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Incorporation of micro/nanoparticles of Polycaprolactone with essential oil of Cymbopogon nardus in bacterial cellulose

Aline Kringes[1], Vanusca D. Jahno[1]*, Fernando D. P. Morisso[1].

[1]*Master's Degree in Materials Technology and Industrial Processes, FEEVALE University, CEP 93525075, Novo Hamburgo, RS, Brazil.*

**Corresponding author: E-mail address: vanusca@feevale.br*

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ABSTRACT

Incorporation studies of particles in different substrates with herbal assets growing. The objective of this work was the preparation and characterization of micro/nanoparticles containing *Cymbopogon nardus* essential oil; and the incorporation of them on bacterial cellulose. For the development of the membranes was used the static culture medium and for the preparation of micro/nanoparticles was used the nanoprecipitation methodology. The incorporation of micro/nanoparticles was performed on samples of bacterial cellulose in wet and dry form. For the characterization of micro/nanoparticles were carried out analysis of SEM, zeta potential and particle size. For the verification of the incorporation of particulate matter in cellulose, analyses were conducted of SEM and FTIR. The results showed that it is possible the production and incorporation of micro/nanoparticles containing essential oil in bacterial cellulose membranes in wet form with ethanol.

Introduction

The essential oil of *Cymbopogon nardus (C. nardus)*, popularly known as citronella, can be used as insect repellent, insecticide and as, for example, larvicidal for *Aedes aegypti*[1-3]. It is also used to calm itching, muscle aches, rheumatic aches, headaches and as antiperspirant. The forms of use may be for massage, compress, bath, cosmetic care, inhalation, dissemination, on a neutral tablet or in food, and may be used for other various purposes[2].

The main chemical components of the essential oil of *C. nardus* are citronellal, geraniol and citronellol. These components have anti-inflammatory, sedative and antiviral properties. Citronella essential oil can contain different levels of the components mentioned by crop factors and planting[3,4]. On the other hand, essential oils are sensitive to the effects of light, humidity and high temperatures, in addition to the volatility. For these reasons, encapsulation is an important method for protect the active ingredients[5]. Thus, the objective of this work was the preparation and characterization of micro/nanoparticles containing *Cymbopogon nardus* essential oil and the incorporation of these particles on bacterial cellulose membranes with the intent to facilitate the dissemination of mentioned therapeutics characteristics.

Materials and methods

Preparation of micro-and nanoparticles containing citronella essential oil

Micro and nanoparticles were prepared in triplicate with 40% essential oil of *C. nardus* (WNF), and were kept under exhaustion during 4:00 with constant magnetic stirring for evaporation of acetone (Quimis PA), which was used as organic phase in the nanoprecipitation method. The organic phase was obtained by dissolution of 0,115g of polycaprolactone (PCL, Sigma-Aldrich, 45,000 Mw g/mol), 0,0546g of Span® 80 surfactant (Sigma-Aldrich, Mw: 428,62 g/mol) and 0,020g of citronela oil in 30 mL of acetone by magnetic stirring under temperature of about 30°C. In turn, the aqueous phase was prepared with approximately 50 mL of distilled water and 0.08g surfactant Tween ® 80 (Sigma-Aldrich, Average Micellar Weight 79.000) also by magnetic stirring under approximately 30°C. After the two solutions (organic and aqueous phase) reached the same temperature of about 30°C, the organic phase was added drop by drop with Pasteur pipette to the aqueous phase under agitation provided by ultra-turrax (20,500 rpm). At the finish of the addition of organic phase on the aqueous phase, the newly formed dispersion was lead to the acetone evaporation during two different

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times under magnetic stirring, 4 and 24 hours. Then, it was stored in amber bottles and stored away from light, at ambient temperature of about 25°C.

Incorporation of particles containing Citronella essential oil in the membranes of bacterial cellulose

The incorporation of micro/nanoparticles was performed in triplicate for each type of in bacterial cellulose (BC), dry and wet form. BC was prepared according to the method described recently[6]. The samples of BC, receipt the amount of dispersion of micro/nanoparticle containing citronella oil and impregnation aid agent shown in Table 1.

The dry BC was obtained starting from the wet BC after this being dried for 48h at 25°C. Each formulation shown in Table 1 was prepared with 3.0 x 3.0 cm samples of BC. The Petry dishes containing the BC membranes impregnated with 3 mL of micro/nanoparticles dispersion with or without impregnation aid agent were left for 72 hours in ambient temperature until complete drying by natural evaporation of water coming from the dispersions.

