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PRODUCTION AND CHARACTERIZATION OF HYDROGEL WITH CHIA SEED OIL-LOADED NANOCAPSULES[1]

PRODUÇÃO E CARACTERIZAÇÃO DE GEL HIDROFÍLICO CONTENDO NANOCÁPSULAS DE ÓLEO DE SEMENTE DE CHIA

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ABSTRACT

The present work aims to develop and characterize a hydrophilic gel containing nanocapsules within chia oil for topical application of natural products as a promising alternative to prevent and treat wound. The chia oil-nanocapsules (NCCO) suspension was developmented and a hydrogel formulation was produced. The gel was tested for characterization and stability for 90. Toxicity tests were carried out for NCCO, chia oil in natura and free chia oil-nanocapsules in murine B16F10 skin melanoma cells. The NCCO showed a particle diameter of 246.23 ± 1.53 nm and a polydispersity index of 0.148 ± 0.08. The nanocapsules within hydrogel formulation displayed pH 4.80 ± 0.07, inicial viscosity 14960 ± 426.66 9 cP and spreadability 4599.05 ± 1304.27 mm². The hydrogel under low temperature (± 4ºC) maitained stability for 90 days without changes on odor, color and visual aspect. The NCCO suspension was not able to induce cytotoxicity in tumoral lineage cells. After all, the conclusion is that the hydrogel containing NCCO showed feasibility to be applied dermatologically on skin care.

Keywords: essential fatty acids, nanotechnology, prevention, treatment, wounds.

RESUMO

O presente trabalho objetiva desenvolver e caracterizar um gel hidrofílico para servir como veículo tópico para nanopartículas contendo óleo *de chia, visando uma aplicação tópica na prevenção e tratamento de feridas. Para isso, foram desenvolvidas suspensões com nanocápsulas contendo* óleo *de chia (NCOC), e após estas foram incorporadas em gel hidrofílico, onde foi realizado testes de caracterização e estabilidade do gel por 90 dias. Foram realizados testes de citotoxicidade com as suspensões de NCOC* óleo *de chia in natura e nanocápsulas brancas, utilizando linhagens de células de melanoma murino B16F10. O diâmetro médio das nanocápsulas contendo* óleo *de chia foi de 246 ± 1,53 nm e* índice *de polidispersão de 0,148 ± 0,08. O gel com as NCOC apresentou um pH inicial de 4,80 ± 0,07, viscosidade inicial de 14960.00 ± 426,66 9 cP e espalhabilidade inicial de 4599,053 ± 1304,27 mm². Os géis mantidos em baixa temperatura* (± 4ºC) *permaneceram estáveis durante todo o experimento, não apresentando qualquer alteração de odor, coloração e aspecto. Nos ensaios de citotoxidade todas as concentrações foram iguais ao controle de sobrevivência. Desta forma, conclui-se o hidrogel com NCOC apresentou potencialidade para aplicações dermatológicas no cuidado à pele.*

Palavras-chave: *ácidos graxos essenciais, nanotecnologia, prevenção, tratamento, feridas.*

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INTRODUCTION

The treatment of wounds involves a wide therapeutic arsenal of synthetic substances. The development of new technologies aimed at accelerating the cicatricial process, as well as reducing health skin complications absolutely necessary (FRANCO; GONÇALVES, 2008).

According the World Health Organization (WHO), herbal medicines have been used to improve health and to treat pathological symptoms (WHO, 2014). The use of natural medicine has been highlighted due to the absence or reduction of side effects (NASRI; SHIRZAD, 2013). In this context, it is observed the resumption of therapeutic practices sometimes considered as popular or "non-scientific", which include the use of medicinal herbs and natural products as an alternative or therapeutic complement. Herbal medicine has been used and assayed to treat wounds with promising results, as: *Aloe vera*, *Carica papaya L*., *Calendula officinalis*, *Stryphnodendrom adstrigens*, among others (GARROS et al., 2006; PIRIZ et al., 2014).

