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Attainment of hydrogel-thickened nanoemulsions with tea tree oil (*Melaleuca alternifolia*) and retinyl palmitate

Juliana Soares Oliveira, Tatiana Araujo Aguiar, Hygor Mezdri, and Orlando David Henrique dos Santos*

Department of Pharmacy, School of Pharmacy, Federal University of Ouro Preto, Ouro Preto, MG, Brazil.

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We propose the development of hydrogel-thickened nanoemulsions (HTNs) with tea tree essential oil (*Melaleuca alternifolia*) and vitamin A palmitate (retinyl palmitate). Nanoemulsions and HTNs were produced by the use of phase inversion method, employing non-ionic surfactants. As surfactant phase were used sorbitan esters and hydrogenated/ethoxylated castor oil. HTNs with drops size ranging between 86 to 96 nm were obtained, depending on the polymer used, with increased viscosity. Rheological profile of developed HTNs was determined, showing the influence of the kind of polymer used, here all presented pseudo-plastic behaviour, with different viscosity measurement.

Key words: Nanoemulsion, hydrogel-thickened nanoemulsion, essential oil, phase inversion, vitamin A, retinyl palmitate, tea tree oil.

INTRODUCTION

Many natural products have increased their popularity as an alternative medicine in recent decades. One of such product is tea tree oil (TTO), the volatile essential oil derived from the Australian native plant *Melaleuca alternifolia*. Used mainly for its antimicrobial properties, TTO is incorporated as the active ingredient in many topical formulations used to treat several skin diseases. It is widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments. TTO is a remarkable phytomedicine, with many described actions, specially antibacterial, antifungal, antiviral, antiprotozoal and anti-inflammatory activities (Carson et al., 2006).

An emulsion is defined as a heterogeneous system consisting by a close mixture of two immiscible liquids, one of which is dispersed in the other in drop form. The liquid contained in the interior of drops is called dispersed phase (internal phase), whereas that one which contain the drops is known as continuous or dispersant phase (external phase). Such systems possess a minimum stability, which can be increased by additives as the active agents of surface, solids finely divided and others

(Friberg et al., 1988). These systems are thermodynamically unstable and they are not formed spontaneously (Forgiarini et al., 2001).

Nanoemulsions are special emulsions that consist in very small drops with size between 20 to 500 nm (Solans et al., 2003; Nakajima et al., 1993) and 50 to 200 nm (Tadros et al., 2004). Unlike microemulsions, nanoemulsions are metastable systems, whose structure depends on the process used to prepare them and on the steric stabilization when it is used non-ionic surfactants and/or polymers (Tadros et al., 2004; Fernandez et al., 2004).

The nanoemulsions are interesting as vehicle for cosmetic products, such as skin cares and health care products (Tadros et al. 2004), due to the following:

a) Drops size: Very small particles cause reduction of the gravitational force and consequently the expiration of this force by the Brownian motion indicates that creaming or sedimentation processes will not occur; it prevents flocculation process, keeping the system dispersed; it prevents the coalescence process: the thickness of the film prevents any rupture or thickening of the film between the particles; it allows the uniform deposition in the substrate.

b) Release systems: Used as release systems of

*Corresponding author. E-mail: orlando@ef.ufop.br. Tel: (5531)3559 1038. Fax: (5531)3559 1628.

Table 1. Non-ionic surfactants used to develop the nanoemulsion and HTNs with Vitamin A.

Chemical denomination	Commercial name	HLB
Sorbitan monostearate	Crill [®] 3	4.3
PEG-40 Hydrogenated Castor Oil	Croduret [®] 50	14.0

HLB (hydrophilic/lipophilic balance).

fragrances or the same can be an option as substitute for liposome and carried systems to carry lipophilic composites.

c) Low concentration of surfactants (5.0 to 10.0%): In contrast to microemulsions, they do not need high concentration of them.

d) Sensorial aspect: They have transparency or fluidness, a very valued characteristic in cosmetic products;

e) Low interfacial tension: They increase the wettability, spreading and penetration proprieties.

Though, nanoemulsions as the topical carrier are rarely studied, it might offer several significant advantages including low skin irritation, powerful permeation ability and high drug-loading capacity for topical delivery when compared with the other carriers such as microemulsions, liposomes or solid lipid nanoparticles (Tadros et al., 2004; Solans et al., 2005; Sonnevile-Aubrun et al., 2004). However, the low viscosity of nanoemulsion restrains its clinical application due to inconvenient use (Mou et al., 2008).

