. Weak gel model parameter in terms of network strength (A) and extension (B). Samples that share a letter are statistically equal (Tukey test: $p < 0.05$).

Bigels showed higher viscosities than control emulsions, especially when 12HSA was used. Macroscopically, it was possible to observe that those formulations (12HSA:VO:VE, 12HSA:MO:VE, 12HSA:MO and 12HSA:VO) presented an elastic appearance. Higher viscosities may be linked with higher number of gelled globules dispersed in the aqueous medium of O/W type dispersions [33]. Despite the "z" parameter earlier calculated did not demonstrate higher number of interactions within the gel network, the "A" parameter for 12HSA samples showed stronger interactions that seemed to affect viscosity. No changes were observed in viscosity upon vitamin E usage.

The maintenance of a certain viscosity when no shear is applied may be measured by rheological models and the property estimated is known as consistency [2]. Besides stability correlation, consistency is a sensory attribute, since it is the primary contact between skin and formulation [2]. For better understand the consistency, the Carreau-Yasuda viscosity model (Eq. 4) was chosen as it showed the best fit coefficient ($R^2 > 0.99$) for all samples in our work. The model uses the flow curve between shear viscosity (Pa.s) and shear rate (s^{-1}) to calculate the parameters presented in the Eq 4.

$$\eta(\gamma) - \eta_\infty = (\eta_0 - \eta_\infty)[1 + (\lambda\gamma)^a]^{\frac{(n-1)}{a}} \quad (4)$$

where, η is the shear-dependent viscosity, η_0 is the zero-shear viscosity, η_∞ is the infinite shear viscosity, λ is the relaxation time, "a" is a parameter describing the rate of transition from Newtonian plateau to the power law region and n is the power law index [34]. Bigels showed higher consistency than control emulsions when 12HSA was used as organogelator, but the same behavior was not observed for CW (Table 1). Higher consistency may promote higher stability and alterations in sensory pattern of dispersions. In order to verify this hypothesis, stability tests were carried out with rheological and centrifuge analysis. We observed, from microscopy photographs, the presence of large aggregates in 12HSA bigels (Fig. 1A), which were the samples with higher zero-shear viscosity (Table 1). When a shear stress is applied, aggregated systems show strong shear thinning behavior due to the gradual breakup/deformation of aggregates induced by shear [35]. In

Table 1
Thixotropy (hysteresis) and Carreau-Yasuda parameters estimation for bigels and control emulsions. Values represent the mean of triplicate. Samples that share a letter within the same column are statistically equal (Tukey test: $p < 0.05$).

Sample	Hysteresis (Pa.s)	η_0 (Pa.s)	η_∞(Pa.s)	λ (s)	n	a
12HSA:VO:VE	5875.4^{a} (±467.84)	1719.41^{a} (±35.02)	−0.65^{a} (±0.56)	0.12^{a} (±009)	−0.21^{a} (±0.34)	0.24^{a} (±0.36)
12HSA:MO:VE	7217.6^{a} (±1724.38)	219.34^{b} (±0.27)	1.68^{b} (±0.04)	3.27E-04^{a} (±0.00)	−10.17^{b} (±3.25)	0.42^{a} (±0.00)
CW:VO:VE	2442.1^{b,c,d} (±466.48)	88.88^{c} (±15.53)	0.12^{c,d} (±0.20)	0.77^{b} (±0.00)	0.22^{a} (±0.08)	19.80^{b,c} (±0.08)
12HSA:MO	2471.7^{b,c} (±686.95)	127.87^{d} (±6.79)	1.87^{b} (±0.11)	3.19E-05^{a} (±0.00)	−26.55^{c} (±9.44)	0.43^{a} (±0.00)
12HSA:VO	3757.5^{b} (±342.59)	87.21^{c} (±4.68)	0.78^{d} (±0.21)	0.35^{c} (±0.05)	−0.06^{a} (±0.08)	1.21^{a} (±0.12)
CW:VO	3889.8^{b} (±222.66)	97.99^{c} (±1.82)	−0.36^{a,d} (±0.16)	0.95^{d} (±0.01)	0.21^{a} (±0.03)	1.57^{a} (±0.48)
MO:VE	582.3^{e} (±171.46)	90.71^{c} (±17.58)	0.24^{c} (±0.01)	0.79^{b} (±0.00)	0.22^{a} (±0.00)	22.00^{b,c} (±0.54)
VO:VE	627.7^{d,e} (±300.31)	69.88^{c} (±3.96)	0.27^{c,d} (±0.03)	0.80^{b} (±0.03)	0.22^{a} (±0.00)	17.03^{b} (±0.32)
MO	1127.3^{c,d,e} (±224.40)	66.95^{c} (±9.61)	0.35^{c,d} (±0.11)	0.79^{b} (±0.08)	0.19^{a} (±0.04)	23.76^{c} (±6.28)
VO	880.9^{c,d,e} (±25.42)	61.91^{c} (±0.65)	0.24^{c} (±0.00)	0.77^{b} (±0.01)	0.26^{a} (±0.00)	19.86^{b,c} (±0.20)

our 12HSA bigels, the large aggregates seemed to show a significant resistance over initial shear, leading to higher η_0. The artifacts in Fig. 1A suggested that 12HSA organogels aggregates may have deformed under the applied stress (sample preparation) which contributed to the thixotropy estimated by hysteresis (Table 1). We suggest that aggregates have deformed under shear (decreasing the apparent viscosity), but the higher network strength (Fig. 1A) of 12HSA bigels led to a gradual recovery when the shear rate was removed. This behavior was not observed at the same extent for the other formulations (Fig. 4). In our flow curve experiments, we applied 60 s intervals between each curve, which was not enough to fully reforming the structure [36].

Smaller relaxation times showed higher mobility of the inner structure for 12HSA bigels when compared with CW bigels and emulsions [37]. The reduced oily phase particles observed in CW bigels and emulsions (Fig. 1C, 1 G e 1 H) probably impacted mobility of the polymer into the hydrogel. We believe the physical organization governs the mobility since the "A" parameter (Fig. 3A) showed a stronger network for 12HSA bigels. Despite that all samples showed shear thinning behavior (n<1 at Table 1), the 12HSA bigels shortened the Newtonian region and encouraged the shear thinning behavior ("a" values at Table 1). However, CW bigels showed a similar behavior with emulsions, increasing the width of the transition region between Newtonian and power-law trends. Shear-thinning behavior is also strongly linked with spreadability [38] and corroborates with the hysteresis outcome previously reported.

1.1. *Stability of bigels and emulsions*

The stability of bigels has been related with the organogel/hydrogel ratio [3,6,7]. At high ratios (e.g. 20/80 and 50/50), organogel may lead to a decrease in the integrity of the generated gel matrix or inability to incorporate successfully the organogel phase within the hydrogel matrix [3,6,7]. Rheology-based methods have been recently used as potential alternatives over traditional stability procedures, especially dynamic temperature ramp tests [2]. We conducted five cycles of temperature ramps (20–50 °C) and all samples showed maintenance of the G' and G" behavior. This constancy of the viscoelastic properties of the dispersions during all temperature cycles indicated thermal stability of the samples [39]. Bigels and emulsions had the same behavior, indicating that hydrogel properties overcome organogel thermo-reversibility. To corroborate with rheological findings, we conducted the analytical centrifuge stability test with LUMiSizer® and similar results were obtained. The pattern of sedimentation or coalescence was not observed for any of the samples, which presented instability indexes below 0.1.

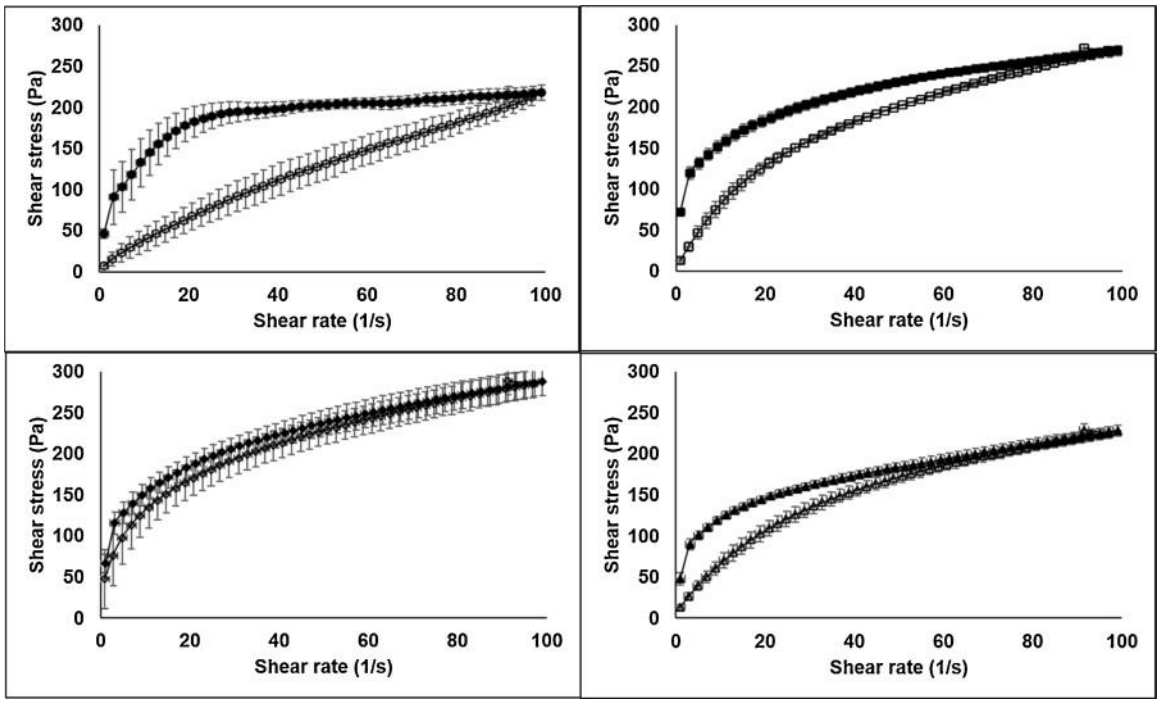

Fig. 4. Flow curve for formulations 12HSA:VO:VE (●), CW:VO:VE (■), MO:VE (◆) and MO (▲). Closed symbols for increase (S1 – transient curve) and open symbols for decrease (S2 — steady state curve).