Chia (*Salvia hispânica L*.) is a plant that is native in Mexico. It has seeds with special properties used to treat skin lesion, mainly due to its composition rich in oil containing unsaturated fatty acids (omega 3 and 6) (IXTAINA et al., 2011; TIMILSENA et al., 2016). In clinical practice, it has been investigated the use of products based on fatty acids, in which essential polyunsaturated fatty acids (PUFAs) alpha-linoleic (ALA, 18:2, omega 3) and linolenic (18:3) acids are the most important (FERREIRA; GAMA; VILANOVA, 2013; TIMILSENA et al., 2017). These PUFAs are precursors of bioactive long-chain fatty acids (LCPUFAs) as arachidonic, eicosapentaenoic and docosahexaenoic acids which may acts as regulators of physiological functions of the skin such as inflammatory responses, platelet aggregation, vasodilation, and cell proliferation (MACHADO et al., 2017).

Nowadays, various products and technologies have been studied aiming to improve time and quality of wound healing. Nanostructured formulations may be developed to provide the basis for innovation in new applications and products, especially in the area of health (LOURO; BORGES; SILVA, 2013). Nanoproducts have reduced size with new physical, chemical and biological properties that do not exist in bigger sizes. This is the reason why it can be used in innovative therapies (DEVI; BHIMBA, 2012). When natural oil is introduced to nanoformulations in hydrogel (nanogel), it provides the benefits of protection to the oxidative degradation, biocompatibility, versatility, hydrophilicity and other ones conferred to nanoparticles (HAMIDI et al., 2008). Additing all theses properties, nanogels may have potent skin permeation profiles and high drug-loading capacity for both transdermal and topical drug delivery systems (CHOUDHURY et al., 2017).

In this study, NCCO was introduced to a hydrogel matrix and characterized to a topical system of PUFAs delivery into the skin. For this, cytotoxicity in melanoma tumoral lineage cells were evaluated.

MATERIAL AND METHODS

MATERIALS

Chia seed oil was purchased from Pazzer®. Pluronic® F-127, polysorbate 80, sorbitan monooleate, MTT and DMEM were purchased from Sigma Aldrich. Ethanol was purchased from Synth and cetyl palmitate was purchased from Volp. Carbopol Ultrez 10 NF and Cosmoguard® were obtained from Nova Derme and trietanolamina was purchased from Via Farma.

METHODOLOGY

Production and characterization of suspensions of chia seed oil-loaded nanocapsules

NCCO suspensions were prepared by interfacial deposition of preformed polymer method (BALEST, 2013). Briefly, an organic solution composed of chia seed oil (0.25 g), cetyl palmitate (0.191 g), sorbitan monooleate (0.775 g), the polymer Pluronic® F-127 (0.25 g) and ethanol (66.75 mL) was added to an aqueous solution containing polysorbate 80 (0.191g) and ultrapure water (133.25 mL) and kept under magnetic stirring for 10 min. Then, ethanol and excess of water were removed by rotary evaporator at 60 rpm and 40°C under reduced pressure to the final volume of 25 mL. Nanoformulations were prepared in triplicate and the physicochemical analysis were evaluated along 120 days, at different temperatures: 4 ± 2 °C (samples kept in refrigerator), 25 ± 2 °C (samples kept in room temperature) and 40 ± 2 ºC (samples kept in a climatic chamber). The analyzes were performed at the day of production (t = 0) and at 7, 15, 30, 60, 90 and 120 days. The free chia oil-nanocapsules were produced in the same way, but chia oil was replaced in the same proportions on the formulation by Crodamol® GTCC (caprylic and capric acid triglycerides).

The basic physicochemical characteristics evaluated were pH, particle sizes and polydispersity indices. The NCCO formulation was diluted in purified water to measure particle sizes and polydispersity indices by photon correlation spectroscopy (3 measures/batch; 2 runs of 30 s/measure at 25 ◦C) (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK). The size of the particles was measured by laser diffraction (Microtrac®) and pH was performed in NCCO suspension. The zeta potential values were determined (3 measures/batch; 10 runs/measure at 25 ◦C) after dilution (1:500) of the suspensions in 1 mM NaCl (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK). All measures were performed at room temperature.

