Production, characterization and impregnation of nanostructured lipid carriers of *cupuaçu* butter (*T. grandiflorum*), alpha-bisabolol, tea tree oil (*M. alternifolia*) and citric acid in nonwoven fabric

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***Abstract*— The present study aimed to add nanostructured lipid carriers (NLCs) of *cupuaçu* butter, alpha-bisabolol, tea tree essential oil (M. alternifolia) and citric acid into nonwoven fabrics (NWFs). This procedure allows the benefits of this substrate to be extended, as it combines the characteristics of the fabric with the properties of active components present in the impregnated formulation. For impregnation, different procedures were carried out to determine the most appropriate methodology to address the materials under study. The analysis of the NLC characterization made it possible to verify whether the formulation produced was physically stable 15 days following its preparation. It was found that the substrate used for impregnation was made of polypropylene and manufactured by spunbond and pointbonding. The study suggests that the most effective method for impregnation of nanostructured lipid carriers in nonwoven fabric is spraying, without prior treatment.**

***Index Terms* — characterization, impregnation, nanostructured lipid carrier, nonwoven fabric**

I. INTRODUCTION

The textile industry is increasingly interested in developing fabrics characterized by different properties. In recent years, researchers have investigated the use of functional textiles in personal health, hygiene and beauty products. Fabrics that contain elements of body care, fitness and health in their composition are classified as cosmetic textile. On contact with the skin, the fabrics release active substances that are slowly absorbed into the skin [1-8].

To achieve this effect, a viable alternative is the use of nanotechnology. This technique improves the properties of the textile, making it longer durable, (mainly of volatile substances, such as essential oils) and also enables controlled release of active ingredients. Several studies have been performed on the impregnation of micro- and nanoparticles in textile [9-16]. To increase the effectiveness of the incorporation of these substances, it is critical to know the substrate to be used. In fact, the property of the woven hydrophilic (such as cotton) and hydrophobic (such as polypropylene) threads can affect the absorption of substances [17].

Therefore, the present study aimed to incorporate nanostructured lipid carriers of *cupuaçu* butter (*T. grandiflorum*), alpha-bisabolol, tea tree oil (*M. alternifolia*) and citric acid into nonwoven fabrics. This procedure allows the benefits of this substrate to be extended, as it combines the characteristics of the fabric with the properties of active components present in the nanostructured lipid carriers. Among these properties, there are the moisturizing power of *cupuaçu* butter [18], the bactericide and fungicide action of alpha-bisabolol [19, 20] and tea tree oil [21-38], and the capacity to regulate the pH of the citric acid [39, 40].

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II. MATERIALS AND METHODS

The lipid matrix of NLC aqueous dispersion is composed of *cupuaçu* butter. The tea tree oil and alpha-bisabolol were the active substances used. Other components were added: citric acid, as pH adjuster; butylated hydroxy-toluene (BHT), as antioxidant; polysorbate 80 (Tween 80), as surfactant; imidazolidinyl urea, as preservative; and distilled water as vehicle. The concentration of each component was determined according to studies reported by other authors, as shown in T**able I.**

Table I Concentration of components of the NLC.

Phase	Component	Conc (%p/v)	Author
Lipidic	*Cupuaçu* butter	8	Colomé et al. [41]
	BHT	0.05	Bueno et al. [42]
	Tea tree oil	1.5	UnivNorthwest A & F [43]
	α-bisabolol	0.1	Feedback Trayer SL [44]
Aqueous	Polysorbate	2	Bueno et al. [42]
	Imida. urea	0.1	Beraldo [45]
	H2O qs	100	-
-	Citric acid	1	Medicis Pharm Corp et al. [46]

To obtain the aqueous dispersion of nanostructured lipid carriers, the high-pressure homogenization (HPH) technique was used [41, 42, 47]. First, the lipid phase was obtained by placing the *cupuaçu* butter in a water bath at 40°C up to its complete melt. Secondly, while the temperature was kept constant, the BHT was added, which remained in a magnetic stirrer until it was completely dissolved in *cupuaçu* butter. Finally, the tea tree oil and alpha-bisabolol were incorporated. The aqueous phase began with the dissolution of Tween 80 in distilled water at 40° C. The temperature was then increased up to 45-50° C, and imidazolidinyl urea was added. The solution was magnetically stirred until all components were evenly dispersed. Afterwards, the aqueous phase was added to the lipid phase and homogenization was carried out using a homogenizer IKA T-25 Ultra-Turrax (Ika, Germany);

centrifugation at 11,000 rpm for 1 min, followed by another cycle of 13,000 rpm for 1 min more. The emulsion was prepared by high pressure homogenization in a Panda 2K NS1001L homogenizer (Niro Soavi, Italy) with operating pressure value of 220 bar. The citric acid was then poured into the emulsion.

The textile substrate used for impregnation of aqueous dispersions of NLC was the nonwoven fabric (NWF). These materials are widely applied in personal care and hygiene products due to their ability to absorb high amounts of moisture. NWFs are comfortable and classified as disposable or durable based on usage and can be fabricated faster. Although the NWFs are made up of fibers and filaments, these materials differ from textile fabrics, because their fibers are not knitted, woven or plaited [17, 48-50]. In accordance to NBR-13370:2002 [51], "the nonwoven is a flat, flexible and porous structure consisting of a mat or veil of fibers or filaments directionally or randomly oriented, consolidated by mechanical means (friction) and / or chemical (adhesion) and / or thermal (cohesion) and combinations thereof".