Negative charges of the polymer used in this work could assist in the stabilization of samples, not only by the gelation of the aqueous phase, but also by the electrostatic repulsion around the oil globules [40]. Those results indicated that bigels could not increase stability facing thermal or centrifuge stress when compared to control emulsions. However, the stability was not decreased either, indicating that the 5/95 organogel/hydrogel ratio may be used to compare bigels and emulsions on further investigations when stability is not addressed as an isolated issue.

1.1. Size and pH value

The inner phase size and the pH value are parameters used to verify the quality of topical products (Table 2). We obtained a range of pH values between 5.90 and 6.05, which is appropriate to skin applications [41]. The size of dispersed inner phase of formulations was affected by the presence of organogel and vitamin E. Bigels showed higher diameter when compared to control emulsions and when vitamin E was present, the size was even bigger and more polydisperse. Bigels containing 12HSA were the ones with the biggest inner phase diameter and it can be linked with viscosity and gel strength parameters earlier demonstrated in this work. Samples with higher diameter in area ($D_{3,2}$) presented higher contact surface with the hydrogel and exhibit stronger networks (Fig. 2A). On the other hand, samples with higher diameter in volume ($D_{4,3}$) presented higher zero shear viscosity probably due higher energy required for big particles to flow.

The unimodal diameters distribution was observed mainly for control emulsions. Bigels showed a broader size distribution (Fig. 5). The distribution of particle sizes showed that the large aggregates observed in microscopy were deformed and enlarged by sample preparation (artifacts). For 12HSA bigels, a tendency of a bimodal distribution in one wide peak was observed, which contributed to rheological behavior, especially flow curves used to viscosity measurements [42]. It is important to notice that even with those aggregates, the stability of samples was not impaired. These results confirmed the observation of the distribution of the globules performed by light microscopy and suggested that vitamin E have improved the size of the inner phase of O/W dispersions [27]. Narrow curve distributions are probably linked with more homogeneous shapes of the aggregates [43], better seen for control emulsions (smaller span values). The shape of particles was not evaluated with the Fraunhofer model used in the size analysis.

1.2. Antioxidative evaluation (DPPH)

We used DPPH test to verify if bigels could increase vitamin E oxidative stability. However, the positive controls reached almost the same free radical scavenging (FRS) than samples, ranging between 90.0 and 95.0 % throughout 90 days in all storage conditions. We excluded method disfunction since negative controls indicated FRS between 1.0 and 2.0 %. During storage at 40.0 °C, samples became yellowish, however, the oxidative scavenge capacity were just slightly different from T_0 FRS values. For that reason, we noticed that neither bigels nor emulsions were able to increase vitamin E stability, since the vitamin E itself was stable under the conditions investigated. Our first hypothesis was that the FRS would decrease under heating, however, most of the literature about oxidation instability of vitamin E is over food processing with higher temperature requirements (approximately 110–140 °C) [44,45]. Our experimental design for stability tests used the average storage conditions for topical products in cosmetics and pharmaceutical industries, which did not reach the point when vitamin E starts the degradation process.

Table 2
Size and pH value parameters. Samples in the same column that share a letter are statistically equal (Tukey test: $p < 0.05$).

Sample	$D_{4,3}$ (μm)	$D_{3,2}$ (μm)	Span	pH
12HSA:VO:VE	7.70^{a} (± 0.04)	3.58 (± 0.01)	2.27	6.05 (± 0.03)
12HSA:MO:VE	7.49^{a} (± 0.16)	3.17 (± 0.02)	2.75	5.92^{c} (± 0.02)
CW:VO:VE	3.56 (± 0.01)	2.35 (± 0.01)	1.95	$5.95^{b,c}$ (± 0.02)
12HSA:MO	9.37 (± 0.18)	2.90 (± 0.01)	4.46	$5.94^{b,c}$ (± 0.02)
12HSA:VO	3.92 (± 0.05)	2.40 (± 0.00)	2.46	$5.95^{b,c}$ (± 0.01)
CW:VO	3.30 (± 0.00)	2.32 (± 0.00)	1.81	$5.95^{b,c}$ (± 0.01)
MO:VE	5.92 (± 0.02)	2.68 (± 0.01)	3.02	$5.95^{b,c}$ (± 0.01)
VO:VE	4.55 (± 0.01)	2.49 (± 0.00)	2.68	$5.96^{b,c}$ (± 0.01)
MO	2.92^{b} (± 0.01)	2.13 (± 0.00)	1.62	5.98^{b} (± 0.01)
VO	2.72^{b} (± 0.01)	2.11 (± 0.01)	1.42	5.90^{c} (± 0.05)

1.3. Biological behavior

The biological behavior evaluated were hyperpigmentation analysis by Fontana-Masson Technique and inflammatory marker quantification (interleukin 6: IL-6) by Enzyme-linked Immunosorbent Assay (ELISA). We chose samples to represent bigels and emulsions with and without vitamin E. Our hypothesis was that samples with greater thixotropy and consistency (bigels with 12HSA) would protect skin against hyperpigmentation and inflammation due better film formation. However, the results showed that samples were not statistically different (Fig. 6), even in the presence of vitamin E. The evaluation of antimelanogenic agents using Fontana-Masson and inflammatory interleukins are widely used [46]. However, each molecule may act at different stages of melanogenesis, such as inhibiting the tyrosinase or its maturation and the melanosome transference. Antioxidants decrease the reactive oxygen species (ROS) that activate tyrosinase. ROS are naturally produced in skin by mitochondria, peroxisomes and enzymes activity and may be increased by external stressors (e.g. radiations and pollutants). Despite the benefits of vitamin E as antioxidant in the hyperpigmentation cascade [47], we suggest that we could not observe differences due the small contribution of ROS in samples. We believe the use of stress would increase ROS and highlight the vitamin E action.

2. Conclusion

The bigels and emulsions obtained led to oil-in-water (O/W) dispersions with crystals located at the interface of phases. Rheological characterization indicated that all samples had viscoelastic weak gel behavior, with physical gel interactions strengthen when 12HSA was used, as organogelator. The type of polymer used in the hydrogel seemed to hide organogel influence over bigels in rheological properties, except of sensory parameters, such as thixotropy, shear-thinning and consistency. All samples showed excellent thermal and centrifuge-stress stability, indicating that 5/95 organogel/hydrogel ratio may be used to compare bigels and emulsions on further investigations. Vitamin E showed little influence over rheological, biological and physical parameters, except for the size of oil globules and maintained its oxidative stability in bigels and emulsions for 90 days in 5, 24 and 40 °C of storage. This was the first study comparing bigels and their emulsions and the 5/95 organogel/hydrogel ratio. The influence of mineral and vegetable oily phases, 12HSA and CW organogelators and vitamin E, as a model drug, was not yet explored for bigel preparation. We brough one perspective of the dependence of rheological properties of bigels on the particle size distribution, however better models to accurately predict the properties of these systems are still needed. Further investigation about topical delivery of vitamin E from these formulations in *ex vivo* model would present a strong argument for the use of the bigels over the emulsions used to compare in this investigation. We encourage the use of stress, such as irradiation or pollutants, to increase the differences among samples. Future exploration of different organogel/hydrogel ratios would also bring important data about each phase contribution to rheology and microstructure.