Preparation and characterization of hydrogel containing chia seed oil-nanocapsules

Carbopol® Ultrez 10 NF (polymer of acrylic acid) at 0.5% (w/w) was dispersed in the suspensions containing NCCO resulting in a concentration of 10 mg/mL (OURIQUE et al., 2011). The polymeric dispersion was neutralized with triethanolamine (0.2%, w/w) to a suitable pH (≈ 5.0) to obtain a proper consistency of the hydrogel and pH applicable to the skin. Methyl bromoglutaronitrile and phenoxyethanol (Cosmoguard®) were added as a preservative (0.1%, w/w). Based on these same criteria, hydrogels using free chia oil-nanocapsules were also tested. The physicochemical characterization of the hydrogel formulation was evaluated as pH, viscosity and spreadability were evaluated. All measures were performed at room temperature.

Determination of pH

The pH was measured using a calibrated potentiometer (Digimed®). The measurements were carried out by immersing the electrode directly into the semi-solid formulations. The results were expressed as the mean of three measurements from different batches.

Determination of viscosity

The viscosity measures of the semisolid formulations were determined using a rotational viscometer (RV DV-1+, Brookfield®). The measures were performed at the speed of 100 rpm using spindle 07, placed directly in the semi-solid formulations. The results were expressed as mean of cP (centipoise) three formulation batches.

Determination of spreadability

The spreadability measures were performed in hydrogel samples at the day of production (t = 0) and at 7, 15, 30, 60 and 90 days, in triplicate, according to the methodology described by De Paula et al. (1996). This method uses a circular mold plate of glass (diameter = 20 cm, width = 0.2 cm) with a central hole of 1.2 cm diameter, which is placed on a glass support plate (20 cm x 20 cm) positioned over some millimetric graph paper. Each sample was introduced into the hole of the die plate and the surface leveled with a spatula. The plaque mold was carefully removed and a glass plate of known weight was placed over the sample. After one minute, the diameter in opposing positions (as covered by the sample) was read with the aid of the graph paper scale. Subsequently, the diameter was calculated. This procedure was repeated successively adding other plates in one-minute intervals. The results were

expressed as spreadability of the sample due to the applied weight, according to the equation below, which corresponds to the mean of three determinations.

$$Ei = (d^2.\pi)/4 \quad (1)$$

where: Ei = spreadability of the sample weight for a given i ($mm^{2)}$; d = diameter (mm).

Determination of organoleptic characteristics

The organoleptic characteristics evaluated were odor, visual macroscopic aspects, precipitation, turbidity, phase separation and the color change, as recommended by Brazilian Health Regulatory Agency (ANVISA) (BRASIL, 2008). The results were expressed as mean of three determinations.

Gel stability

All nanoformulations were prepared in triplicate and stored protected from light and temperature at 4 ± 2 ° C (samples kept in refrigerator), 25 ± 2 ° C (samples kept in room temperature) and 40 ± 2 ºC (samples kept in a climatic chamber). The analyzes were performed on the day of production (t = 0) and at 7, 15, 30, 60, 90 days. The parameters pH, viscosity, spreadability and organoleptic characteristics were measured.

Cell viability assay

In vitro cytotoxicity tests were performed on murine B16F10 skin melanoma cells (ATCC CRL-6475) analyzed by reducing MTT. Melanocytic cells were cultured in DMEM medium with 10% fetal bovine serum, penicillin and streptomycin (100 IU/50 µl). The cells were grown as monolayers in tissue culture flasks at 37°C under 5% CO^2 /95% O^2. The cells were released from the inner surface of the culture flask, centrifuged for 5 min at 1,100 rpm. After centrifugation, the supernatant was discarded and the cells were resuspended in DMEM and counted in Neubauer's chamber. The cells were seeded at the density 1 x 10^4 cells/well in 96-well culture dishes. Twenty-four hours after seeding, the cells were adhered and incubated with the NCCO, free chia oil-nanocapsules suspensions or chia oil *in natura*. All determinations were performed in triplicate.