The zeta potential (ZP) was observed by Phase Analysis Light Sctattering (PALS). The particle size was observed by Dynamic Light Scattering (DLS). Polydispersion (PDI) was a consequence of observation of DLS. These tree properties were measured in a NanoBrook equipment, model 90 Plus/Pals. The analyses were performed in triplicate at 25°C in polystyrene cuvettes with 1 cm of optical path and volume of 4.5 mL. The light scattering was observed with an angle of 90°. As the analyses were performed in triplicate, the results are expressed as simple average and the standard deviation. The standard deviation was calculated by standard procedure.

Scanning electron microscopy (SEM) images were obtained in a Field Emission Scanning Electron Microscope JEOL JSM-6510L. Samples were coated with tick gold layer following the standard procedure.

The infrared spectra were obtained in a Perkin Elmer Spectrum Two Spectrometer, with Universal ATR accessory (UATR), in the range of 4000 to 450 cm^{-1} with resolution of 32 cm^{-1} and 4 scans per spectrum.

Acronym	Formulation	Incorporation
BCD-3	1	Dry bacterial cellulose with 3 mL dispersion
BCM-3	2	Moist bacterial cellulose with 3 mL dispersion
BCD-33	3	Dry bacterial cellulose with 3 mL dispersion + 3 mL of ethyl alcohol
BCM-33	4	Moist bacterial cellulose with 3 mL dispersion + 3 mL of ethyl alcohol
BCD-12	5	Dry bacterial cellulose with 1 mL dispersion + 2 mL of purified water
BCM-12	6	Moist bacterial cellulose with 1 mL dispersion +2 mL of purified water

Table 1 - Description of the content of each sample of BC with incorporation of micro/nanoparticles.

Results and Discussion

Characterization of micro/nanoparticles

In order to check the morphology of the micro/nanoparticles prepared in this work, micrographs from dry material were obtained. Figure 1 presents micrographs of three dispersions of micro/nanoparticles with citronella essential oil formulations (a, b and c), with 4 hours of solvent evaporation, which shows high similarities. Figure 1 denotes the formation of micro/nanoparticles in all three formulations, most of them exhibiting spherical and uniform shapes. In these micrographs, also could be seen that the size of the particles features inhomogeneity points.

This inhomogeneity could be related to the organic phase droplet size dispensed into the aqueous phase[7], which was controlled manually. Thus, these differences in the size (nano and micro) of particles were expected. It is worth to note that the solvent evaporation time apparently does not affect significantly the shape of the particles, as can be seen in the Figure 2.

Figure 2 presents micrographs of three dispersions of micro/nanoparticles with citronella essential oil formulations (d, e and f), with 24 hours of solvent evaporation which, also, shows high similarities.

The particle size may vary depending on the amount of oil in relation to the polymer and, in some cases, the same can also occur with the rise of oil/polymer ratio

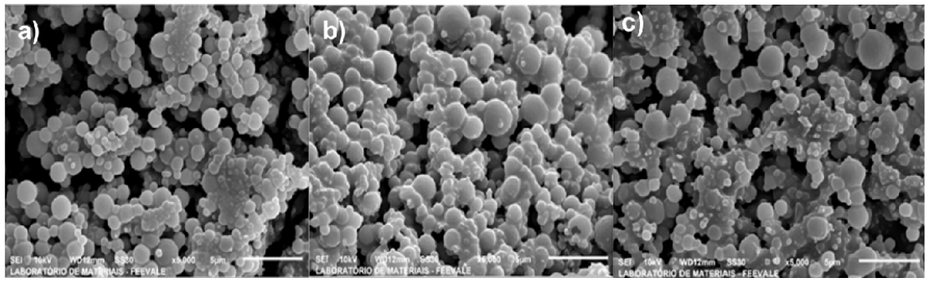

Figure 1 - Micrographs of micro/nanoparticles formulations (a), (b) and (c), with 24 hours of solvent evaporation (5000 x).

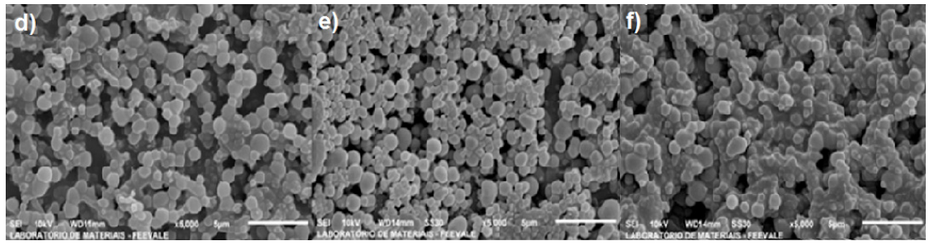

Figure 2 - Micrographs of micro/nanoparticles formulations (d), (e) and (f), with 24 hours of solvent evaporation (5000 x).

