Copaiba oil microemulsions: A new system for future use in inflammatory diseases.

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INTRODUCTION

Colloidal drug delivery systems are becoming more and more interesting because they enable controlled drug release and improved bioavailability[1]. Microemulsions (MEs), optically transparent systems with low viscosity, are thermodynamically stable dispersions of oil and water. They are stabilized by an interfacial film of a surfactant, usually in combination with a cosurfactant. In pharmaceutics, MEs are used as vehicles to deliver a number of drugs due to their thermodynamic stability, simple preparation, and good appearance. In general, MEs can be divided into 3 types: water-in-oil (w/o), bicontinuous, and oil-in-water (o/w)[2, 3]. MEs have shown several advantages such as enhanced drug solubility, good thermodynamic stability and enhancing effect on transdermal ability over conventional formulations[2]. These properties may find applications in food, cosmetic and pharmaceutical industry when solubilization of lipophilic or hydrophilic ingredients, bio-separations, and enhancing rates of chemical and biochemical reactions are required[4].

Phase diagram (PD) is described as an experimental tool used to obtain MEs. The limit regions can be classified as emulsion, separation of phases and MEs. The pseudoternary PD is constructed to determine the composition of polar, nonpolar, and surfactant phases that will yield a ME. The combination of the MEs components can be plotted as a percent on a pseudoternary diagram [5].

The oleo-resin (copaiba balsam) obtained through small cuts on the stem bark of this plant is used as a popular medicine in its natural form as an anti-inflammatory and anti-infective agent to treat various diseases, such as sore throat, urinary and pulmonary diseases, ulcers, and wounds. Phytochemical studies on oleo-resin showed presence of essential oils (β-caryophylline, caryophylline oxide, β-elemane, α-cis-bergamotene, ar-curcumene, and α-trans-bergamotene) and a mixture of diterpenes (kaurenoic and polyalthic acids). Previous studies established the anti-inflammatory, gastroprotective and wound healing activities of the oil-resin[6,8] and the antinociceptive, antimicrobial, cytotoxic and smooth muscle relaxant effects of the kaurenoic acid [9,11].

The aim of this work was to prepare ME systems of copaiba oil for the treatment of anti-inflammatory diseases by construction of PDs. The stable ME systems consisting of copaiba oil, Tween 20, Span 80 and water were prepared, and their physicochemical properties and stability were evaluated.

MATERIAL AND METHODS

The Copaiba oil was obtained from Flores & Ervas (Piracicaba, SP, Brazil), Span 80 was purchased from Sigma Aldrich Inc (St Louis, MO, USA), and Tween 20 was purchased from VETEC (Rio de Janeiro, RJ, Brazil). Distilled water was used throughout the experiments. All chemicals were of pharmaceutical grade and used as received without further purification.

Construction of Phase Diagram

In order to find the concentration range of all components (copaiba oil/water/Tween 20: Span 80) in which MEs could be formed, various pseudoternary PDs were constructed using the water titration method. Tween 20:Span 80 were mixed at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 to obtain the surfactant mixture (Smix). Copaiba oil and the Smix were then mixed at the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. These mixtures were diluted dropwise with water, under moderate stirring using vortex. The samples were classified as MEs when they appeared as clear preparation and their microscopic appearance using cross-polarized light microscopy (Olympus BX 41, Shinjuku-ku, TOY, Japan) is isotropic. From each PD constructed, different preparations were selected from the ME region.

Microemulsion characterisation

The colour, isotropy and homogeneity of the MEs, and the presence of precipitates or phase separation were scored after visual and cross-polarized light microscopy evaluation. The electrical conductivity of the samples was measured using DM-32 conductivity (Digicrom Analytical, Campo Grande, SP, Brazil), having a cell constant of 0.11 cm^{-1}. The measurements were performed at 25°C ± 2°C. The observed pH values of the samples at 5% were measured by a PG-2000 pHmeter (GEHAKA, Morumbi, SP, Brazil), at 25°C ± 2°C. The refractive index (RI) of formulations was determined using an Abbe-type refractometer (Shijiazhuang Optical Instrument Factory, China), at 25°C ± 1°C.

Stability of microemulsions

The physical stability of MEs with copaiba oil was studied via turbidity and phase separation observation at 4°C, 25°C and 45° C for 15 days. The physical stability of MEs was also assessed by means of centrifugation (BE-5100 centrifuge, BIOENG, Vila Campestre, SP, Brazil – 3,500 rpm for 30 min). The freeze-thawing cycles were perfomed with vial samples filled with the ME and hermetically closed and vertically stored for 24 h in a freezer at −5 °C ± 2,0 and then for 24 h at 45°C ± 2. The MEs were observed and changes were recorded. This cycle was repeated six times.

RESULTS AND DISCUSSION

A pseudoternary PD of the studied quaternary system made of Copaiba oil / Tween 20/ Span 80/Water is presented in Figure 1. Formation of ME systems (the shaded area) was observed at room temperature. The other regions on the PD represent turbid and conventional milky emulsions. No liquid crystalline structure was observed using a cross polarizer. Phase behavior evaluation of this system demonstrated to be a suitable approach to determine the compounds concentration in which the ME system is formed.