A stock solution was prepared with 100 µL of the free chia oil-nanocapsule suspension and another with NCCO and 900 µL of DMEM/10% fetal bovine serum. From this solution, dilutions were prepared for toxicity analysis in cells: 10, 20, 30, 50, 70 or 90 µg/mL. To measure the effect of free chia oil-nanocapsules, the cells were incubated with the volume equivalent to 90 µg/mL NCCO.

To measure the effect of chia oil *in natura*, an oil solution was prepared with 10 mg chia oil solubilized in 1 mL ethanol (0.01%). Also, the effect of ethanol 0.01% was evaluated in the cells.

Some cells were incubated with solutions for 24 or 48 hours. To induce death, some cells were incubated with 100 mM hydrogen peroxide (H_2O_2). The viable control cells were incubated with DMEM/10% fetal bovine. After the cell incubation period, formazan was measured by the colorimetric method proposed by Mosmann (1983).

Melanocytic cells were incubated with 20 µl of MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide), the culture environment was homogenised and the cells were incubated again in an oven for 4 hours. After the incubation period, each 0.5 mg/mL MTT of dye was added to the cells. Insoluble formazan produced after 4h was dissolved with dimethyl sulfoxide (DMSO) and purple color detected at 570 nm in micro-plate reader (Fisher Bio-Tek BT2000). The arbitrary values of optical density corresponding to cell viability was expressed as mean of measures from independent experiments.

Statistical analysis

All formulations were prepared and analyzed at least in triplicate. The results were expressed as mean and standard deviation (SD). The data were pre-treated with the Shapiro-Willk method to define the normal distribution. A statistical analysis for parametric data was performed by one-way ANOVA followed by Dunnett's post hoc. It was considered $p < 0.05$ as statistically significant. Data were analyzed using GraphPad Prism® software (version 7.0).

RESULTS AND DISCUSSION

PRODUCTION OF NANOCAPSULES CONTAINING CHIA SEED OIL

The NCCO, exhibited a milky appearance, macroscopically homogeneous, with opalescent bluish aspect resulting from the *Tyndall* effect that is characteristic of nanometrics particles (MORA-HUERTAS; FESSI; ELAISSARI, 2010).

The analysis of the size of particles by the refractometer showed that the NCCO showed a size of 231 ± 0.02 nm (Table 1). It should be noted that there was only one population of particles, indicating that the production of nanoparticles formulation was adequate.

The technique of photon correlation spectroscopy, also known as dynamic light scattering, the mean diameter of the NCCO was 246 ± 1.53 nm and polidispersity 0.148 ± 0.08 (Table 1).

The NCCO showed an unimodal distribution and 0.25 IPD, which indicates homogeneity of the particles, an important property to ensure homogeneity of the diameter of particles and it is an

indicator of the stability of the formulation when values are in the range from 0.15 to 0.3 (MOHANRAJ; CHEN, 2006). The present results were also found by the two techniques of analysis of particle size (laser diffraction and photon correlation spectroscopy).

Faria (2014) showed that the characterization of nanocapsules containing oil of baru (*Dypterix alata vog*) showed a mean particle diameter of 396.3 nm. The polymer used was the poly(Ɛ-caprolactone) and the method was the precipitation of the polymer pre-formed, similar to the present study. According the author, the concentration of the active or the oil in that it will be diluted can influence the size of the particle.

The pH of the suspensions is another important characteristic to be evaluated since its variation over time is an important factor to predict the stability of the formulations (KISHORE et al., 2011). The initial pH was 6.51 ± 0.12 similar to the values of free chia oil-nanocapsules (Table 1), demonstrating that chia oil did not show effect on this parameter of nanoformulation. The pH close to neutrality are adequate to topical administration of active, since skin pH range 4 to 7 (ALVES et al., 2007).