In this work, we developed hydrogel-thickened nanoemulsions (HTN) system with Vitamin A (retinyl palmitate) and tea tree essential oil with good stability, small drop size and increased viscosity, being able to be used as a drug delivery system for topical application.

MATERIALS AND METHODS

Tea tree essential oil (*M. alternifolia*) was used as internal phase and was supplied by Galena-Brazil. The water phase used was distilled water. The surfactants were supplied by Croda do Brasil[®]; chemical and commercial name, characteristic and respective HLB number is in Table 1. Four polymers were tested: Carbomer 940 (Carbopol[®] 940 – Lubrizol-USA); Acrylates/C10-30 Alkyl Acrylate Crosspolymer (Carbopol[®] Ultrez 20 – Lubrizol-USA); Acrylates/C10-30 Alkyl Acrylate Crosspolymer (Pemulen[®] TR-1 NF – Lubrizol-USA); Hydroxyethylcellulose – HEC (Natrosol[®] - Aqualon-UK). Vitamin A was used as its palmitate ester (retinyl palmitate) and was supplied by Emfal Ltda-Brazil. All raw materials were used as received, without further purification.

Preparation of nanoemulsions

The emulsification method used was phase inversion, where the oil phase with the surfactants was heated up to 75 +/- 5°C. The water phase was heated to the same temperature and flown into the oil phase remaining the agitation speed at 600 rpm until complete

cooling (25°C, 30 min) with Fisaton agitator model 713R (Morais et al., 2006). Vitamin A was added into the oil phase, previous to water phase addition. Composition of the nanoemulsions was: Tea tree essential oil (5.0%w/w), sorbitan monoestearte (2.0%w/w), PEG-40 hydrogenated castor oil (3.0%w/w), Vitamin A (1.0%w/w), polymer dispersion (when added) (10.0%w/w) and purified water (SQ 100%w/w). Special care was taken during heating to avoid the evaporation of the essential oil that could change its concentration in the product. The samples were covered with a plastic film and any sign of evaporation or condensation of the oil on its surface were controlled.

Preparation of HTNs

Polymers were added to water for swelling to form the hydrogel dispersion. Carbopol[®] 940, Carbopol[®] Ultrez 20 and Pemulen[®] TR-1 NF dispersions had concentration of 0.5 and 1.0%, giving a final concentration on the HTNs of 0.05 and 0.1%, respectively. Natrosol[®] dispersions had concentration of 2.5 and 5.0%, providing final concentration of 0.25 and 0.5%, respectively. After swollen, the gel matrix was mixed with nanoemulsions and HTN was prepared after pH adjustment with sodium hydroxide solution (10%w/v) for that made with carbomers derivatives or light heating (60+/- 2°C) for Natrosol[®].

Characterization of nanoemulsions and HTNs

Intrinsic stability of the nanoemulsion

For each formulation, three test tubes had been filled with 15 ml of the same one and sealed with plastic film. They had been stored vertically to ambient temperature (21+/-2°C) and observed in 1; 2; 4; 6 and 24 h. Any change was observed as: separation or creaming (Roland et al., 2003).

Preliminary stability test

Preliminary stability test consisted of two different tests: centrifugation and thermal stress test.

Centrifugation test: 24 h after preparation, samples (5.0 g) were submitted to 3 different rotation speeds corresponded to 70, 440 and 863G during 15 min for each cycle and at room temperature (25±2°C) (Excelsa Baby II - Fanem Ltd. Brazil).

Thermal stress test: Samples were submitted to thermal stresses in a thermostatic bath. The temperature range used was from 40 up to 70+/-2°C, increased by 5°C. Emulsions were kept at each temperature during 30 min (Thermomix B. Braun - Germany).

Drops size and polydispesity index

The mean size of the nanoemulsions and HTNs was determined by photon correlation spectroscopy. This method allows the

determination of the mean diameter of the particle and the polydispersity index (PI), which is a dimensionless measure of the broadness of the particle size distribution. Particle size was evaluated in a Nanosizer 5 Plus (Beckmann Coulter - USA). Samples were analyzed after appropriate dilution in ultra-pure Milli-Q water.