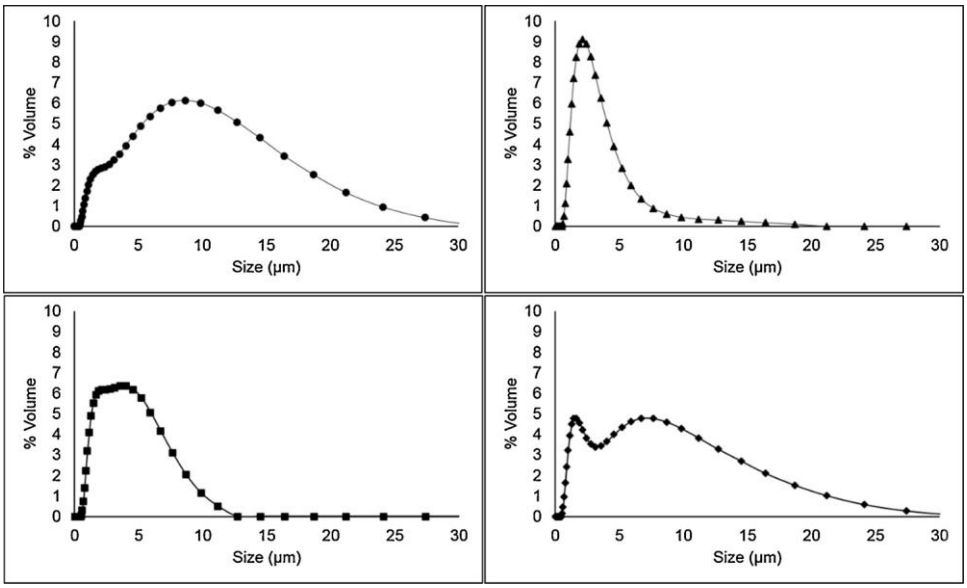

Fig. 5. Size distribution in volume for formulations 12HSA:VO:VE (●), CW:VO:VE (■), MO:VE (◆) and MO (▲).

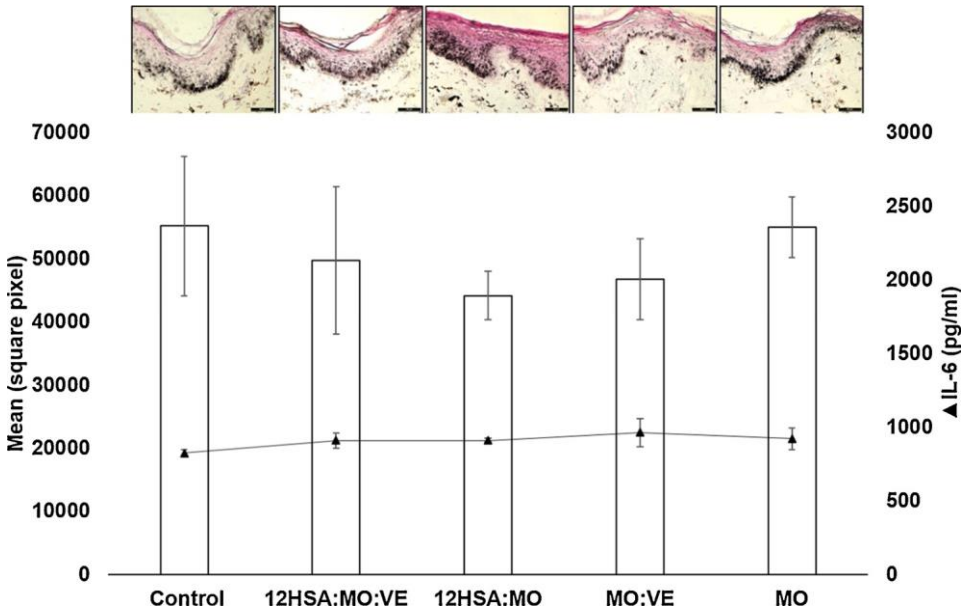

Fig. 6. Effect of the samples 12HSA:MO:VE, 12HSA:MO, MO:VE and MO over the semi-quantification of the melanin pigments formation (bars) and the synthesis of IL-6 (▲) in the supernatant of human tissue macerated. The data represent the mean ± standard deviation. **P < 0.05, in relation to EPS control (ANOVA, Dunnett). At the top, the evaluation of the respectively fragments of human skin (*ex vivo)* by Fontana-Masson in microscope images at 400x magnification.

CRediT authorship contribution statement

Renata Miliani Martinez: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Wagner Vidal Magalhães:** Methodology, Writing - review & editing, Funding acquisition. **Bianca da Silva Sufi:** Methodology, Formal analysis, Investigation, Writing - original draft. **Giovana Padovani:** Formal analysis, Investigation, Writing - original draft. **Lucas Idacir Sbrugnera Nazato:** Formal analysis, Investigation, Writing - original draft. **Maria Valéria Robles Velasco:** Supervision, Funding acquisition. **Suzana Caetano da Silva Lannes:** Supervision, Funding acquisition. **André Rolim Baby:** Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors are greatly thankful to São Paulo Research Foundation (FAPESP, Grant number 2016/24360-4) and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, Grant number 305250/2019-1).

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Article 3

Influence of the Mixtures of Vegetable Oil and Vitamin E over the Microstructure and Rheology of Organogels

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Influence of the Mixtures of Vegetable Oil and Vitamin E over the Microstructure and Rheology of Organogels

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Abstract: Candelilla wax (CW) and 12-hydroxystearic acid (12HSA) are classic solid-fiber-matrix organogelators. Despite the high number of studies using those ingredients in oily systems, there is scarce literature using a mixture of oil and antioxidants. Vitamin E (VE) is an important candidate for its lipophilicity and several applications on pharmaceutical, cosmetics, and food industries. In this work, we investigated the influences of mixtures between vegetable oil (VO) and VE on the microstructures and rheological properties of CW and 12HSA organogels. A weak gel ($G'/G'' > 0.1$) with a shear-thinning behavior was observed for all samples. The presence of VE impacted the gel strength and the phase transition temperatures in a dose-dependent pattern. Larger and denser packed crystals were seen for 12HSA samples, while smaller and more dispersed structures were obtained for CW organogels. The results obtained in this work allowed the correlation of the structural and mechanical properties of the organogels, which plays an important role in the physical-chemical characteristics of these materials.

Keywords: organogel; candelilla wax; 12-hydroxystearic; vitamin E; biomaterials; rheology; SAXS

Citation: Martinez, R.M.; Oseliero Filho, P.L.; Gerbelli, B.B.; Magalhães, W.V.; Velasco, M.V.R.; da Silva Lannes, S.C.; de Oliveira, C.L.P.; Rosado, C.; Baby, A.R. Influence of the Mixtures of Vegetable Oil and Vitamin E over the Microstructure and Rheology of Organogels. *Gels* **2022**, *8*, 36. https://doi.org/10.3390/gels8010036

Received: 1 December 2021
Accepted: 2 January 2022
Published: 5 January 2022

1. Introduction

Organogels are tridimensional structures formed by the organization of molecules in order to hold an organic solvent. Gel formation can be investigated by crystal network observation via polarized microscopy, rheological viscoelastic properties, and/or physical structure at the atomic scale via small-angle X-ray scattering (SAXS) [1]. The type of organogelator and the production process allow the classification of organogels into three categories: (a) fluid filled matrix; (b) solid fiber matrix; and (c) chemical organogels [1]. Low-molecular-weight organogelators (LMWOs), such as candelilla wax (CW) and 12-hydroxystearic acid (12HSA), can self-organize within an organic system, forming a thermoreversible organogel [2]. The gelation occurs upon the total melting of organogelators, followed by a cooling process as the driving force for the nucleation of the organogelator

molecules. This mechanism, known as solid fiber, leads to a three-dimensional fibrillar network structure that holds the organic phase [1].

The crystallization pattern (the shape, size, and distribution of crystals) of organogels is a manifestation of several interaction forces between organogelators and the organic solvent and is affected by many variables, such as the physical and chemical properties of the components, the organogelator/solvent ratio, the total melting of the organogelator, and the cooling rate [3]. The starting point for organogelification is the nuclei formation that grows into the crystalline network. Heterogeneous nucleation is the most favorable type of process, when the nuclei are formed by a substrate that provides the orientation for the crystalline network. The substrate can be any surface, such as the walls of the container or even impurities present in solutions [4]. Therefore, every organogel component is a potential nucleation agent, especially if it is solid or crystalline by itself. For that reason, most organogel studies are conducted with one simple organic phase for each organogelator, such as sunflower oil and CW [5,6] or canola oil and 12HSA [7]. However, for practical applications, more complex matrices are often used. For instance, when oil is used as the organic phase of organogels, the presence of a lipophilic antioxidant could protect against degradation. Despite robust information regarding the organogel's structure using mixed sterols [8] and lecithin blended with other materials [9], few information about mixtures with antioxidants is available.

Our research group has been studying the influence of antioxidants on colloidal systems for the past decade and has recently started exploring organogels and bigels with vitamin E (VE) [10]. Despite beneficial to humans, VE is not naturally produced by the human body and must be topically or orally delivered. This antioxidant is involved in cutaneous protective pathways against aging, atopic dermatitis, and melanoma, for instance [11–13]. It also plays an important role in food industry as a shelf-life enhancer of oily products or to increase oxidative stability in heated oils [14]. Therefore, it is used in a wide range of concentration into pharmaceutical, cosmetic, and food products, with several applications. Despite the wide use, VE was still not explored in organogel systems. The popularization of organogels to replace trans-fat contents in foods [15] and the benefits over the sensory and transdermal delivery of molecules [16] drive the necessity to understand the effect that additives have over the gel formation. The variation of organogels' structures may be very detrimental to the formulation development.

In this research work, the influences of mixtures between vegetable oil (VO) and VE on the microstructures and rheological properties of CW and 12HSA organogels were verified. The VE at several concentrations in CW and 12HSA organogels were investigated using polarized microscopy, rheology, and SAXS, providing detailed structural and mechanical information.