Table 1 - Chemical physical analysis of NCCO and NCFO.

Formulation	Particles size (nm) in laser diffraction	Particles size (nm) in photon correlation spectroscopy	PDI	Zeta potencia (mV)	pH
NCCO	231 ± 0.02	246 ± 1.53	0.148 ± 0.08	-4.31 ± 1.54	6.51 ± 0.12
NCFO	198 ± 0.01	205 ± 2.2	0.08 ± 0.01	-3.84 ± 0.05	6.40 ± 0.09

PDI: polydispersity index; NCCO: chia oil-nanocapsules; NCOF: free chia oil-nanocapsules

Regarding the preliminary stability studies, the NCCO formulation maintained at heated temperature (± 40ºC) showed a change in color and rancid odor after 30 days of analysis, characteristic of formulations prepared with oil. The samples maintained at ± 4ºC or ± 25ºC remained without visual alterations during 120 days.

During the study of thermal stability during 120 days, it was observed that the best conditions of storage for the NCCO suspensions retain their physicochemical characteristics in relation to the initial value, which was the temperature of ± 4°C. Up to 60 days, the suspensions remained the same values to the initial, after that there was a significant variation in pH. There was an increase in the acidity of 6.49 ± 0.18 (60 days) to 5.30 ± 1.00 (120 days), however, this value is still suitable for topical applications.

PRODUCTION OF HYDROPHILIC GEL WITH NCCO

The gel with NCCO initially presented a pleasant odor, white coloration (due to the milky appearance of suspensions of nanocapsules) and a characteristic aspect of gel (Figure 1).

Figure 1 - Macroscopic aspect of the hydrogel containing NCCO.

Representative photography of the hydrogel prepared with nanocapsules.

CHARACTERIZATION HYDROGEL WITH CHIA OIL-NANOCAPSULES

The hydrogel containing NCCO presented an initial pH of 4.80 ± 0.07, initial viscosity of 14960.00 ± 426.669 cP and initial spreadability 4599.05 ± 1304.27 mm².

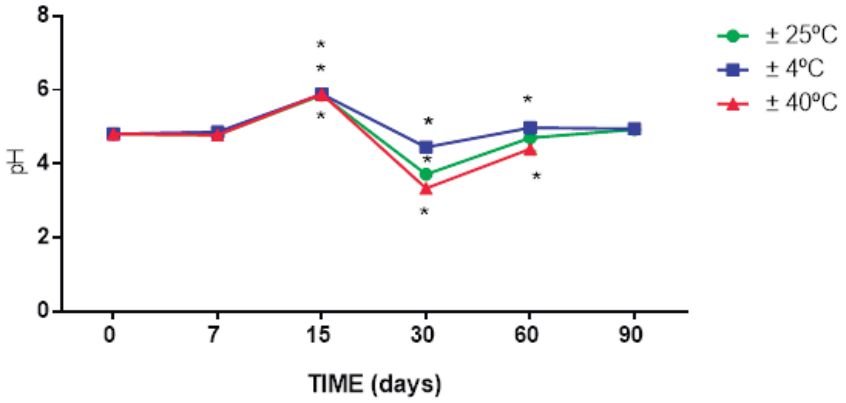
These characteristics are in agreement with the polymers carbomer (Carbopol®), which are gelling agents that offer numerous advantages such as: high viscosity in low concentrations; wide range of viscosity; compatibility with many active ingredients; bioadhesive properties; good thermal stability; sensory characteristics, excellent and good topical acceptance. The gels of Carbopol® are prepared from the polymer dispersion in water, where it can increase by up to a thousand times in the original volume (HURLER et al., 2012).

STABILITY OF THE GEL

To determine the stability of the nanogel triplicate, some samples were assayed. Each sample was divided into three bottles that were maintained at different temperatures: ± 4ºC ± 25ºC and ± 40ºC for 90 days. For the tests, samples were withdrawn before the storage conditions and left to rest until they reach the room temperature (±25ºC).