A. Characterization of NLC aqueous dispersion

The pH of the dispersion was determined by using a digital Digimed pH meter (model DM-22). The pH was measured at selected times (0, 7, 15, and 30 days). The samples were stored in a controlled room temperature environment (25 ± 2 ºC; 65 ± 5% relative humidity) and protected from light.

The average diameter volume-based (D [4,3]) was used as a parameter for the particle size distribution. Particle diameter values corresponding to cumulative size distribution at 10%, 50%, or 90% ($d_{0.1}$, $d_{0.5}$ and $d_{0.9}$, respectively) were also calculated. These values were used to determine the span, which is a common calculation to quantify distribution width, with definition shown in the equation 1 [52]:

$$Span = \frac{D_{v0.9} - D_{v0.1}}{D_{v0.5}} \quad (1)$$

The particle diameter and polydispersity index (PDI) were determined by means of dynamic light scattering (DLS). The following parameters were used: the refractive index of 1.47 for butters in the 0.02 and 2000 µm spectral range. Both analyses were performed in a Zetasizer Nano ZS 3600 equipment (Malvern Instruments, UK). The mixture dispersion was diluted 1,000 times (v/v) with distilled water and filtered through a 0.45-µm membrane.

The electrophoretic mobility measurements determined the zeta potential (Zetasizer® Nano ZS 3600, Malvern). The mixture was diluted 1,000 times (v/v) with filtered solution of NaCl (0.45-µm membrane).The results were calculated based on the mean of three determinations.

The formulations were maintained within a MA835/F-172 climatized chamber (Marconi Equipmentos para Laboratórios Ltda), and measured at selected times (0, 7, 15, and 30 days). According to the guidelines for Accelerated Stability Tests for Cosmetic Products [53], the formulations should be stored in a controlled room temperature environment (37 ± 2 ºC; 65 ± 5% relative humidity). However, the temperature parameter was changed to 25 ± 2° C, since the melting point of *cupuaçu* butter is 33° C and high temperatures could alter the physical properties of the formulation.

NLC dispersions were subjected to stability based on the particle diameter (measured by laser diffractometry), the average particle diameter (measured by DLS), as well as zeta potential and pH.

B. Characterization of nonwoven fabrics

The analysis of the chemical composition was performed using Fourier transform infrared-attenuated total reflectance (FTIR/ATR) spectroscopy (PerkinElmer® Spectrum 100).

The analysis of the substrate morphology was performed with the images obtained using a scanning electron microscope (SEM) (Hitachi®) TM 3000). The magnified images (X50 and X250) allowed the analysis of the structure and manufacture aspects of the material under study.

The rate of capillary absorption of water is among the tests prepared by the Brazilian Standards (Standard number: NBR-13735:2006) that proved to be adequate to evaluate this parameter in nonwoven fabric. This material is often used in products that are directly in contact with the skin, therefore, it is of fundamental importance that the liquid moisture (mucous, urine, blood or sweat) flows quickly through the nonwoven fabric, reducing the moisture built up in the microclimate. The NWFs samples were partially immersed in distilled water at selected times (10, 30 and 60 seconds) to measure the height attained by the water.

C. Impregnation of NLC dispersions in nonwoven fabric

The nonwoven fabric was previously subjected to impregnation treatment. The fabric was prepared according to the NBR standards (NBR-13735:2006). The fabric samples, measuring 30 x 100 mm, were divided into three groups identified for further evaluation. Group 1 was not submitted to any treatment; Group 2 was washed with distilled water in ultrasound device for 12 hours [12, 54, 55]; and Group 3 was washed with 95% alcohol solution in a magnetic stirrer for 12 hours [56, 57]. All the samples were kept at room temperature for 24 hours and were subsequently subjected to the exactly same impregnation processes. Thus, it was possible to determine whether the pretreatment results showed significant differences with the addition of NLCs in nonwoven fabrics.

The first technique used was impregnation bath [13, 14]. NWFs samples were immersed in 50 mL (enough to cover the samples) of aqueous dispersion of NLCs and maintained under magnetic stirring. For Assumption [13], the immersion time required was 0.25 hours, and for Rossi [14], it was 24 hours. In the present study, both impregnation times mentioned above were assessed. The samples were dried at room temperature for 24 hours.

The second technique used was compressed air spray [16]. The concentration of the original solution was used and 8 mL were sprayed on the samples with a pressure of 116 psi (= 8 bar). The samples were dried at room temperature for 24 hours.

III. Results and Discussion

A. Characterization of NLC aqueous dispersion

The physico-chemical properties of the NLC aqueous dispersion are shown in Table II.

Table II Physico-chemical stability of the NLC aqueous dispersion

	0 day	7 days	15 days	30 days
D [4,3]	195.5±27.22	200±22.86	193.5±27.07	191.5±24.82
span	195.5±27.22	200±22.86	193.5±27.07	191.5±24.82
ZP	195.5±27.22	200±22.86	193.5±27.07	191.5±24.82
Ø	195.5±27.22	200±22.86	193.5±27.07	191.5±24.82
PDI	195.5±27.22	200±22.86	193.5±27.07	191.5±24.82
pH	195.5±27.22	200±22.86	193.5±27.07	191.5±24.82

Note: Data expressed as average and standard deviation of the three batches on days 0, 15 and 30.