1. Results and Discussion

The macroscopic evaluation showed rigid and transparent gels with little syneresis for 12HSA organogels, while soft and opaque organogels were observed for CW samples (Figure S1). The polarized microscopy observation (Figure 1) explained those findings, since a heterogeneous and discontinuous crystal distribution was observed for CW organogels (Figure 1A), instead of dense and homogeneous crystals in 12HSA samples (Figure 1B). Similar patterns were observed previously in other organogels with the same concentrations of those organogelators [4,5]. However, for CW organogels, a tighter pattern with more evenly distributed crystals may be seen since the natural origin and the variable composition of the CW [17].

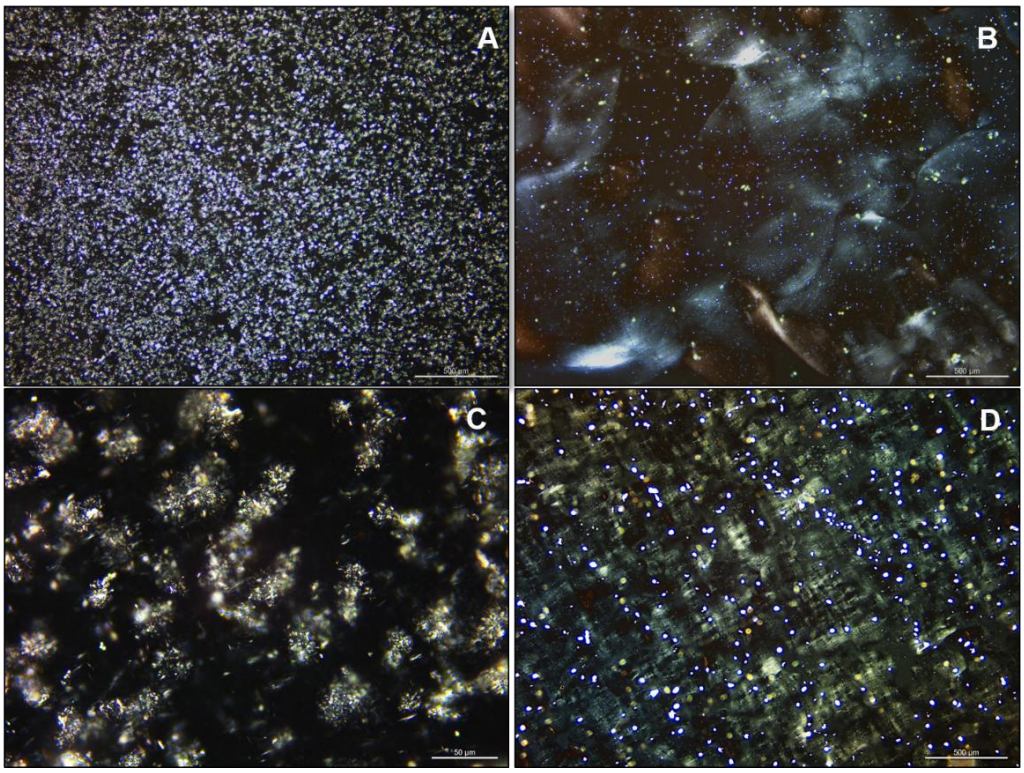

Figure 1. Polarized photomicroscopy images of organogels: (**A**) candelilla wax 2 (CW2):vegetable oil 97 (VO97):vitamin E 1 (VE1); (**B**) 12-hydroxystearic acid 2 (12HSA2):VO96:VE2; (**C**) CW2:VO98; and (**D**) 12HSA2:VO78:VE20. The scale bars are 500 μm in (**A**,**B**,**D**), and the scale bar is 50 μm in (**C**).

Throughout the concentration range of VE tested (0–20%), the size and distribution of crystals were the same under polarized microscopy. The methodology was not a statistical image analysis; therefore, the rheology and SAXS investigations were used for better understanding the crystallization pattern of the organogels.

For all samples, in the frequency sweep measurements, the elastic moduli (G′) dominated the viscous moduli (G″), i.e., G′ > G″, throughout the range where the applied shear was evaluated (Figure S2), which marked solid-like behaviors of the gels [18]. We investigated the contribution of the VE concentration to the complex modulus (G*)—a combination of the G′, the G″, and the phase angle—at 1 Hz for a comparison [19]. For the CW organogels, the G* increased exponentially with the increasing of the VE content, while the exact opposite effect was observed for the 12HSA organogels. Apparently, the composition of the oily phase (VE and VO) affected the organization of the organogels, increasing the G* values for CW and decreasing the G* values for 12HSA (Figure 2). Higher G* values were related with higher network gel strengths [20]. The phase angle was lower than the unity in the whole range of frequency investigated (Figure 2). Those results contributed to verifying the solid-like behaviors of all organogels [17]. The loss tangent was the tangent of the phase angle denoted as: $\tan\delta = G''/G'$. The organogels containing 12HSA showed values of the loss tangent higher than 0.1 ($\tan\delta > 0.1$), typical of the so-called weak gels, throughout the whole range of frequency tested [21]. However, the CW organogels presented an elastic behavior ($\tan\delta < 0.1$) at lower frequencies (<1 Hz) from 0 to 5% VE concentrations [22]. When higher VE amounts were used (20%), the behavior changed to weak gels ($\tan\delta > 0.1$). The VE hydrophilic area can impact somehow the arrangement of crystals, as described earlier, in combination with lecithin organogels [23]. In addition, when increasing the VE concentration, we proportionally reduced the amount of VO, which impacted the oily phase composition and could have changed its physical properties for crystal binding.

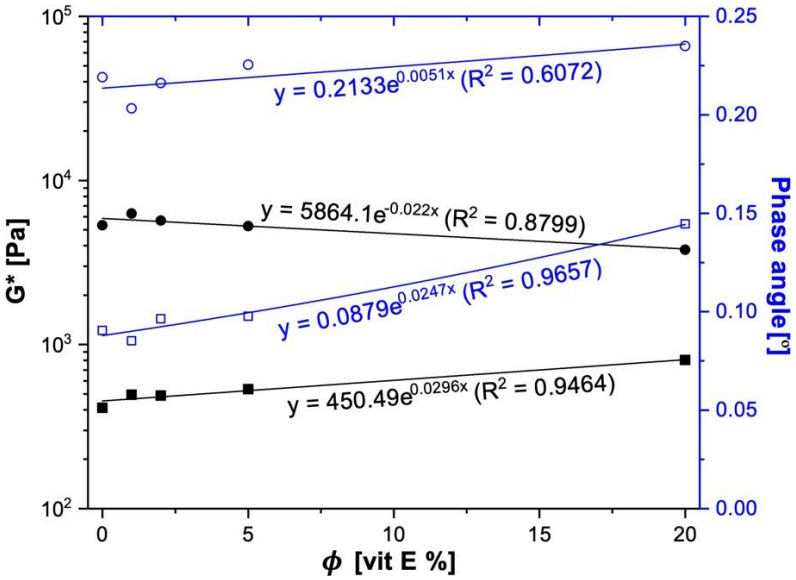

Figure 2. Frequency sweep measurements results for formulations with 12HSA:VO (•) and CW:VO (■) in terms of G* (closed symbols) and the phase angle (open symbols) at 1 Hz. The experimental data are represented by symbols, and the fitting model is represented by lines according to the percentage of VE available (Φ).

The flow curve measurements showed a non-Newtonian shear-thinning behavior for all samples, characterized by a decrease in the viscosity as the shear rate increased (Figure 3A,B). The values of the parameters obtained using the Carreau–Yasuda model (Equation (13)) are shown in Table 1 and confirmed the shear-thinning behavior ($\eta < 1$). Moreover, because of the smaller relaxation time (λ) values, no restriction of mobility was observed for the crystal network inside the organogels [24]. Closer crystals showed slower relaxation times, as observed for the CW organogels. The shear-thinning property is particularly interesting for applications that require the material to flow under an applied force, such as implants [25], topical products [26], injectable products [27], and foods [28]. The viscosity was higher for 12HSA organogels than that of the CW ones, which corroborated the microscopy findings earlier described. A more densely packed crystal network, such as seen for 12HSA organogels, can reduce the mobility of the components inside the organogels by increasing the tortuosity of the system [29]. Crystals acted as barriers against the organogel flow during the viscosity analysis.

The zero-shear viscosity (η_0) is an important parameter to evaluate the consistency (the initial viscosity when the sample starts to flow). Consistency can be is translated as a physical attribute of the first contact between skin and formulation for topical products [30], stability [31], and package design [32]. For all compositions, an initial plateau was observed followed by a large drop in viscosity (shear-thinning region) and finally a second plateau (infinite shear viscosity). This behavior is characteristic of shear-thinning fluids, and it was more accentuated for 12HSA organogels (Figure 3B). The composition of the oily phase affected the consistency of the samples. When higher amounts of VE (and proportionally lower amounts of VO) were used, we observed an increase in the consistency for CW organogels and a decrease in the consistency for 12HSA organogels. However, the 12HSA organogels showed naturally much higher η_0 values. Along with the G* results, the consistency suggested that the composition of the oily phase affected crystal organization into the organogels. Earlier studies showed that the 12HSA network can be disrupted when lecithin is used in association as a co-oleogelator [33]. Likewise, lecithin showed a synergic effect with candelilla and other natural waxes on oleogels formation [15]. In our case, VE played a role as a co-organogelator, along with the reduction of the VO availability. Less oil could hinder the fibrous network for 12HSA, while alleviating the necessity of oil support

for the CW network. Another hypothesis is that the combination of VE and VO presents singular physical-chemical properties for organogelation.