in the organic phase. This correlation could be a factor responsible to increase the resistance to diffusion of the organic phase into the aqueous phase, allowing a greater association of the active/oil in the nanoparticles[8,9], but the huge difference, nano and micro, could not be attibuted to a factor like this. On the other side, it is well known that the measures of size performed by SEM morphology analysis always exhibit sizes greater than those measured by DLS[10]. In this sense, the difference in particle size by DLS and by SEM can be related to the fact that the preparation of the latter requires a drying procedure and sample preparation prior to analysis, which are conducted, as well as own analysis, under high vacuum. This vacuum exposure during the preparation and analysis should be responsible for enforcing essential oil volatilization and consequently, dilatation of the particles[11].

Thus the quantitative analises of particle size by DLS should provides better results. The particle size and standard deviation of each of the six formulation, measured by DLS, are presented in Figure 3.

Figure 3 shows the particles sizes of the formulations *a* to *f*. Samples *a, b* and *c* were submitted to 4 hours of solvent evaporation, while samples *d*, *e* and *f* were submitted to 24 hours of solvent evaporation.

This result suggests that a factor such as the solvent evaporation time at room temperature could be responsible for the difference in the size of these particles, since all six formulations were prepared with almost the same oil / polymer ratio, 1/5, 1 / 4 and 2/5, respectively, considering the pairs a/d, b/e and c/f. The particle size corroborates the literature[11], which mention that whichever method is adopted to prepare polimeric nanoparticles, generally the size of the particles varies between 100 and 300 nm.

It is worth mention that in some cases, the size of particle may be less than the minimum limit of the described range due to choice of oil, which can modify the characteristics of viscosity and hydrophobicity among other aspects[11]. In this study, neither of the two characteristics was evaluated because just the citronella essential oil was used.

Associated to the particle size measures, the PDI values of each formulation are presented in Figure 4.

The PDI values shown in Figure 4 to all six formulations, are close to 0.3, which represents a moderate and relative homogeneity in the distribution of particle size. Table 2 presents the values of ZP of micro/nanoparticle formulations.

Table 2 shows the values of ZP for the formulation a to f. It could be seen that formulations a to c presents ZP near to the -15 mV, while formulation d to f, around the -10 mV, with some deviation of this value for formulations d and e. According to the literaure, the higher the value of the ZP (less negative) the greater the amount of particle greater aggregation trend[12,13]. In this respect, nanoparticles with ZP above ± 30 mV are stable suspensions which prevents the aggregation of nanoparticles[14].

Characterization of bacterial cellulose membranes containing micro/nanoparticles with essential oil of C. nardus

Figure 5 shows the micrographs of the bacterial cellulose membrane impregnated with micro/nanoparticles of PCL containing citronella essential oil.

The micrographs in Figure 5, from dry and wet cellulose, does not show the bacterial cellulose fibers, except in the sample BCM-33, but with 11,000 times of magnification,

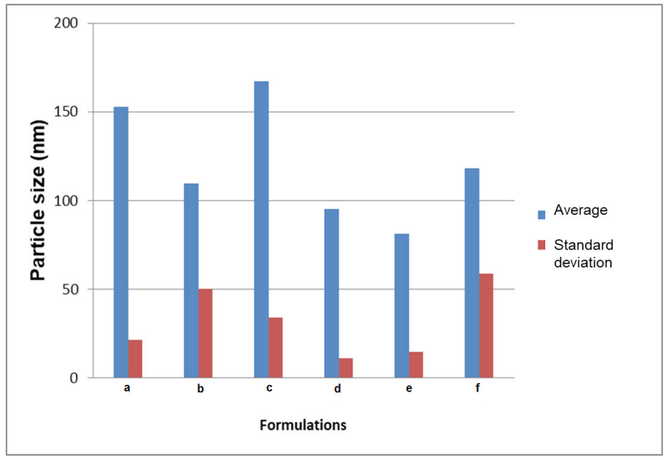

Figure 3 - Particle size of the formulations *a* to *f* measured by DLS.