Abbreviations: D [4.3]: equivalent spherical diameter (nm); ZP: zeta potential (mV); Ø: z-average (nm); PDI: polydispersity index.

The data obtained by laser diffractometry showed that there was no significant variation over the period of analysis, which was considered a sign of good physical stability of the formulations. The volume mean diameter (D [4.3]) and the span displayed a monomodal distribution, without the presence of micrometric populations of particles as contaminants (Fig 1).

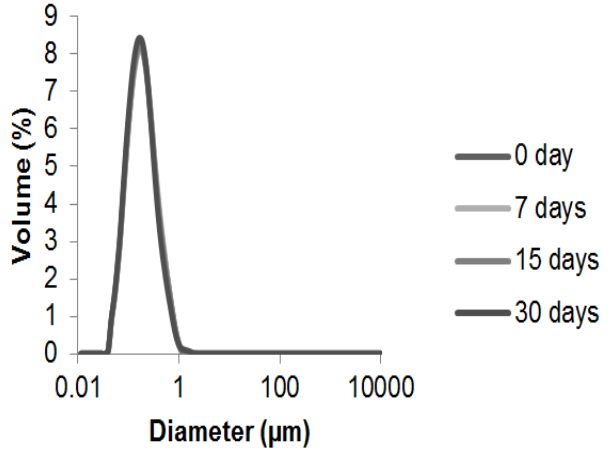

Fig. 1 Particle size distribution by laser diffractometry of the NLC aqueous dispersion. The formulation assessments were carried out on day 0, 7, 15 and 30. Data expressed as average of the three batches.

The pH variations were negligible during the four analyses carried out. For the particle diameter (ø), the dynamic light scattering gave a Z-average of 170 nm and a polydispersity index of 0.15, indicating a homogeneous particle size distribution.

All formulations had zeta potential values that varied from -8.37 to -6.09 mV during the period time of analysis. In the Fig 2, the graph shows bimodal distribution of micrometric populations on the 30th day. Stability testing was conducted to determine the maximum time interval between the production and application of NLCs in fabrics.

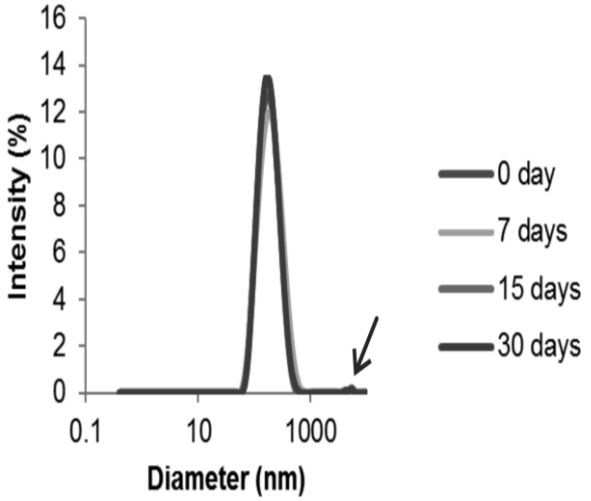

Fig. 2 Particle size distribution by dynamic light scattering of the NLC aqueous dispersion. The graph shows bimodal distribution (peak indicated by arrow) of micrometric populations on the 30th day. The formulation assessments were carried out on day 0, 7, 15 and 30. Data expressed as average of the three batches.

B. Characterization of nonwoven fabric

The analysis of chemical composition of the nonwoven fabric was performed via FTIR/ATR for polymer identification. The spectrum generated showed that the substrate was composed of polypropylene (Fig 3). The interpretation was based on the scheme prepared by Lopes; Fascio [58], which corroborate the one used as reference.

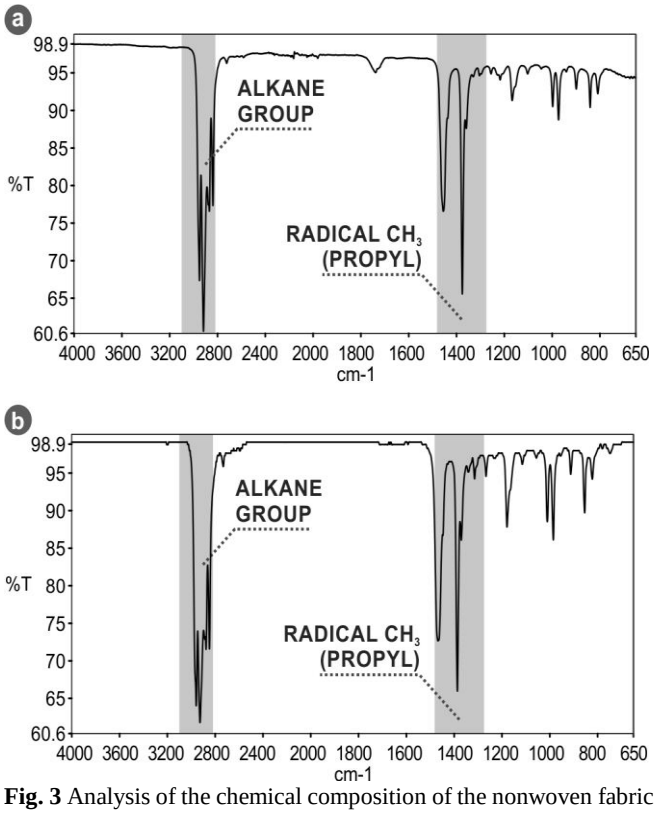

Fig. 3 Analysis of the chemical composition of the nonwoven fabric generated by FTIR/ATR. The spectrum generated (a) showed that the substrate was composed of polypropylene (PP). The interpretation was based on the scheme prepared by Lopes; Fascio [59], and corroborate the PP spectrum used as reference (b).