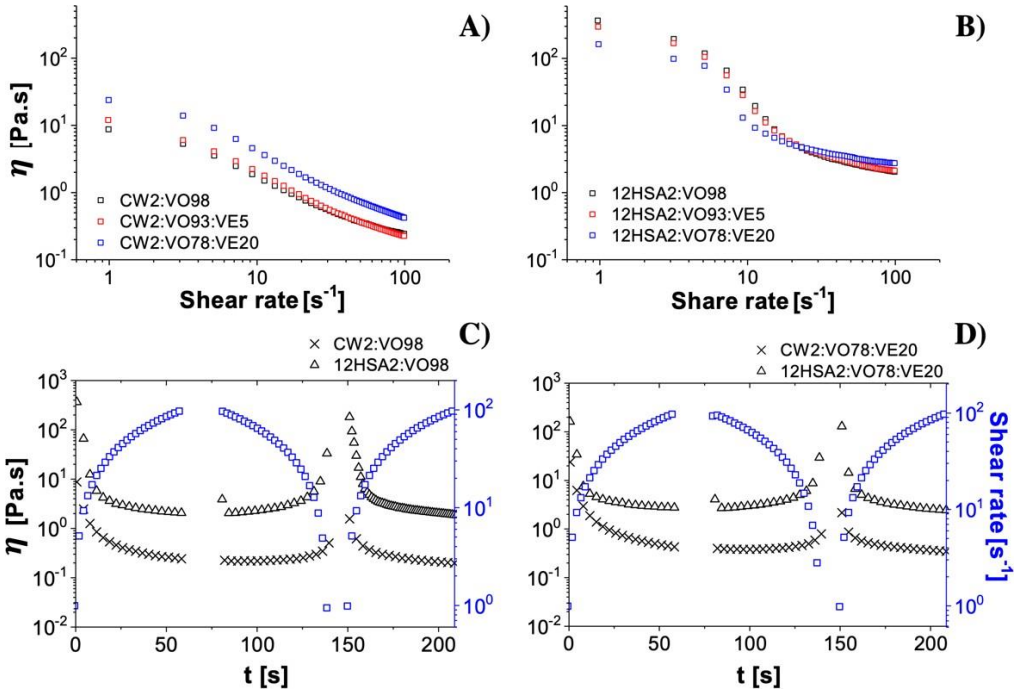

Figure 3. Viscosity (η) versus shear rate for CW organogels (**A**) and for 12HSA organogels (**B**). Structure recovery after shear stress (black dots) for the organogels without VE (**C**) and with VE (**D**).

Table 1. Fitting parameters of the Carreau–Yasuda equation for flow curves and approximate phase transition temperatures Tmelt (melting temperature) and Tgel (gelation temperature).

Code	η_0 (Pa·s)	η_∞ (Pa·s)	λ (s)	n	a	Tmelt (°C)	Tgel (°C)
CW2:VO98	9.37 ± 0.03	0.15 ± 0.01	0.39 ± 0.01	−0.26 ± 0.01	2.15 ± 0.03	55	34
CW2:VO93:VE5	16.06 ± 1.26	0.14 ± 0.01	0.38 ± 0.02	−0.44 ± 0.33	1.24 ± 0.03	55	38
CW2:VO78:VE20	24.93 ± 0.18	0.22 ± 0.01	0.41 ± 0.01	−0.29 ± 0.02	2.49 ± 0.04	50	39
12HSA2:VO98	350.08 ± 8.80	2.19 ± 0.01	0.27 ± 0.01	−1.65 ± 0.01	3.01 ± 0.01	72	66
12HSA2:VO93:VE5	284.41 ± 7.25	2.21 ± 0.01	0.29 ± 0.01	−1.40 ± 0.01	3.63 ± 0.01	63	58
12HSA2:VO78:VE20	150.89 ± 4.09	2.94 ± 0.01	0.29 ± 0.01	−1.39 ± 0.01	4.82 ± 0.01	59	46

The time-dependency behavior and the structure recovery after stress are the two phenomenon involved with thixotropic fluids [34]. Thixotropy plays an important role in the sensory and stability features of topical and oral formulations. It is related with the spreadability of topical formulations over skin [35] and to predict the deformation of foods during the processing and handling operations [36]. The flow curves showed a recovery in the 12HSA viscosity after shearing at the same extend, despite the alterations of the oily phase. However, no recovery was observed for the CW organogels (Figure 3C,D). When a shear force is applied over oleogels, the ability to retain oil decreases since small crystals are formed [29]. In our experiment, the shear applied over CW organogels, which naturally showed a small crystals distribution, probably promoted an oil migration and increased the difficulty in recovering the original structure.

The thermal behavior of the gel–sol or sol–gel phase transition domains (PTDs) of organogels was evaluated using a temperature ramp test [37]. All PTDs shifted to smaller temperatures proportionally to the addition of VE (Figures 4 and 5). However, the shift was more accentuated for the 12HSA organogels. The melting temperature (Tmelt) for the

12HSA organogels without VE was close to 79 °C, as reported by Esposito and coworkers [38] at the same concentration, but higher than 37 °C for the gelation temperature (Tgel). Besides the different rheometer geometries and oily phases, the authors used strategies that could impact the crystallization pattern, such as a slower cooling rate compared to in our experiment (1 °C/min), a surfactant, and a co-solvent [3,37]. They pointed that the addition of the surfactant disrupts the crystalline network, causing the reduction of the gel density, which was similar with our findings for VE. VE also impacts more complex systems, such as nanoemulsions. A decrease in the temperature of fat crystallization in oil-in-water stearin-rich milk fractions was observed for sodium caseinate-stabilized nanoemulsion containing VE [39]. Several works have studied the impact of VE in cellular membrane models using phospholipids. Alpha tocopherol, specifically, works in bilayer membranes' de-stabilization by forming complexes with lipid components [40]. This mechanism is plausible to explain the reduction of PTD temperatures in our results; nevertheless, the specific interaction between VE and the organogelators is currently unknown. In this sense, another hypothesis is that VE modifies the oily phase properties, consequently impacting the rheological properties of the organogel itself.

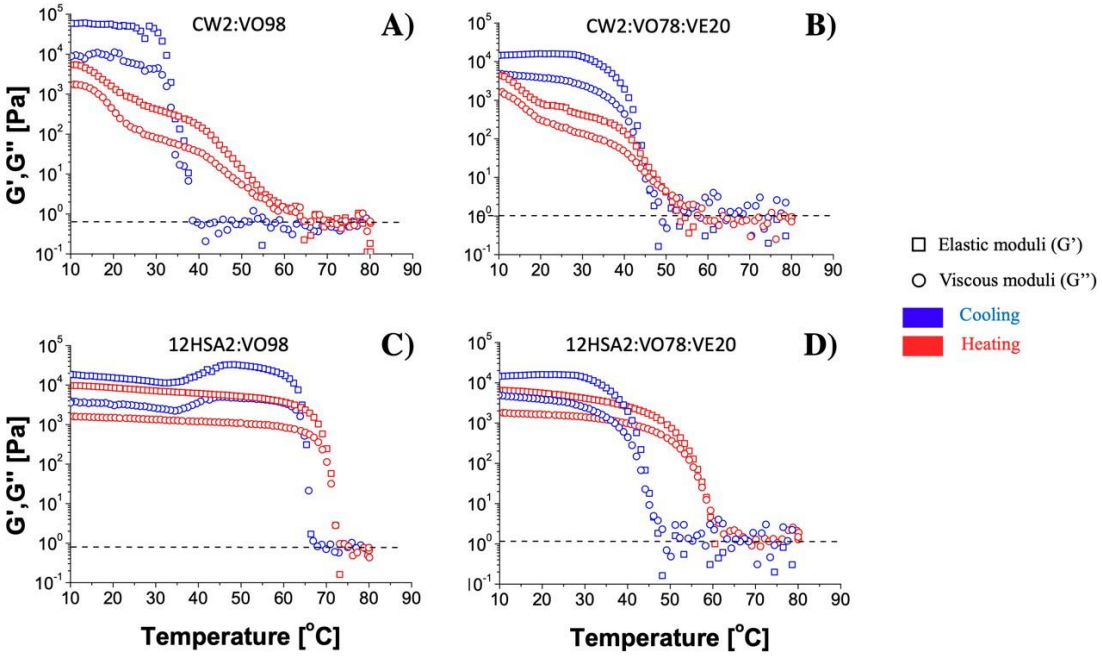

Figure 4. Temperature ramp test results for CW2:VO98 (**A**), CW2:VO78:VE20 (**B**), 12HSA2:VO98 (**C**), and 12HSA2:VO78:VE20 (**D**) organogels. In Table 1, the organogel melting temperature and the gelation transition temperature obtained from the ramps where G ≈ G are presented.