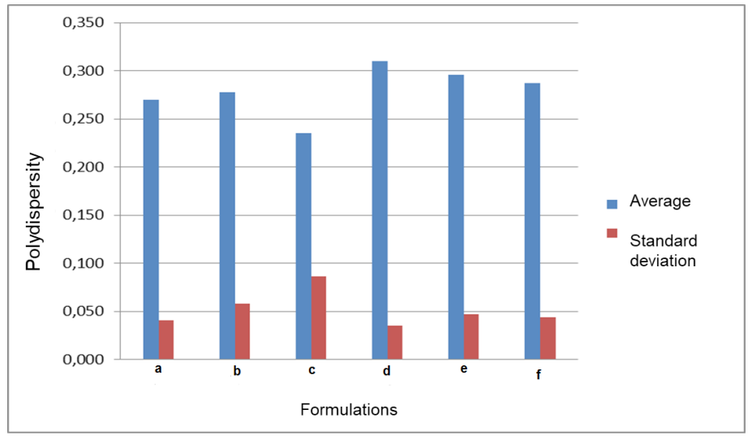

Figure 4 - Polydispersity (PDI) of the formulations a to f measured by DLS.

PZ (mV)		
Formulation	**Average**	**Standard Deviation**
a	-13,940	9,74
B	-14,710	6,76
C	-14,940	5,06
D	-8,71	7,69
E	-7,18	8,98
F	-12,31	8,94

Table 2 - Zeta potential (PZ) of 1, 2, 3 formulations, 4, 5 and 6.

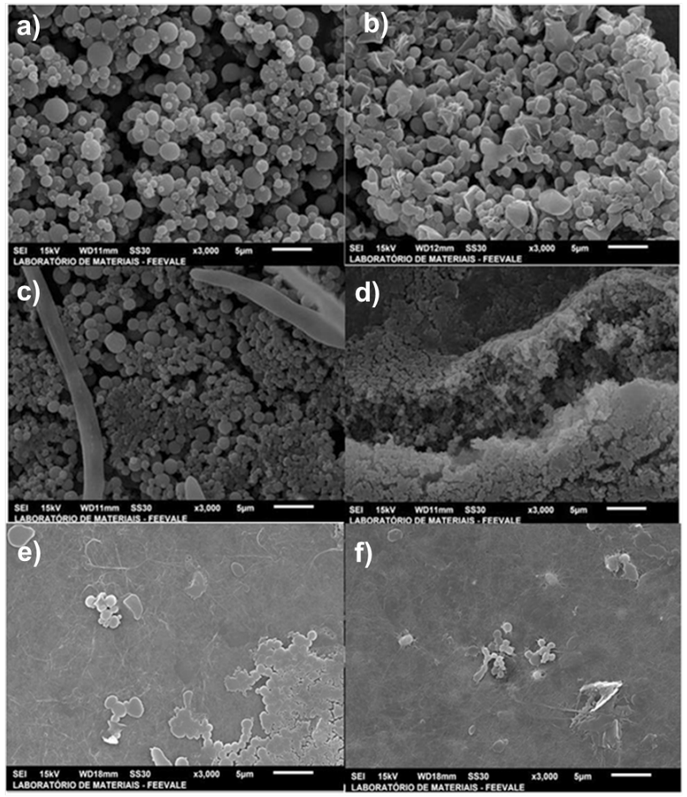

Figure 5 - Micrograph (a) BCD-3 (b) BCM-3 (c) BCD-33, (d) BCM-33, BCD-12 (e) and (f) BCM-12, 3000 x.

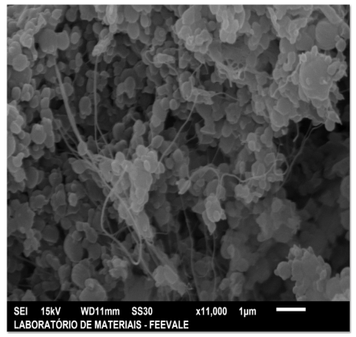

Figure 6 - Micrograph of sample BCM-33 (11,000 x).

as in Figure 6, possible to verify the presence of filaments, suggesting be CB fibers with micro/nanoparticles.

To analyze the presence of fibers between the micro/nanoparticles, Figure 7 shows the cross-section of the BCD-3 samples, BCM-3, BCD-33, BCM-33, BCD-12 and BCM-12 with magnification of 11,000 x.

In Figure 7, is possible to observe the deposition of micro/nanoparticles on the surface of the membranes (a) BCD-3, (b) BCM-3, (c) BCD33, (e) BCD-12 and (f) BCM-12. The cross section images, do not make clear the presence of micro/nanoparticles in the middle of the membrane fibers. Already in the sample (d) BCM-33 is possible to noted the presence of micro/nanoparticles between the fibers of the BC. In this case, the moist membranes allow more easily a deposition and, apparently, less locally thick, giving the impression of absorption of the dispersions.