Plan view photomicrographs of the nonwoven fabric magnified 50X and 250X (Fig 4) showed that the analyzed material was manufactured by spunbonding and point bonding processes, as described by Gupta; Smith [59]. The bond spots are some of the features of both processes.

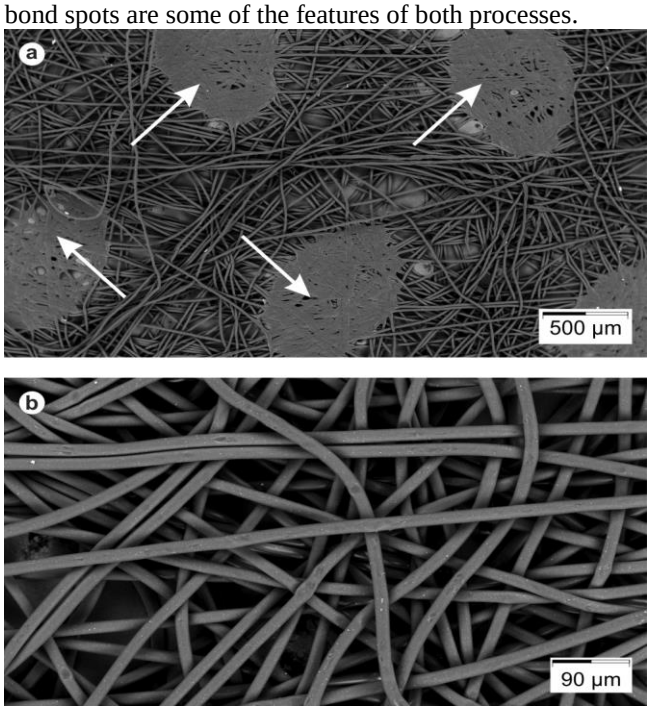

Fig. 4 Photomicrographs of the NWF used in the present study. The images were magnified 50X (a) and (b) 250. Bond spots (arrows) can be seen and are typical features of both manufacturing processes.

The capillary flow rate was measured using six NWF samples. Results showed that this fabric did not absorb water.

C. Impregnation of NLC dispersion in nonwoven fabric

The samples were divided into three groups: Group 1 did not receive prior treatment; Group 2 was sunk into ultrasound in water for 12 hours; and Group 3 remained immersed in water under magnetic stirring for 12 hours. After this procedure, the samples were dried for 24 hours at room temperature and the mass of each sample was measured in digital scale accurate to 0.0001 g. Following, the samples were submitted to three methods of impregnation: (i) immersion bath for 15 min; (ii) immersion bath for 24 hours; (iii) and spraying. Once dried, the sample mass was measured again. The mass measurements provided data to help select the appropriate treatment and impregnation technique (Fig 5).

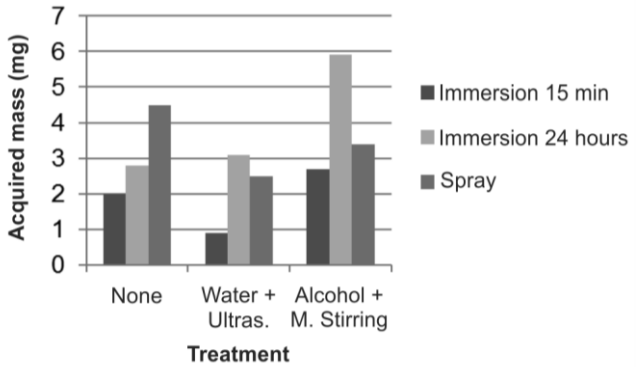

Fig. 5 Acquired mass of nonwoven fabric samples after impregnation. Each bar corresponds to the mean mass of the three samples.

In terms of pretreatment, the samples that were sonicated with water acquired less mass compared to the other two samples. For the impregnation method, a water bath for 15 min resulted in less acquired mass when compared to the samples from the same group. The sample mass submitted to a water bath for 24h and previously sunk in ultrasound with water was greater than the mass of the samples of the same impregnation technique that were kept in alcohol and magnetic stirring. However, an absolute difference of 0.03 mg between the two treatment groups was considered negligible.

Although the best result obtained was the previous treatment of alcohol plus magnetic stirring combined with a water bath for 24 hours, both methods can be highly costly if included in a large-scale production. Therefore, the most appropriate impregnation technique was determined through the evaluations of SEM photomicrographs and the measurement of penetration rates.

The scanning electron microscopy (SEM) was used to verify the presence of NLC in fabrics. The sample photomicrographs were magnified 400X and revealed the presence of suspended fibers of nonwoven fabric (Fig 6).