For the CW organogels, the pattern of the heating curve showed a gradual melting process, different from the sharp decrease in G' for 12HSA. CW is a mixture of n-alkanes, esters of acids, alcohols, sterols, and free acids [9]. Each component has its own melting point and impacts the total organogel Tmelt, as well as in the interactions with VE, if they exist. Interestingly, the cooling ramp seemed to organize the crystals, since the cooling rate was controlled (5 °C/min), opposing to the organogel production when it was naturally cooled. This may explain the higher G' values after the temperature ramp test. Regarding this aspect, the 12HSA samples recovered approximatively their initial G' values (Figure 4C,D), while the CW organogels increased 10 times the initial G' magnitude (Figure 4A,B), reassuring a thixotropic effect for the 12HSA organogels but not for the CW organogels. No significant changes were observed when changing the oily phase, except for the discrete reduction of PTD temperatures previously described.

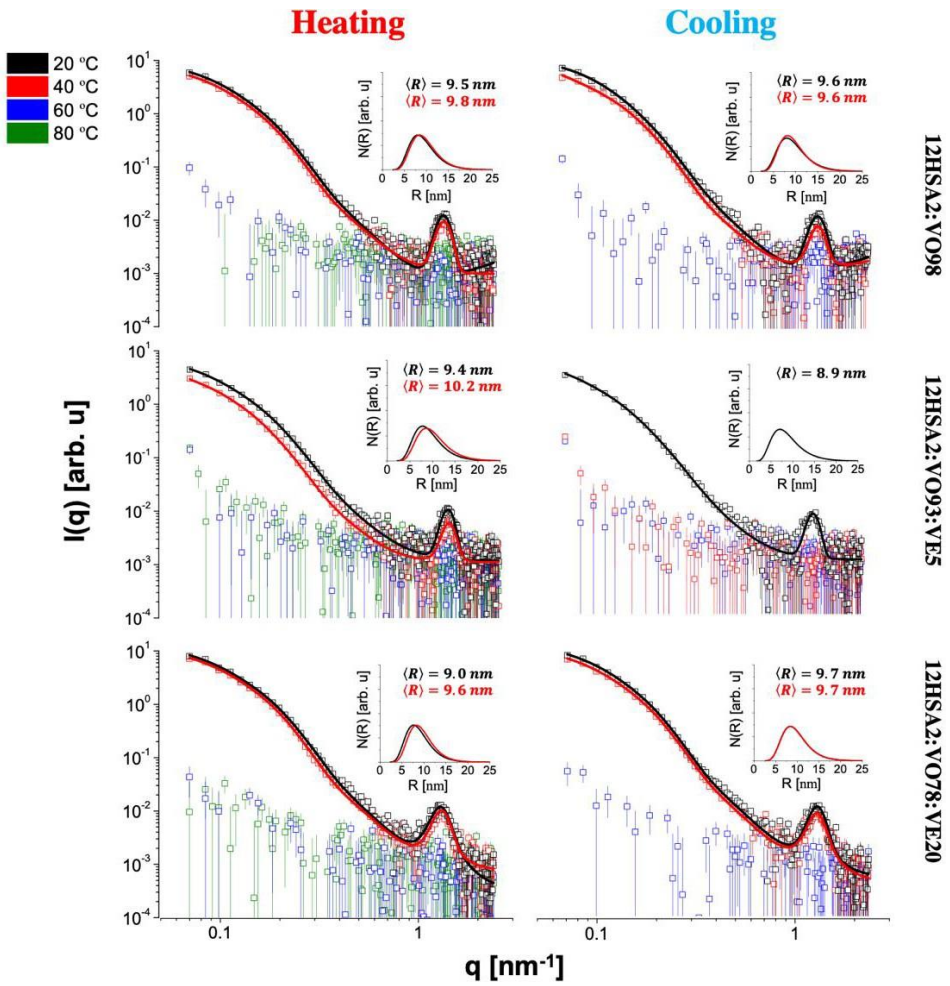

Figure 5. Small-angle X-ray scattering (SAXS) data for the 12-HSA organogels on heating and on cooling (open symbols) and fitted with Equation (12) (continuous lines). The insets correspond to the cylinder radius size distributions. The average radius for each distribution is shown as well.

To better understand the effect of the oily phase over the network structure at a nanoscale, SAXS experiments were performed, which brought important information about the shape and size of the gel nanostructure [41]. The SAXS data for the 12HSA and CW organogels (open symbols) at all investigated temperatures for heating and on cooling are shown in Figures 5 and 6, respectively. In the studied length scale, the profiles of the curves obtained for all organogelators were different, suggesting nanostructures with a distinct size and/or shape. A peak at q of approximately 1.3 nm^{-1} was observed for the 12HSA organogels and was attributed to the (001) Bragg reflection of the 12HSA crystal [42]. It is interesting to note that even CW also has a crystalline structure according to the X-ray diffraction data from previous works [43,44], which was not observed in our data. Possibly, its neatly ordered molecular arrangement was disrupted in the organogel formation, since its main component hentriacontane (~79%, w/w) is highly soluble in organic solvents, such as VOs [5].

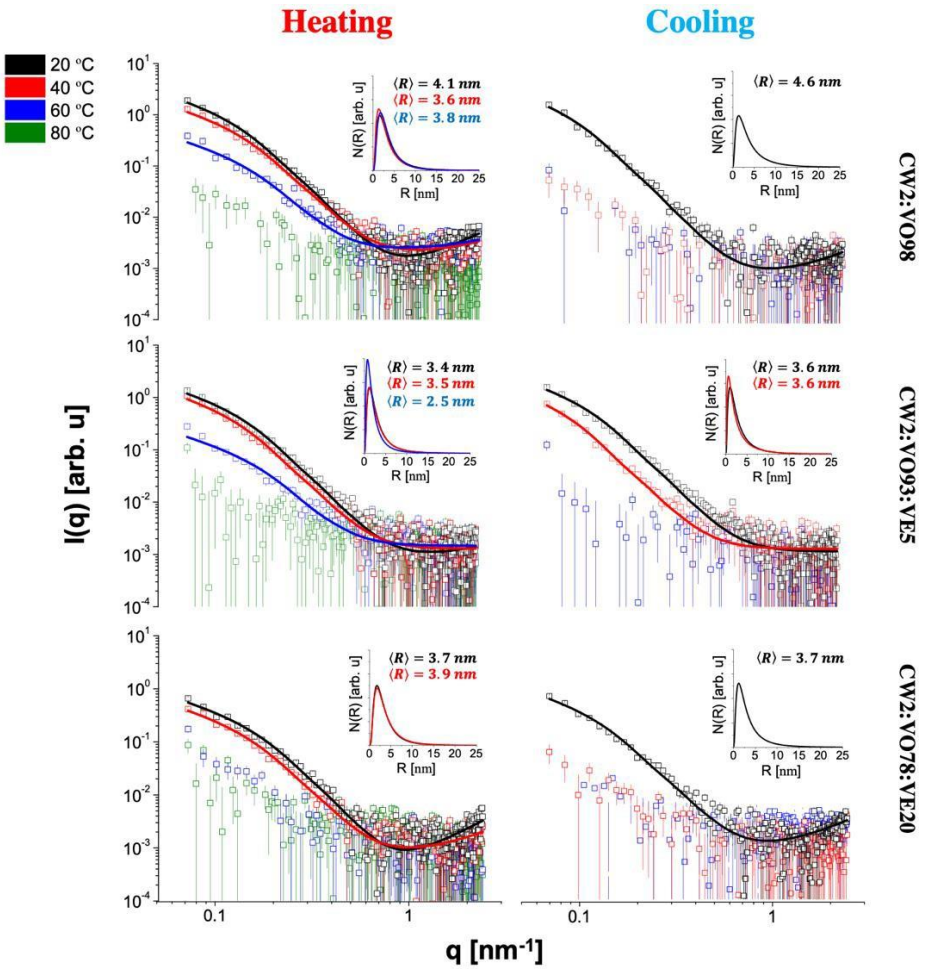

Figure 6. SAXS data for the CW organogels on heating and on cooling (open symbols) and fitted with Equation (12) (continuous lines). The insets correspond to the cylinder radius size distributions. The average radius for each distribution is shown as well.

On heating, a structural gel-to-sol transition was clearly seen for all materials. After this event, the sample scattering curve practically coincided with the sunflower oil curve (Figure 7), the major component used in the sample preparation, and the data reduction led to very noisy curves. This fact made it easier to identify, in Figures 5 and 6, the mentioned transition, which occurred at $T \geq 40$ °C for all 12HSA compositions and CW2:VO78:VE20 and only at $T \geq 80$ °C for all CW compositions, except CW2:VO78:VE20, in agreement with the temperature ramp tests. Furthermore, the CW organogels seemed to be more thermal-resistant than the ones composed of 12HSA, in the sense that they kept their nanostructures at high temperatures up to, at least, 60 °C, except CW2:VO78:VE20. For this particular composition, it was unclear if the melting of the gel at lower temperatures (relative to the other compositions) was caused by a specific effect of VE over the organogel structure or if it was a direct result of change in the oily phase properties due to the addition of VE followed by the reduction of VO. Nevertheless, from all these observations, one can argue that the sol–gel transition is composition-dependent.