PCL is a hydrophobic polymer that has application in preparation of hydrophilic polymer composites as BC[14]. The BC has great affinity with polar solvents like water, and lends itself to the preparation of composites, with, for example, PCL. It is suggested, therefore, that the absence of micro/nanoparticles in the BCD-3 samples, BCM-3, BCD33, BCD-12 and BCM-12 is not connected

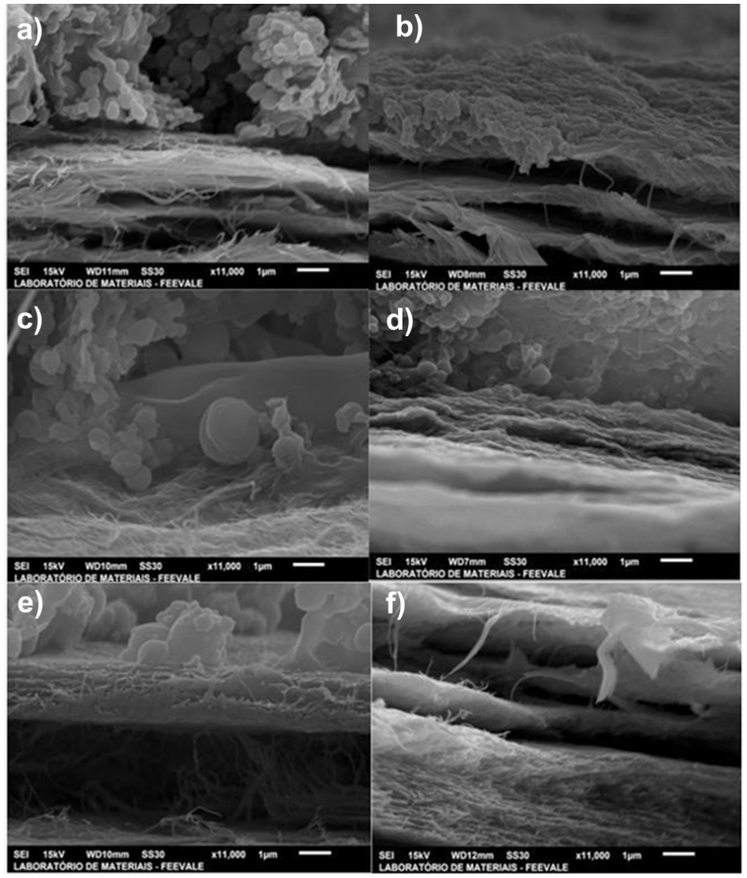

Figure 7 - Micrograph Cross (a) BCD-3 (b) BCM-3 (c) BCD-33, (d) BCM-33, (e) BCD-12 and (f) BCM-12, 11000 X.

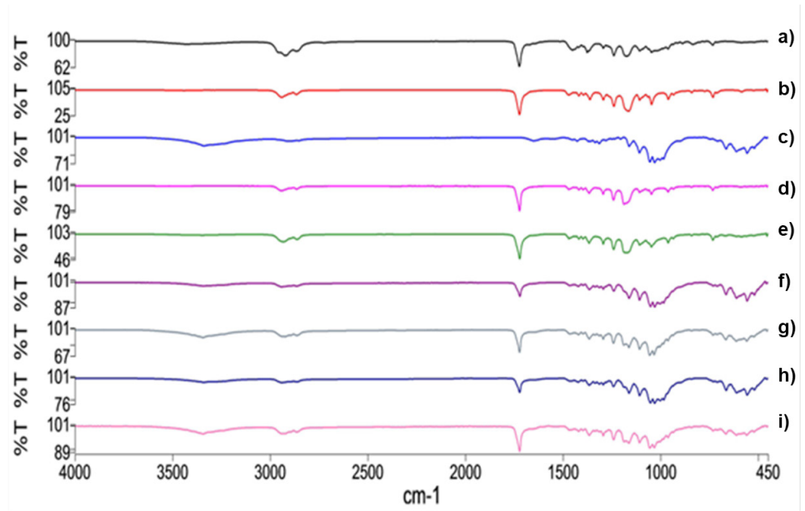

Figure 8 - Infrared spectra: (a) essential oil, (b) PCL, (c) BC, (d) BCD-3 (e) BCM-3 (f) BCD-33 (g) BCM-33, BCD-12 (h) and (i) BCM-12.

of BC. This formulation impregnated on BC in wet form using impregnation aid agent ethanol was the sample that presented the most satisfactory result, micro/nanoparticles between the fibers of the BC.

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