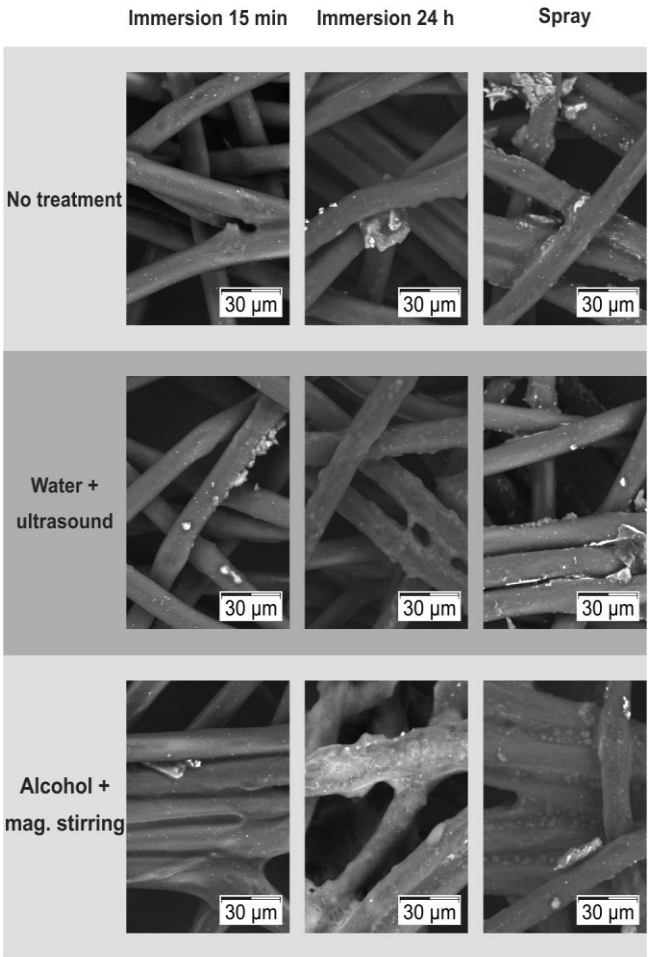

Fig. 6 Photomicrographs of NWF samples after being submitted to different impregnation techniques of NLC dispersions.

Note that the formulations are presented in the form of film and fiber. The disintegration of lipid nanocarriers, which formed this film, could have been caused by SEM. The principle of SEM is based on the use of a small-diameter beam of electrons to explore the surface of the sample, point by point, along successive lines, and transmit the detector signal to a cathodic screen whose scan rate is perfectly

synchronized with that of the incident beam [60]. It uses a tungsten filament as the electron source, heated and accelerated by a voltage of 1-50 kV. When the electron beam focused on the lipid nanocarriers (consisted of *cupuaçu* butter and melt when exposed to a temperature above 33° C) disintegration of particles may have occurred forming a film over the fibers.

In addition, the amount of formulation was lower in the samples impregnated in a water bath (15 min), when compared to other impregnation methods. This data has already been found when the mass acquired after the impregnation rate was measured. In turn, the sprayed samples showed greater amount of NLCs adhered to the fibers, even in those with no previous treatment. In tests that measure the penetration rate of the pure nonwoven fabric samples, there was no water absorption. In samples impregnated with NLCs, there was an improvement of the fabric ability to absorb water. The *cupuaçu* butter possesses both hydrophilic (water-loving, polar) and lipophilic (fat-loving) properties Therefore, the water absorption after immersion could be explained by the amphiphilic character of the *cupuaçu* butter.

Although the visual analysis made through the photomicrographs revealed a greater amount of impregnated material, no significant improvement was observed in water absorption between the samples with no treatment and those who were treated before impregnation. This aspect was confirmed by comparing the mass weight before and after impregnation (Fig 7). Regarding the feasibility of processing coatings for large scale production, the inclusion of a pre-treatment process is highly costly and may even become unfeasible.

There were no significant differences between the rates of capillary flow of the fabrics that were impregnated using different methods. When the higher (mean 0.83 mm, obtained with 15 min of immersion) and the lower scores (average of 0.5 mm, obtained by spray) were compared, an absolute difference of 0.3 mm was observed. In relative terms, this could be considered a negligible value, probably due to measurement errors. In addition, the NWF samples immersed in dispersion for 24 hours were wrinkled after being stirred for a long time. This result can be considered unsatisfactory, since a pleasant visual appearance is a decisive factor for the acceptance of hygiene products by consumers. In terms of costs, it is worth mentioning that it was necessary to use 50 mL of solution to keep the samples fully immersed. In contrast, only 8 mL were enough to impregnate the NWF samples using spray.