The gel produced with NCCO lost its stability when stored at ± 40ºC during 60 days. Also, nanogel showed without viscosity, became liquid with rancid odor and color slightly yellowish, evoking interruption of study in this temperature. The initial pH of the nanogel was 4.80 ± 0.07. The gels maintained at room temperature (± 25°C) showed a significant variation at 15 and 30 days, where the pH value changed to 5.86 ± 0.08 and 3.71 ± 0.08, respectively, but the pH of nanogel reduced under ± 4°C to final value 4.97 ± 0.07 after 60 days. Also, at heated temperature (± 40°C) pH reduced to 4.4 ± 0.13 after 60 days (Figure 2).

Figure 2 - Effect of temperature on pH of hydrogel with NCCO.

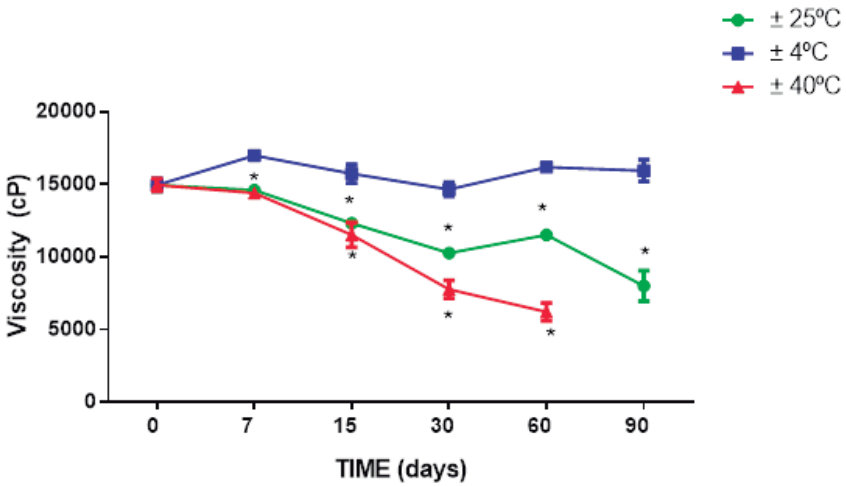


The pH values of nanogel exposed to different temperatures during 90 days.
*p < 0.05 compared to the initial pH.

The decrease on pH from nanogel can be related to the degradation of polymers that constitute the hydrogel, influencing the parameters spreadability and viscosity in the gel (OLIVEIRA, 2009). Aulton (2005), observed that stratum corneum is considerably resistant to changes in pH, tolerating a variation of 3 to 9. According to the author, the pH values between 5 and 7 in topical formulations avoid possible skin irritation. Thus, the gel with NCCO is in agreement with the pH values indicated for topical system to drug delivery.

In relation to the viscosity, the initial value of the nanogel was 14960 ± 560 cP. The gel stored at ± 4ºC did not alter its viscosity throughout the analysis period. The nanogel samples maintained at ± 25ºC or ± 40ºC showed significant decrease in viscosity after 15 days (Figure 3).

Figure 3 - Effect of temperature on viscosity of hydrogel with NCCO.



The viscosity measures of nanogel exposed to different temperatures during 90 days.
*p < 0.05 compared to the initial value.

The nanogel did not show changes in the viscosity parameter when exposed to ± 4ºC, but showed a significant reduction during the first week when exposed to ± 25ºC or ± 40ºC (Figure 3).