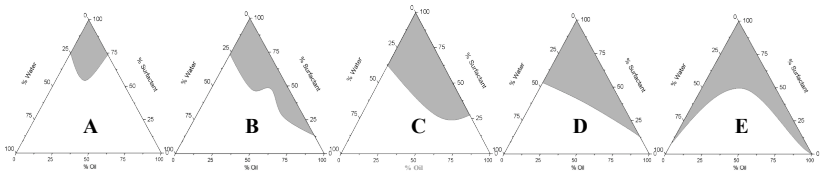

Figure 1 - PD of a system containing the following components: oil = copaiba oil, surfactant/ cosurfactant (Smix) = Tween 20/Span 80. Smix ratio of A is 6:4, B is 7:3, C is 8:2, D is 9:1 and E is 10:0.

The selected MEs were clear yellowish products that appeared dark under cross-polarized light microscopy (no birefringence) and were, therefore. classified as isotropic dispersion of spherical droplets (Table 1).

Table 1 - MEs composition.

Sample	Oil (%)	Tween 20 (%)	Span 80 (%)	Water (%)
1	76	11.4	7.6	5
2	76	13.3	5.7	5
3	63	27	0	10
4	66.5	25.7	2,9	5
5	40	36	4	20
6	45	31.5	13.5	10
7	21	49	0	30
8	8	57.6	14.4	20
9	8	64.8	7,2	20
10	8	72	0	20
11	9.5	68.4	17.1	5
12	54	28.8	7.2	10
13	14	56	0	30
14	6	54	0	40

Table 2- Physicochemical properties of MEs

Sample	pH	Conductivity	Refractive Index
1	4.750	0.000	1.489
2	4.548	0.000	1.489
3	4.445	1.123	1.479
4	4.483	0.181	1.482
5	4.570	3.783	1.458
6	4.440	0.071	1.473
7	4.478	26.210	1.438
8	4.295	2.087	1.448
9	4.300	6.200	1.447
10	4.250	9.093	1.445
11	3.910	1.282	1.461
12	4.435	0.556	1.476
13	4.335	25.986	1.434
14	4.278	46.862	1.417

The physicochemical properties of MEs are reported in Table 2. The conductivity values of the studied samples were quite variable. However, it is well-known that low conductivities occur in systems whose water fraction was smaller than the oil fraction, as in w/o MEs. When high conductivities occur, it indicates that the water fraction was bigger than the oil fraction, as in o/w MEs. The increase in conductivity at $30\%_{(w/w)}$ water content is most likely caused by a transition from an oil-continuous ME system to a water-continuous ones. In spite of the pH values found in the samples were different; they remained within the range of 4 a 5. The pH values of the MEs remained close to the pH of the skin, suggesting that the system has an optimum pH for a topic formulation. The RI of the developed systems remained about 1.45. Some samples showed RI similar to the water (1.333), this value reveals the transparency of the system.

The changes like phase separation were observed for formulation 5 in 15 days. The MEs stored at 4ºC and 25°C showed no change in clarity and phase behavior. The formulations 6, 7 and 13, stored at 45ºC, showed changes in phase behavior and clarity. The centrifugation tests showed that all MEs had good physical stability, except for samples 5, 6 and 13. The freeze/thawing cycles study reveals that despite the presence of phase separation on the first cycle for some MEs, after the six cycles only the samples 5 and 8 showed separation of phases.

CONCLUSIONS

The pseudoternary PD is an important method to determine regions of MEs and other colloidal systems. The MEs are of great interest to the pharmaceutical industry not only for their extended use world-wide, but also because they are easy to prepare, and present low-cost as well as high stability. This study revels that MEs prepared with surfactants, copaiba oil and water were easily produced, using little thermodynamic energy, and remained stable after submission of physicochemical stress.

REFERENCES

1 Podlogar, F.et al. (2004) *Structural characterisation of water-Tween 40((R))/Imwitor 308((R))-isopropyl myristate microemulsions using different experimental methods.* International Journal of Pharmaceutics 276 115-28.

2 Lawrence, M. J.et al. (2000) *Microemulsion-based media as novel drug delivery systems.* Advanced Drug Delivery Reviews 45 89-121.

3 Paul, B. K.et al. (1997) *Microemulsions: An overview.* Journal of Dispersion Science and Technology 18 301-67.

4 Kogan, A.et al. (2006) *Microemulsions as transdermal drug delivery vehicles.* Advances in Colloid and Interface Science 123 369-85.

5 Tenjarla, S. (1999) *Microemulsions: An overview and pharmaceutical applications.* Critical Reviews in Therapeutic Drug Carrier Systems 16 461-521.

6 Fernandes, R. M.et al. (1992) *Anti-inflammatory activity of copaiba balsam (copaifera cearensis, Huber).* . Rev. Bras. Farm 73 53–56.

7 Paiva, L. A. F.et al. (1998) *Gastroprotective effect of Copaifera langsdorffii oleo-resin on experimental gastric ulcer models in rats.* Journal of Ethnopharmacology 62 73-78.

8 Paiva, L. A. F.et al. (2002) *Investigation on the wound healing activity of oleo-resin from Copaifera langsdorffi in rats.* Phytotherapy Research 16 737-39.

9 Velikova, M.et al. (2000) *Antibacterial ent-kaurene from Brazilian propolis of native stingless bees.* Fitoterapia 71 693-96.

10 Costa-Lotufo, L. V.et al. (2002) *The cytotoxic and embryotoxic effects of kaurenoic acid, a diterpene isolated from Copaifera langsdorffii oleo-resin.* Toxicon 40 1231-34.

11 Cunha, K. M. D.et al. (2003) *Smooth muscle relaxant effect of kaurenoic acid, a diterpene from Copaifera langsdorffii on rat uterus in vitro.* Phytotherapy Research 17 320-24.