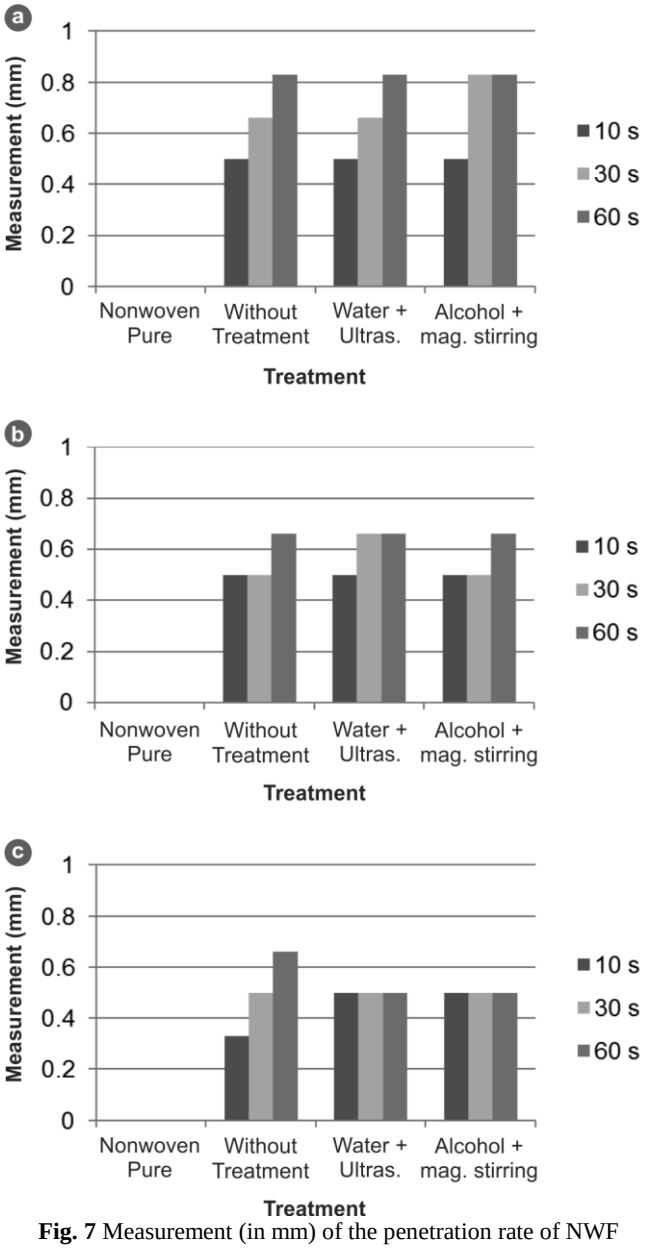

Fig. 7 Measurement (in mm) of the penetration rate of NWF samples before and after being impregnated with nanostructured lipid carriers. Graph (a) corresponds to the impregnation made in a water bath for 15 min; Graph (b), impregnation in a water bath for 24 hours; and Graph (c), impregnation by spray. In the horizontal axis of the graph, the space of pure NWF refers to the rates of capillary flow of the fabric before impregnation (0 mm). Each bar corresponds to the average mass of the three samples.

IV. Conclusions

The present study aimed to analyze the feasibility of adding nanostructured lipid carriers (NLCs) of *cupuaçu* butter (*Theobroma grandiflorum*), alpha-bisabolol, tea tree oil (*M. alternifolia*) and citric acid into nonwoven fabrics (NWFs). The analysis of the NLC and NWF characterizations allowed a better understanding of the physical and chemical properties of materials.

During the evaluation period of the NLCs, the diameter of the particles and the polydispersity index obtained showed homogeneous distribution of particle size. Data obtained by laser diffractometry revealed no significant changes over the period of analysis, which can be considered a good sign of system stability. However, when the particles were analyzed by means of light scattering, it was found that the absolute value of the zeta potential remained low. This result suggests that the formulation particles may aggregate fast. In fact, a micrometric population was observed on the 30th day. Therefore, this formulation is considered physically stable up to 15 days after its preparation. The pH value did not vary significantly (pH 2.4).The spectrum generated by the analysis of NWF by FTIR/ATR showed that the material was composed of polypropylene. The SEM photomicrographs revealed that the NWF were manufactured by spunbond and pointbonding. While determining the rate of capillarity flow in pure, nonwoven fabric, it was observed that there was no water absorption.During the evaluation period of the NLCs, the diameter of the particles and the polydispersity index

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According to the measurement of the mass acquired after impregnation, the best result was the previous treatment of alcohol + magnetic stirring combined with impregnation by bath for 24 hours. The analysis of SEM photomicrographs showed that the best results were obtained by spray impregnation, regardless of whether the samples were treated previously or not.

When the capillarity flow rates in pure NWF (0 mm) were compared to those of impregnated nonwoven fabric (up to 0.83 mm), it was observed that the presence of NLCs indicated the water absorption ability of nonwoven fabrics. Additionally, there was no significant difference in water absorption between the samples submitted to the different processes tested. Thus, this easy spray-on application method, with no previous treatment, is a good way to start potential applications of the impregnation process of nanostructured lipid carriers in nonwoven fabrics, as described by the present study.