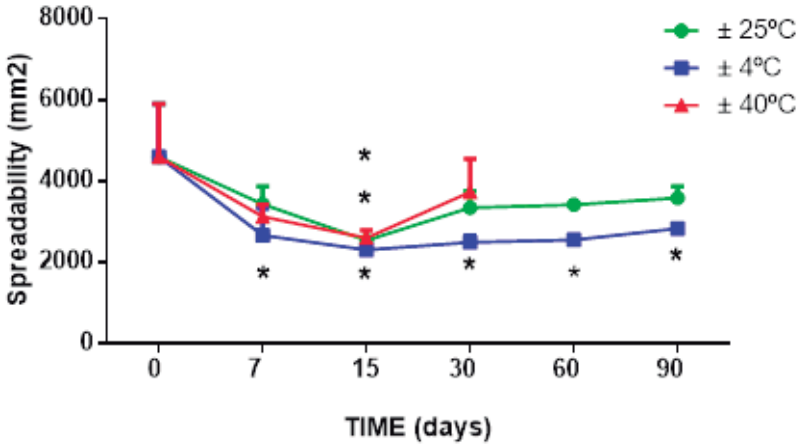
It is important to note that, neutral pH is related to higher values of viscosity (WELIN-BERGER; NEELISSEN; BERGENSTAHL, 2001) which is related to increment active release rates, increasing transdermal absorption (DI GIUSEPPE et al., 2015).

Although, the determination of viscosity is an appropriate criterion to evaluate the formulations stability for topical application, its use in stability studies is not related with absolute values of viscosity, but with the evaluation of this property along the time (OLIVEIRA, 2009).

Pillai and Panchagnula (2003) conducted in vitro studies of physicochemical stability of gel using Pluronic® as the carrier of insulin, and this formulation was unchanged in 45 days. When stored under refrigeration, the content of insulin was ≥ 98% up to 30 days and reduced to 89% after 60 days. The Pluronic® is a good carrier for delivery and can avoid degradation of proteins, preserving their biological activity. Kant et al. (2014) conducted a study in Wistar rats aiming verify the wounds healing using only Pluronic® F-127 in gel for topical application. The animals showed an increase in the contraction on wound border, improvement of the tissue granulation following early maturation of tissue. Thus, due to properties of the Pluronic®, the healing process is more effective when compared to the wounds treated with a saline solution.

The spreadability is a parameter of hydrogel defined as the expansion of a semisolid formulation on the surface along the time. It is an essential characteristic of semisolid formulations for topical use, because spreadability is related to its local of application and desired action of gel (ZANIN et al., 2001). The spreadability of nanogel was altered when exposed to different temperatures. The stability study demonstrated that the nanogel stored at ± 25°C or ± 40°C had spreadability reduced after 15 days; the last sample was discarded after 30 days because it became liquid with a rancid odor. The gel maintained at ± 4ºC had the measures reduced for all periods (Figure 4). In relation to the organoleptic characteristics, the gels produced with NCCO kept on room temperature during 60 days showed their principal characteristics preserved: pleasant odor, white coloration and physical aspect of gel.

Figure 4 - Effect of temperature on spreadability of hydrogel with NCCO.



The spreadability values of nanogel exposed to different temperatures during 90 days.
*p < 0.05 compared to the initial value.

The hydrophilic gels, as Carbopol®, have been widely used as dermatological bases, due its properties, besides less greasy, easy spreadability and can carrier different bioactive compounds (CORRÊA et al., 2005). Probably Carbopol® contributes to preservation of spreadability in hydrogels containing nanoparticles, because when dexamethasone-loaded nanocapsules were added to gel was observed that the presence of nanoparticles did not change the spreadability when compared with gel containing the drug in free form.