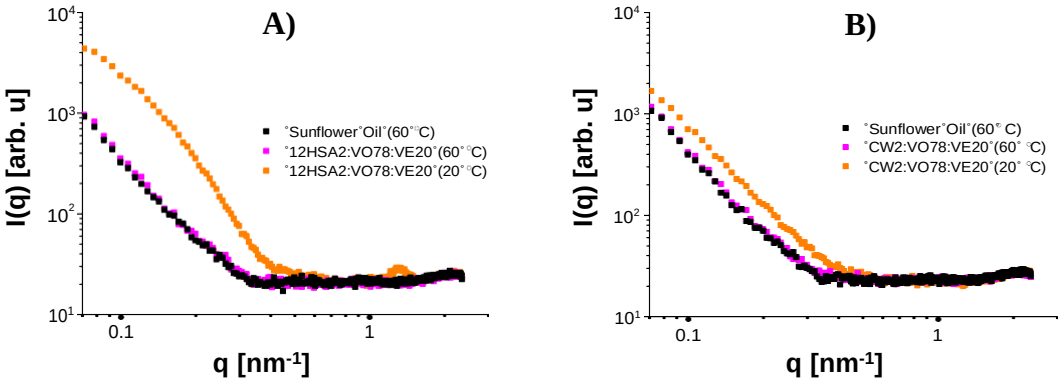

Figure 7. Comparison between the SAXS curves of sunflower oil at 60 ˚C, the major component of the organogels presented in this manuscript, and the samples containing 12HSA (**A**) and CW (**B**), at 60 and 20 ˚C. In this example, the curves collected at 60 ˚C are almost perfectly overlapped. This fact makes some of the treated curves presented in Figures 5 and 6 very noisy in all q values. For the other curves, like the ones collected at 20 ˚C, only their high q regions are noisy.

On cooling, the sol-to-gel transition was, in general, distinct from the one observed on heating, except for 12HSA2:VO98 and 12HSA2:VO78:VE20. Gels were formed at $T \leq 60$ ˚C for all 12HSA compositions (except 12HSA2:VO93:VE5) and only at $T \leq 20$ ˚C for all CW organogels (except CW2:VO93:VE5). The combination 93% VO + 5% VE seemed odd in both types of materials: with 12HSA, the gel formation was difficult, whereas with CW the exact opposite occurred. This fact corroborated once more that the particular composition of the gel had a great influence on its properties, including the sol-to-gel transition temperature. It is quite interesting to observe that the materials containing CW were less prompt to form gels on cooling, whereas the same gels on heating lasted longer at more high temperatures than the ones composed of 12HSA, as discussed before.

Aiming to retrieve quantitative information on the organogel nanostructure, the SAXS data were satisfactorily fitted using Equation (13) (continuous lines of Figures 5 and 6). From the fittings, the average radius, $\langle R \rangle$, and the size distributions of R values (graphs insets in Figures 5 and 6) were obtained. For the 12HSA organogels $\langle R \rangle$ was approximately 10 nm, in agreement with the value reported for 12HSA gel in toluene [45], whereas for the CW materials, $\langle R \rangle$ was approximately 4 nm. This difference in $\langle R \rangle$ might be directly associated to the differences observed in the polarized microscopy results. With the temperature increase, $\langle R \rangle$ slightly increased for all compositions containing 12 HSA, whereas there was no apparent pattern for the CW organogels. In general $\langle R \rangle$ values on cooling were different compared to the ones obtained on heating. This sort of hysteresis was also observed in the measured rheological properties. Regarding the size distributions, they were positively skewed, i.e., the distributions had more data on the right tail. A dispersion of R values around 30% was found in all cases, which was ~1.5 times higher than the value found for 12HSA gel in toluene [45], likely related to the presence of sunflower oil. To the best of our knowledge, there is no reference value for CW organogels, and this is the first time that this kind of SAXS analysis is applied to this material. All in all, the SAXS data for both types of organogel pointed to the structural differences between them, which were satisfactorily quantified by the use of an advanced analysis. Moreover, the gel-to-sol transition on heating and the sol-to-gel transition on cooling were quite evident in the presented curves and strongly dependent on the composition of each investigated organogel.

SAXS Model

SAXS data were modeled assuming non-interacting randomly oriented cylindrical particles with radius R and length L. In this case, the theoretical scattering intensity is given by:

$$I(q) = sc \cdot P(q, R, L) + back, \quad (1)$$

where the parameter *sc* is a scale factor and $P(q, R, L)$ is the cylinder normalized form factor [46]:

$$P(q,R,L) = \int_0^{\frac{\pi}{2}} \left[\frac{2J_1(qR\sin\alpha)}{qR\sin\alpha} \frac{\sin(0.5qL\cos\alpha)}{0.5qL\cos\alpha} \right]^2 \sin\alpha d\alpha, \quad (2)$$

where $J_1(x)$ is the first-order Bessel function and α is the angle between the axis of the cylinder and the scattering vector $\vec{q}$. In most of the samples was observed a non-flat curve at high q values, related to a gel–buffer mismatch in data reduction. To compensate this effect, a non-linear background, *back*, was introduced. In our case, a simple second-order polynomial function satisfactorily fit this region of the SAXS curves.

Under the hypothesis, the cylinders are sufficiently long ($L \gg R$), used in previous works [45]. Equation (2) can be approximated as a product of the longitudinal factor, $P_{rod}(q, L)$, parallel to the cylinder axis, and the scattering cross-section function, $P_{CS}(q, R)$ [47]:

$$P(q,R,L) = P_{rod}(q,L)P_{CS}(q,R), \quad (3)$$

where

$$P_{rod}(q,L) = \frac{Si(q\cdot L)}{0.5\cdot qL} - \left[\frac{\sin(0.5\cdot q\cdot L)}{0.5\cdot q\cdot L}\right]^2, \quad (4)$$

$$Si(x) = \int_0^x \frac{\sin t}{t} dt, \quad (5)$$

$$P_{CS}(q,R) = [A_{CS}(q,R)]^2, \quad (6)$$

$$A_{CS}(q,R) = \frac{2\cdot J_1(q\cdot R)}{q\cdot R}. \quad (7)$$

In this study, after several tests, L was fixed at 200 nm, which provided the best fittings and fulfilled the above hypothesis regarding long cylinders. Taking the R polydispersity into account, the normalized form factor was written as [48]:

$$\langle P(q,R,L)\rangle = \frac{\int_{-\infty}^{\infty} N(R)V(R,L)^2 P(q,R,L)dR}{\int_{-\infty}^{\infty} N(R)V(R,L)^2 dR}, \quad (8)$$

where $P(q, R, L)$ is defined by Equation (2) or Equation (3) and $V(R, L) = \pi R^2 L$ is the cylinder volume. In this study, $N(R)$ was a log-normal distribution [49,50]:

$$N(R) = \frac{1}{R\omega\sqrt{2\pi}} \exp\left(-\frac{1}{2}\left(\frac{\ln R - \theta}{\omega}\right)^2\right), \quad (9)$$

where θ and ω are the average and the standard deviation, respectively, of a Gauss distribution of $\ln R$. The expected (average) value for the variable R and its standard deviation were described as [49,50]:

$$\mu = \exp\left(\theta + \frac{\omega^2}{2}\right), \quad (10)$$

$$\sigma = \sqrt{\exp(2\theta+\omega^2)\cdot(\exp(\omega^2)-1)}. \quad (11)$$

Therefore, rewriting Equation (1), the final fitting equation was expressed as:

$$I(q) = sc\cdot\langle P(q,R,L)\rangle + back. \quad (12)$$

For the organogels containing 12HSA, its crystal structure contributed with a peak at q of approximately 1.3 nm^{-1} (see Figure 5). This feature was taken into account by introducing a Gauss function term in Equation (1), as performed in previous works [45].

1. Conclusions

The design of the organogel's composition plays an important role in the physical-chemical properties of materials. We found distinct structures for all organogels' compositions. While 12HSA built a highly packed network, CW showed sparse crystals observed with polarized microscopy. All organogels were characterized as weak gels with a shear-thinning behavior, but only 12HSA organogels showed thixotropy. The gradual replacement of VO by VE in the oily phase showed an improvement of the gel strength for CW, but a reduction for 12HSA organogels. Likewise, all phase transition temperatures were reduced in a dose-dependent pattern, especially for 12HSA. Larger crystals that slightly increased upon heating were observed via SAXS for 12HSA when compared with for CW. Phase transition was strongly dependent on the composition of the organogel. Further investigation regarding the deeper structure of organogels could clarify whether VO and VE form a new oily phase with different properties or if VE and organogelators compete for VO availability. Those data would be especially beneficial for pharmaceutical, cosmetics, and food exploitation of organogels.

2. Materials and Methods

2.1. Organogel Preparation

Sunflower (Helianthus annuus) oil (VO) with a high oleic content, was purchased from Agri Pure 80, Cargill Agrícola S/A, São Paulo, SP, Brazil. The organogelators CW and 12HSA were purchased from Double Refined Candelilla Wax 102P, Koster Keunen Inc, Wartertown, CT, USA, and A. Azevedo, São Paulo, SP, Brazil, respectively. VE (dl- α-Tocopherol) was purchased from DSM, Parsippany, NJ, USA. All materials were used as received. The production of organogels consisted in the mixing of VO with CW or 12HSA at 85 °C upon continuously stirring at 200 rpm (RW 20; IKA-Werke, Staufen, Germany), followed by the addition of VE once the organogelator was completely melted. The component amounts of each sample are shown in Table 2. At this point, the mixing was continued for 5 min, followed by rest and cooling at room temperature for 24 h. The batch sizes were standardized at 50 g to insure the same thermal heating transfer characteristics.