Rheological measurements

Rheological determinations were performed in a model DV-III Brookfield rotational rheometer (Stoughton, MA, USA) with a cone-plate configuration, connected to a Brookfield software program, RHEOCALC Version V 3.2. Rheological parameters were determined at 25 °C, using a CP 40 spindle and 0.5 g of each sample, 24 h after preparation and after 7 days of storage time. Rheogram curves constructed with ascendant and descendant segments were obtained with rotation speeds increasing progressively (10 to 50 rpm) and gradually decreasing (50 to 10 rpm). With the results obtained, values for consistency index (related to the system viscosity) and flow index (related to the system pseudoplasticity) were mathematically calculated by the Ostwald law (Guarantini et al., 2006):

$$\tau = \kappa \dot{\gamma}^n$$

Where, τ is the shear stress; κ is the consistency index; $\dot{\gamma}$ is the shear rate and n is the flow index.

RESULTS AND DISCUSSION

The nanoemulsion of tea tree oil and retinyl palmitate was obtained according to the composition previously described. After 24 h, they did not present any sign of instability as phase separation, creaming, sedimentation, etc., and macroscopically they were very fluid with a translucent appearance and slightly green. According to Sonnevile-Aubrun et al. (2004), nanoemulsions have this macroscopic characteristic, which is reflex of their low drops size. This happens because the little droplet size has a low influence on the light beam that passes through the samples, especially that with higher wavelength.

In a previous study, we could attain double emulsions of vegetable oils using the same method and kind of surfactant (Morais et al., 2008). The difference here is that the temperature set for mixing both emulsion phases was quite higher: 75 compared to 70. This little temperature increase could be useful to reorganize the system, especially the intermediary phases that are needed for nanoemulsions formation.

Liu and Friberg, in a recent article (Liu et al., 2009), discussed the formation of an intermediary double emulsion during phase inversion from water in oil emulsion (Inversed micelles of Tween®80 in silicon oil) to oil in water emulsion with high internal phase fraction, accompanied by a lamellar liquid crystal phase. The explanation is that this liquid crystalline phase is able to increase the double emulsion droplet stability, being part of the inversion process.

In our case, the surfactants used can form $L\alpha$ phases depending on their concentration in water and

temperature. Another important intermediary phase that could influence during the phase inversion (D') is associated to the double emulsion formation (Morais et al., 2010), because the increase of water ratio tends to change the surfactant curvature; this is intermediary microemulsion. This curvature change could lead to the small nanoemulsion droplet, especially at higher temperatures, compared to the one that induce only the $L\alpha$ phase formation. The result of this inversion process is that the presence of the microemulsion phase, instead of the $L\alpha$ phase can guide to a complete phase inversion, with the characteristic of the transitional one (Salager et al., 2004), directing the formation of nanoemulsions.

Another point is that in this study, an essential oil was used as internal phase, with composition mainly terpenoids. This can bring new information, because it could solubilize the surfactant in a different manner than other oils, specially hydrocarbons or triglycerides. Apart from that nanoemulsions with this oil could be obtained, indicating that the main item responsible for the phase inversion, in this case, was the surfactant pair.

Results after hydrogel addition can be found in Table 2. All dispersions could be classified as stable nanoemulsions, except that made with HEC at 0.5%w/w addition that presented phase separation. It can be seen that the hydrogel influenced on drops size, showing a slight increase. Droplet sizes for Pemulen HTNs were higher than that for the other polymers. This could be result of its action as polymeric surfactant, being more intensely associated with the droplet interface, increasing their thickness. In our work, droplets size were measured by photon correlation spectroscopy, which means that results depend on the difference of refractive index of the particle and suspension media, so the polymer chain is included in the droplet size. The other polymers were less adsorbed on the interface, so their droplets sizes were smaller. In the case of Carpopol® polymer, probably it has being only dispersed on the external phase (water) of the nanoemulsion, and with the dilution required to measure droplet size it was removed. The reduction of the size could be result of increased viscosity during production, helping on the quality of the phase inversion and interface stabilization. Moreover, PI was smaller, reflecting this action as interface stabilizer and aiding homogenization.