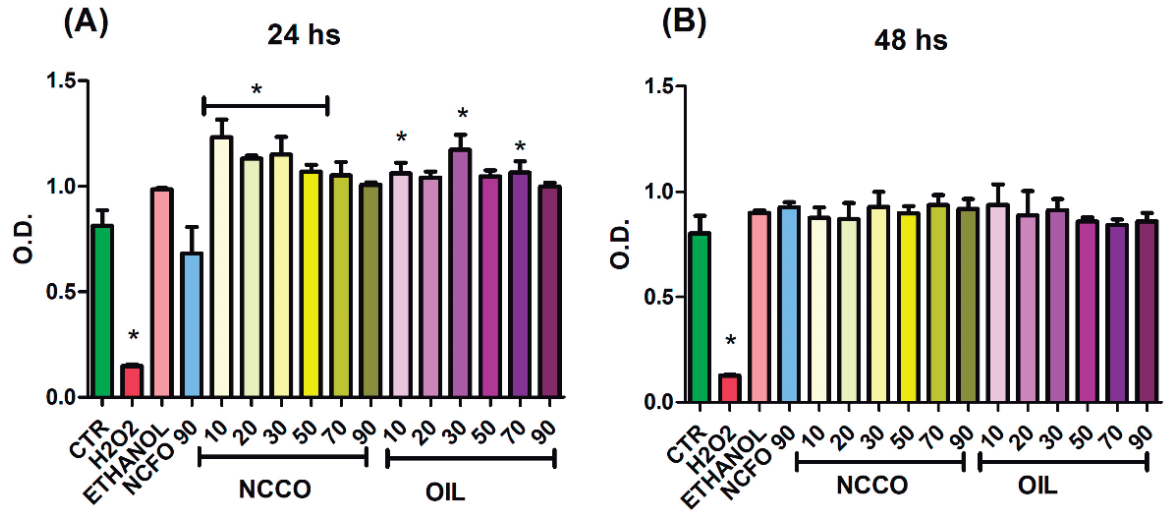
CELL VIABILITY ASSAY

The cytotoxic evaluation by measures of cell viability can be useful initial parameters for nanotechnological products. The screening assay usually applied is the MTT, which is based on the measure of the intracellular dehydrogenase activities in viable cells by conversion of soluble compound MTT to insoluble formazan. In this way, it is possible to establish parameters of cytotoxicity or cell proliferation depending on the compounds used (MIZUNO et al., 2000). The MTT assay was applied in the present study to evaluate if NCCO suspension had effect on viability of murino melanoma skin cells (B16F10) lineage, because this investigation was not found in the literature using chia seed oil.

The MTT assay in melanocytic cells incubated by 24 hours with 10, 20, 30 or 50 μg/mL NCCO demonstrated that low doses of nanocapsules increase the dehydrogenase activities (Figure 5). The observed increase might indicate proliferation, however the effect of NCCO was not measured after 48 hours. Probably, the results indicate transitory metabolic alterations that evoked stimulation on dehydrogenase responsible to transform MTT to formazan (STOCKERT et al., 2012). Other tests should be conducted to evaluate the hypothesis of cell proliferation and/or increase on cell metabolism. The incubation of cells with free chia oil-nanocapsules at concentrations of 10, 30 and 70 μg/mL also induced the transitory stimulatory effect on dehydrogenase. All MTT results showed a reversal of stimulatory effect induced by NCCO and free chia oil-nanocapsules, demonstrating the absence of toxicity by nanoformulation.

Melanocytic lineage cells and Melan-a cells treated with linolenic acid (18:3, omega 3) and linoleic acid (18:2, n-6) were exposed to UVB radiation, however only linolenic acid prevented cells against death when incubated by 24 or 48 hours (VASCONCELOS et al., 2016). This data reinforces the hypothesis that, in the present study, the increased values on MTT reduction could indicate metabolic chances on cells, besides, cell proliferation was not observed in microscopy (data not shown).

Figure 5 - Effect of NCCO on viability of melanocytic cells in culture.



The dehydrogenase activities were measured by MTT reduction in cells incubated during 24 hours (A) or (B) 48 hours as descripted at Materials and Methods. The arbitrary values of MTT reduction are expressed as a mean of optical density ± standard deviation (SD). *$p < 0.05$ compared to control group (CTR). NCFO = free chia oil-nanocapsules.

FINAL CONSIDERATIONS

According to the results presented herein, the hydrogel containing NCCO was stable for up to 90 days, preserving organoleptics characteristics and physicochemical properties at refrigerator temperature. Another advantage of this system was a low toxicity in the culture of melanocytic lineage cells. We conclude that hydrogel with Carbopol® associated to Pluronic® polymer within nanocapsules would make this material a good carrier to bioactive compounds to topical system, besides its pH is compatible to skin and useful to treat wounds.

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