Table 2. Organogels studied in this work. The acronyms "CW", "VO", "12HSA", and "VE" mean candelilla wax, vegetable (sunflower) oil, 12-hydroxystearic acid, and vitamin E, respectively.

Code	% CW (*w/w*)	% 12HSA (*w/w*)	% VO (*w/w*)	% MO (*w/w*)	% VE (*w/w*)
CW2:VO98	2.0	-	98.0	-	-
CW2:VO97:VE1	2.0	-	97.0	-	1.0
CW2:VO96:VE2	2.0	-	96.0	-	2.0
CW2:VO93:VE5	2.0	-	93.0	-	5.0
CW2:VO78:VE20	2.0	-	78.0	-	20.0
12HSA2:VO98	-	2.0	98.0	-	-
12HSA2:VO97:VE1	-	2.0	97.0	-	1.0
12HSA2:VO96:VE2	-	2.0	96.0	-	2.0
12HSA2:VO93:VE5	-	2.0	93.0	-	5.0
12HSA2:VO78:VE20	-	2.0	78.0	-	20.0

2.2. Microscopy Tests

Polarized microscopy was performed in a DM2700, Leica Microsystems, Wetzlar, Germany, with a 40× objective (Leica HI Plan 40×/0.65 POL). The images were captured with a digital camera Leica MC120 HD (Leica Microsystems, Wetzlar, Germany) and analyzed with the LEICA Application Suite Software (Leica, Wetzlar, Germany). All samples were visualized over glass plates without dilution.

2.3. Rheological Characterization

Rheological measurements were performed in a strain rheometer (TA Instruments, DHR-2, New Castle, DE, USA) with the crosshatched parallel plates with a 20 mm geometry (gap: 300.0 ± 0.1 μm) coupled to a Peltier system for temperature control. Approximately

0.5 g of organogels was transferred to the geometry and left still for 3 min to the equilibrium temperature of 25 ˚C before each measurement. We conducted 3 different experiments in order to evaluate the elastic modulus (G′) and the viscous modulus (G″) behavior when submitted to stress: frequency sweep tests, flow curve tests, and temperature ramp tests. All measurements were conducted in triplicates. Frequency sweep tests were carried out in the range of 0.1–100 rad/s at 25 ˚C in the viscoelastic linear region previous evaluated by the amplitude sweep test for each sample. We monitored the G′, the G″, and the phase angle (δ). Three flow curve tests were performed in controlled stress conditions: increasing the stress from 0 up to 100 s^{-1}, decreasing from 100 to 0 s^{-1}, and increasing again from 0 to 100 s^{-1}. Intervals of 60 s were adopted between every curve at 25 ˚C. We monitored the viscosity (η) behavior during shear stress. The Carreau–Yasuda viscosity model [51] was used to fit the flow curves:

$$\eta(\gamma) - \eta_\infty = (\eta_0 - \eta_\infty)\ 1 + (\lambda\gamma)^{a\ \frac{(n-1)}{a}}, \quad (13)$$

where η is the shear-dependent viscosity, η_0 is the zero-shear viscosity, η_∞ is the infinite shear viscosity, λ is the relaxation time, a is a parameter describing the rate of the transition from the Newtonian plateau to the power law region, and n is the power law index.

Dynamic temperature ramp measurements were performed at 1 Hz in the linear viscoelastic regime, ranging from 10 to 70 ˚C and from 70 to 10 ˚C, with intervals of 60 s between curves in a ramp rate of 5 ˚C/min.

1.1. SAXS

SAXS measurements were performed using a Nanostar (Bruker) instrument equipped with a microfocus Genix 3D system (Xenocs). The samples were maintained in quartz capillaries with a mean diameter of 1.5 mm, and the scattered intensity was collected with a 2D Vantec-2000 detector. The sample-to-detector distance was ~1 m, which provided an effective range of the modulus of the transfer moment vector q experimentally accessible of 0.08–2.3 nm^{-1}, with $q = 4\pi \sin(\theta)/\lambda s$, where 2θ is the scattering angle and $\lambda s = 0.154$ nm is the X-ray wavelength from the copper Kα radiation). The sample temperature was varied using a circulating water bath, with a precision of 0.1 ˚C. An equilibration time of 600 s was applied, after the samples reached one of the selected temperatures (20, 40, 60, and 80 ˚C). For the treatment of the 1D data, obtained through the azimuthal integration of the 2D data, the SUPERSAXS package [52] was used and consisted of normalization by the measuring time (1800 s) and sample transmission, followed by the subtraction of the blank scattering. The scattering from the sunflower oil, measured at the same temperatures of the samples, was taken as the blank, since it is the major component of these organogels.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/gels8010036/s1, Figure S1: Macroscopic aspecst of organogels: (A) 12HSA2:VO98; (B) 12HSA2:VO78:VE20; (C) CW2:VO98; (D) CW2:VO78:VE20, Figure S2: Frequency sweep test results: (A) 12HSA2:VO96:VE2; (B) 12HSA2:VO78:VE20; (C) CW2:VO96:VE2; (D) CW2:VO78:VE20.

Author Contributions: R.M.M.: Conceptualization, methodology, formal analysis, investigation, writing—Original Draft, and writing—review and editing; P.L.O.F.: methodology, formal analysis, investigation, and writing—the original draft; B.B.G.: formal analysis, investigation, writing—the original draft; W.V.M.: supervision and funding acquisition; M.V.R.V.: supervision and funding acquisition; S.C.d.S.L.: supervision and funding acquisition; C.L.P.d.O.: writing—review and editing, supervision, and funding acquisition; C.R. and A.R.B.: writing—review and editing, supervision, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: A.R.B. acknowledges Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; process 305250/2019-1) and São Paulo Research Foundation (FAPESP, process 2019/16169-0). R.M.M. acknowledges Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES) Finance Code 001. C.L.P.d.O. acknowledges FAPESP (Thematic Project; 2016/24531-3), INCT-FCx (Instituto Nacional de Ciência e Tecnologia de Fluidos Complexos) and CNPq Scholarship-Brazil (303001/2019-4). P.L.O.F. acknowledges FAPESP (processes 2019/12301-1 and 2020/13204-7). B.B.G

acknowledges FAPESP (processes 2018/05888-3 and 2020/02192-8). C.R. acknowledges Foundation for Science and Technology (FST) projects UIDB/04567/2020 and UIDP/04567/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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4. CONCLUSIONS

Organogels show great potential in cosmetic field for its low-cost, rheological properties and enhanced delivery profiles. It can deliver hydrophilic and lipophilic molecules into the skin in tailor-made formulations. Despite the lack of information on safety and toxicological effects, previous knowledge from food and drug delivery applications may be used to reinforce its use in cosmetics. Regarding the bigels obtained in this research work, they were oil-in-water (O/W) dispersions with crystals located at the interface of phases with viscoelastic weak gel behavior and excellent thermal and centrifuge-stress stability. When 12-hydroxystearic acid was used, physical gel interactions strengthen when compared with candelilla wax. The type of organogelator in the organogel influenced the thixotropy, shear-thinning and consistency. Vitamin E showed little influence over rheological, biological and physical parameters, except for the increase in the size of oil globules. This was the first study comparing bigels and their emulsions and the 5/95 organogel/hydrogel ratio. Further investigation about topical delivery of vitamin E from these formulations in *ex vivo* model would present a strong argument for the use of the bigels over the emulsions used to compare in this investigation. We believe that the use of stress, such as irradiation or pollutants, could increase the potential of vitamin E bigels over *ex vivo* analysis. Further investigation of different organogel/hydrogel ratios could also contribute to highlight the role of each phase in rheology and microstructure.

Despite all organogels studied herein were characterized as weak gels with a shear-thinning behavior, the organogel's composition plays an important role in its physical chemical and phase transition properties. Organogels containing 12-hydroxystearic acid showed highly packed network with thixotropy and a reduction in gel strength in the presence of vitamin E. On the other hand, candelilla wax showed sparse crystals and an increase in gel strength when vitamin E was added. All phase transition temperatures were reduced in a dose-dependent pattern, especially for 12-hydroxystearic acid organogels. Larger crystals that slightly increased upon heating were observed via SAXS for 12-hydroxystearic acid organogels when compared with candelilla wax. We brought two possible mechanisms for vitamin E and the oily phase interaction that required further investigation: (1) vegetable oil and vitamin E form a new oily phase with different properties or (2) vitamin E and organogelators compete for vegetable oil availability.

Our findings suggested that organogels and bigels showed great potential as vitamin E delivery systems for cosmetics. The concentration of vitamin E is an important factor for organogel production and must be addressed properly during the phase of formulation design. The versatility, stability and sensory modulation of organogels and bigels are the main advantages. However, further studies are required to evaluate biological effects under UV and/pollution exposition.

5. SCIENTIFIC PRODUCTION

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