After the intrinsic stability test, all HTNs remained stable. According to Sonnevile-Aubrun et al. (2004), one of the most important instability mechanisms of nanoemulsions is the Ostwald ripening, which is related with the partial "solubility" of the oil. However, this instability happens usually when the emulsion presents a big difference between its drop sizes, shown by high polydispersity index. In this work, all HTNs presented PI lower than 0.5 that represents that the droplet size distribution is homogeneous. In this condition, phase separation promoted by Ostwald ripening is very difficult to occur during the period that the HTNs were tested.

Table 2. Results of stability test of the hydrogel thickened nanoemulsions of tea tree oil and vitamin A.

Formulation	Intrinsic stability	Thermal stress	Centrifugation	Droplet size (nm)	PI
Nanoemulsion without polymer	N	N	N	89.0	0.188
HTN-Carbopol [®] 0.05%	N	N	N	90.8	0.252
HTN-Carbopol [®] 0.10%	N	N	N	96.0	0.189
HTN-Carbopol [®] Ultrez 0.05%	N	N	N	92.2	0.109
HTN-Carbopol [®] Ultrez 0.10%	N	N	N	91.2	0.167
HTN-Pemulen [®] 0.05%	N	N	N	94.6	0.170
HTN-Pemulen [®] 0.10%	N	N	N	86.4	0.182
HTN-Natrosol [®] 0.25%	N	N	N	87.2	0.168
HTN-Natrosol [®] 0.50%	N	PS-40°C	PS	*	*

N – Normal; PS – Phase separation; PI – Polydispersity index; * - data not measured.

Table 3. Results of the rheological analysis after 24 h of preparation.

Formulation	Consistency index (cP)	Flow index	Relative viscosity at 50 rpm (cP)
Nanoemulsion without polymer	2.26	0.92	1.44
HTN-Carbopol [®] 0.05%	5.07	0.95	3.79
HTN-Carbopol [®] 0.10%	2.21	1.04	2.81
HTN-Carbopol [®] Ultrez 0.05%	165.5	0.68	24.98
HTN-Carbopol [®] Ultrez 0.10%	2730.0	0.40	75.8
HTN-Pemulen [®] 0.05%	3.61	0.94	2.55
HTN-Pemulen [®] 0.10%	78.6	0.49	3.99
HTN-Natrosol [®] 0.25%	1.66	1.06	2.35

Table 4. Results of the rheological analysis after 7 days of preparation.

Formulation	Consistency index (cP)	Flow index	Relative viscosity at 50 rpm (cP)
Nanoemulsion without polymer	22.8	0.61	2.55
HTN-Carbopol [®] 0.05%	30.3	0.80	8.83
HTN-Carbopol [®] 0.10%	37.0	0.76	9.22
HTN-Carbopol [®] Ultrez 0.05%	549.7	0.58	47.41
HTN-Carbopol [®] Ultrez 0.10%	1437.0	0.51	87.6
HTN-Pemulen [®] 0.05%	270.2	0.29	4.25
HTN-Pemulen [®] 0.10%	484.5	0.17	4.77
HTN-Natrosol [®] 0.25%	16.4	0.72	3.07

Rheological parameters indicated that addition of vitamin A to the basic formulations did not compromise its structure but altered some of the rheological parameters as shown in Tables 3 and 4. The flow index in all formulations was below 1 indicating pseudoplasticity, which is a desirable rheological property in these preparations. Depending on the polymer added, viscosity and consistency indexes were different. In all cases, they increased compared to the nanoemulsion without any polymer that was previously expected. All HTNs had viscosity and consistency index increased after 7 days, except the one with Carbopol 0.1% that kept values very close to the 24 h. This could happen due to the

organization of the polymer dispersion in the external water phase, associated to their swelling by water. The nanoemulsion without polymer did show this behavior, keeping its parameter without any significant change.

Semenzato et al. (1992) demonstrated that Vitamin A chemical stability strictly depends on the physical stability of the formulations. In this work, we could attain HTNs with different polymer structure, but all of them was able to be used as a vehicle for vitamin A palmitate, being able to be used as a topical drug delivery system specially considering the association of vitamin A with tea tree oil, that have antimicrobial and permeation promoter actions.

Conclusions

Addition of polymers that forms hydrogel did not change the nanoemulsions production, bringing an additional stability for the system. HTNs can be obtained by phase inversion, with different polymers, making these systems more suitable for some specific application, especially as a topical drug delivery system. Vitamin A was introduced in the system without any loss in the stability.

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