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REFERENCES

[1] G. Fisher," Medical and Hygiene Textiles: Continuing in Good Health", in Tech. Textiles Int., n. 11, v. 3, 2002. p. 10-16.
[2] Anon, "Microencapsulation: for Enhanced Textile Performance", in Perform. Apparel Mark., n. 12, 2005. p. 21-39.
[3] R. Czajka, "Development of Medical Textile Market", in Fibres Textiles East. Eur., n. 13, v. 1, 2005. p. 13-15.
[4] C.W. Kan, C.W.M Yuen "Cosmetic Textiles", in Textile Asia, n. 36, v. 6, 2005. p. 29-35.
[5] C.X. Wang, S.L. Chen, "Aromachology and its application in the textiles field", in Fibres Textiles East Eur, v. 13, n. 6, 2005. p. 41-44.
[6] S.Y. Cheng et al, "Systematic characterization of cosmetic textiles", in Textile Research Journal, n. 80, v. 6, 2010. p. 524-536.
[7] M.K. Singh, V.K. Varun, B.K. Behera, "Cosmetotextiles: State of Art", in Fibres & Textiles in Eastern Europe, n. 19, v. 4, 2011. p. 27-33.
[8] D. Bhargava, S. Jahan, "Cosmetic textile: an innovative alliance of textile and cosmetics", in China Textile Science, 2012. p. 41-45.
[9] Boh, Knez, "Microencapsulated antimicrobials on non-woven textiles for shoe insoles", XVth International Workshop on Bioencapsulation, 2007, p. 1-4.
[10] V Salem, "Tingimento Têxtil: fibras, conceitos e tecnologias", São Paulo: Blücher, 2010.
[11] J Falkowski et al, "Method for finishing textiles with skin-care oils". US 7993664 B2, Aug. 9, 2011.
[12] Abou-Okeil et al, "Wound dressing based on nonwoven viscose fabrics", in Carbohydrate Polymers, v. 90, 2012, pp. 658- 666.
[13] E.R. Assumpção, "Impregnação de diferentes tipos de tecidos de vestuário com nanopartículas de permetrina para ação repelente de insetos de longa duração", Dissertação. PPGCF, UFRGS, 2012.
[14] W.S. Rossi, "Estudo de aplicações e testes de durabilidade de microcápsulas em tecidos", Dissertação, PPGDesign, UFRGS, 2012.
[15] J.C. Forgearini, "Desenvolvimento de um spray repelente de insetos de uso têxtil contendo nanocápsulas de núcleo lipídico contendo permetrina", Dissertação, PPGCF, UFRGS, 2014.
[16] E.M. Beretta et al, "Production, characterization and application of eicosane microcapsules in polyurethane foams as a means to improve thermal comfort of wheelchair users", in Journal of Engineering Science and Technology, 2015.
[17] M.R. Alcântara, D. Daltin, "A química do processamento têxtil", in Química Nova, n. 19, v. 3, 1996. p.320-330.
[18] M.B Silva et al, "Development of natural culture media for rapid induction of Fonsecaea pedrosoi sclerotic cells in vitro", in J Clin Microbiol., n. 46, v. 11, 2008. p. 3839-3841.SILVA et al., 2008
[19] B.F. Brehm-Stecher, E.A. Johnson, "Sensitization of Staphylococcus aureus and Escherichia coli to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone", in Antimicrob Agents Chemother, n. 10. 2003. p. 3357-3360.
[20] A. Pauli, "?-Bisabolol from Chamomile - A specific ergosterol biosynthesis inhibitor?", in The International Journal of Aromatherapy, n. 16, 2006. p. 21-25.
[21] P.M. Altman, "Australian tea tree oil - a natural antisseptic", in Australian Journal of Biotechnology, n. 4, v. 3, 1989. p. 247-248.
[22] I.A. Southwell et al, "The search for optimally bioactive Australian tea tree oil", in Acta Hortic, n. 334, 1993. p. 265-275.
[23] C.F. Carson, T.V. Riley, "Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia", in Appl. Bacteriol., n. 78, 1995. p. 264-269.
[24] P, Nenoff, U.F. Haustein, W. BRANDT, "Antifungal activity of the essential oil of Melaleuca alternifolia (tea tree oil) against pathogenic fungi in vitro", in Skin Pharmacol. n. 9, v. 6, 1996. p 388-394.
[25] C.F. Carson, T.V. Riley, B.D. Cookson, "Efficacy and safety of tea tree oil as a topical antimicrobial agent", in Journal of Hospital Infection, v.40, 1998. p;175-178.
[26] J.L. Markham, "Biological activity of tea tree oil", In: I. Southwell, R. Lowe (Org.) Tea Tree, the Genus Melaleuca. Amsterdam: Harwood Academic Publishers, 1999. p. 169-190.
[27] T.A. Syed, et al, "Treatment of toenail onychomycosis with 2% butenafine and 5% Melaleuca alternifolia (tea tree) oil in cream", in Trop Med Int Health, n. 4, 1999. p. 284-287.
[28] J.N. Anderson, P.A. Fennessy, "Can tea tree (Melaleuca alternifolia) oil prevent MRSA?", in Med J Aust, n. 173, 2000.
[29] M Caelli et al, "Tea tree oil as an alternative topical decolonization agent for methicillin-resistant Staphylococcus aureus", in Journal of Hospital Infection, n. 46, 2000. p. 236-237.
[30] E. Ernst, A. Huntley, "Tea tree oil: a systematic review of randomized clinical trials", in Forsch Komplementarmed Klass Naturheilkd, n. 7, v. 1, 2000. p. 17-20.
[31] P.H. Hart et al, "Terpinen-4-ol, the main component of Melaleuca alternifolia (tea tree) oil suppresses inflammatory mediator production by activated human monocytes", in Inflamm Res, n. 2000. p. 619-626.
[32] J. May et al, "Time-kill studies of tea tree oils on clinical isolates", in J. Antimicrob. Chemother., n. 45, 2000. p. 639-643.
[33] S.Y. Zhang, D. Robertson, "A study of tea tree oil ototoxicity", in Audiology Neuro-Otology, n. 5. v. 2, 2000. p. 64-68.
[34] F.D. D'auria et al, "In vitro activity of tea tree oil against Candida albicans mycelial conversion and other pathogenic fungi", in J. Chemother, n. 13, 2001. p. 377-383.
[35] B. Oliva et al, "Antimycotic activity of Melaleuca alternifolia essential oil and its major components", in Letters in Applied Microbiology, v.37, 2003. p.185-187.
[36] C.F. Carson, K.A. Hammer, T.V. Riley, "Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties", in Clinical Microbiology Reviews, n. 1, v. 19, 2006. p. 50-62.
[37] N. Sharma, "Superiority of essential oil mouthrinse when compared with a 0.05% cetylpyridinum chloride containing mouthrinse: a six-mouth study", in Int Dent J. n. 60, v. 3, 2010. p. 175-180.
[38] M.P.V. Charles et al, "Aetiological agents of ventilator-associated

pneumonia and its resistance pattern - a threat for treatment", in Australas Med J., n. 6, v. 9, 2013. p. 430-434.
[39] L.P.S Vandenberghe et al, "Microbial Production of Citric Acid", in Braz. arch. biol. technol., n. 3, v. 42, 1999.
[40] W.B.F. Chair et al, "Final report on the safety assessment of citric acid, inorganic citrate salts, and alkyl citrate esters as used in cosmetics", in Cosmetic Ingredient Review, 2012.
[41] L.M. Colomé et al, "Theospheres based on Theobroma grandiflorum seed butter: development of innovative nanoparticles for skin application", in Soft Materials, n. 8, v. 2, 2010. p. 72-88.
[42] K. Bueno et al, "In vivo gastroprotective effect of nanoparticles: influence of chemical composition and volume fraction", in Current Pharmaceutical Design, n. 19, 2013. p. 7294-7300.
[43] Univ Northwest A & F, "Nanoemulsion composition useful for treating e.g. eczema, herpes, dermatitis or urinary infection, comprises surfactant, auxiliary surfactant, bergamot oil, patchouli oil, tea tree oil, peppermint oil and distilled water", CN102397405-A, 4 abr. 2012.
[44] Feedback Trayer SL, "Intimate feminine hygiene product for application to vaginal region, comprises red myrtle extract, bisabolol and lactic acid", ES2334746-A1, 2010.
[45] E.C.L. Beraldo, "Estudos de pré-formulação e desenvolvimento de preparações cosméticas", Monografia, Faculdade de Ciências Farmacêuticas, UNESP, 2013.
[46] Medicis Pharm Corp et al, "Composition, useful for treating skin condition including dermatosis, pruritis, psoriasis, seborrhea, contact dermatitis, rosacea, atopic dermatitis and skin inflammation, comprises flunisolide and solubilizing agent", WO2014145067-A1, 18 set. 2014.
[47] G. Svetlichny et al, "Solid lipid nanoparticles containing copaiba oil and allantoin: development and role of nanoencapsulation on the antifungal activity", in Pharmazie, n. 70, 2015. p. 155-164.
[48] A.H. Drelich, "Nonwoven Textile Fabrics (Staple Fibers)", in Encyclopedia of Chemical Technology, v. 16, 3a ed, 1981. p. 104-124.
[49] A.T. Purdy, "Developments in Nonwoven Fabrics", in Textile Progress, n. 12, The Textile Institute, 1983.
[50] I.M. Hutten, "Raw materials for nonwoven filter media", in Handbook of nonwoven filter media. Elsevier Science, 2007. p. 103-194.
[51] ABNT. "NBR-13370: Nãotecido - Terminologia". Rio de Janeiro, 2002
[52] J. Chen, S.S. Davis, "The release of diazepam from poly(hydroxybutyrate-hydroxyvalerate) microspheres", in Journal of Microencapsulation, n. 2, v. 19, 2002. p. 191-201.
[53] ANVISA. Guia de Estabilidade de Produtos Cosméticos. Brasília: ANVISA, 2004. 52 p.
[54] H. Yang et al, "New bioactive polyphenols from Theobroma grandilorum ("Capuacu")", in J Nat Prod., n. 6, 2003. p. 1501-1504.
[55] J.M. Yang, H.T. Lin, "Properties of chitosan containing PP-g-AA-g-NIPAAm bigraft nonwoven fabric for wound dressing", in Journal of Membrane Science, n. 243, 2004. p. 1-7.
[56] K.S. Chen et al, "Immobilization of chitosan gel with cross-linking reagent on PNIPAAm gel/PP nonwoven composites surface", in Materials Science and Engineering C, n. 25, 2005. p. 472-478.
[57] K.S. Chen et al, "Long term water adsorption ratio improvement of polypropylene fabric by plasma pre-treatment and graft polymerization", in Polymer Journal, n. 9, v. 38, 2006. p. 905-911.
[58] W.A. Lopes, M. Fascio, "Esquema para interpretação de espectros de substâncias orgânicas na região do infravermelho", in Quim. Nova, n. 4, v. 27, 2004. p. 670-673.
[59] B.S. Gupta, K.K Smith, "Nonwovens in Absorbent Materials", in Chatterjee & Gupta (Org.), Absorbent Technology. Elsevier. 2002. p. 349-388.
[60] B.A. Dedavid, C.I. Gomes, G. Machado, "Microscopia eletrônica de varredura: aplicações e preparação de amostras", Porto Alegre: EdiPUCRS, 2007.