P-039

Phase diagrams of copaiba oil: comparative study on their production process

Silva K.G.H.[1], Xavier-Jr. F.H.[1], Alencar E.N.[2], Morais A.R.V.[2], Araújo, I.B.[1] and Egito E.S.T.[2]*#
[1] Universidade Federal do Rio Grande do Norte (UFRN), Brazil. E-mail: socrates@ufrnet.br
[2] UFRN, Departamento de Farmácia, LaSiD, Natal, RN, Brazil.

INTRODUCTION AND OBJECTIVES

The term microemulsion (ME) was introduced by Hoar and Schulman in 1943 (Hoar 1943). MEs are thermodynamically stable colloidal dispersions of water and oil stabilized by surfactants. They have a droplet diameter usually within the range of 10–100 nm (Tenjarla 1999). MEs have several advantages such as enhanced drug solubility, good thermodynamic stability, ease of manufacturing and enhancement effect on transdermal ability over conventional formulations (Trotta 1997). Several methods can be used to produce MEs. These different methods demand different amounts of energy for system formation. The construction of phase diagrams (PDs), an equilateral triangle limiting the proportion of the ME components, can be an ideal tool to characterize the regions of MEs and describe the structures present in the different regions of the diagrams (Lawrence 2000).

Copaiba oil (*Copaifera langsdorffii Desf)* has been shown anti-inflamatory, and antitumoral effects against Walker sarcoma and melanoma cell line. It also possesses anti-ulcerogenic, antioxidant and anti-lipoperoxidative, cercaricide and antihelmintic, insect repellent and antimicrobial effects (Cunha 2003, Paiva 2004). 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 (Fernandes 1992).

The aim of this work was to perform a comparative study among different methods to produce MEs from the construction of PDs.

MATERIAL AND METHODS

Material

The Copaiba oil was 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). All chemicals were of pharmaceutical grade and used as received without further purification.

Methods

Construction of Pseudoternary Phase Diagrams

In order to find out the concentration range of components for the existing range of MEs, pseudo-ternary PDs were constructed using the H_2O titration method at ambient temperature (25 ºC). Five PDs were prepared for each ME production method. Tween 20:Span 80 were mixed at a weight ratio of 2:8, 5:5, 8:2, 9:1, and 0:10, respectively to obtain the Smix. Copaiba oil and Smix were then mixed at the weight ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1, respectively. The mixtures of oil, surfactant and cosurfactant at certain weight ratio were diluted with H2O drop-wise, under three stirring methods: magnetic stirring, vortex stirring and sonic probe stirring followed by ultrasonic bath cleaner. After equilibrium, the mixtures were assessed visually and their microscopic appearance, using cross-polarized light microscopy (Olympus BX 41, Shinjuku-ku, TOY, Japan), was determined to classify the systems as MEs, crude emulsions, nanoemulsion, gels and phase separation.

Stirring process

Three stirring process were used for MEs production. The first one, mechanical stirring, was generated by using Vortex (IKA, Deutschland, Germany). The second one was magnetic stirring, using the magnetic stirrer (IKA, Deutschland, Germany). The last method was the ultrasonic probe, using the sonicator (Heat Systems, Farmingdale, NY, USA) followed by the ultrasonic bath cleaner (Unique, Indaiatuba, SP, Brazil). All the stirring process, made in duplicates, were performed for 2 minutes for each sample. Also, all the samples were analyzed overnight after their production.

RESULTS AND DISCUSSION

A PD of the investigated quaternary system water/Tween 20/Span 80/copaiba oil is presented in Figure 1. Formation of ME systems was observed at room temperature using three different stirring methods. Vortex shaking, a process made for an internal cyclone that independent on the system viscosity, let not only a great homogenization, but also offers more advantages such as the employment of a small amount of sample at room temperature. The magnetic stirring process also allows the use of a small amount of sample at room temperature. However, for bigger amount of samples and for high viscosity ones this process is not suitable. The highlight effect of ultra-sound is the acoustical effect, where there is the collapse between microbubbles that increase until to implode giving both high pressure and temperatures during the finals collapses stages.

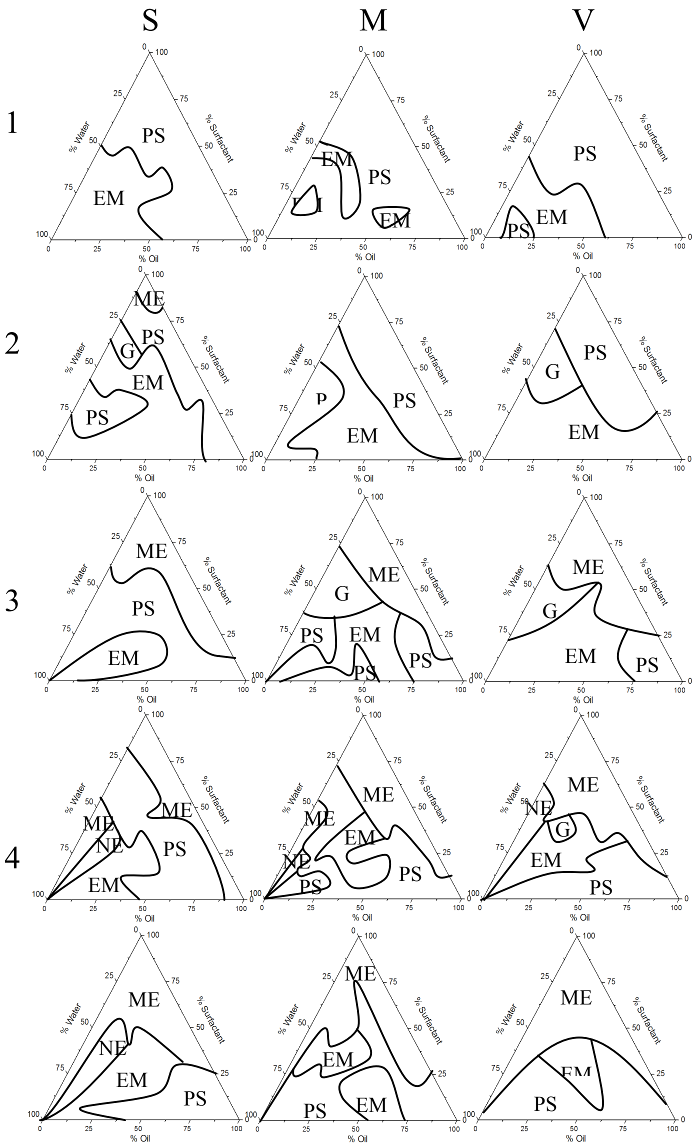

Figure 1- PD of Copaiba oil produced from different stirring process. Where: S, sonic probe stirring; M, magnetic stirring; V, vortex stirring; ME, Microemulsion; EM, emulsion; NE, nanoemulsion; G, gel; PS, phase separation; 1, Smix 2:8; 2, Smix 5:5; 3, Smix 8:2; 4, Smix 9:1 and 5, Smix 0:10

From the analysis of PDs (Figure 1) different colloidal structures were produced, depending on the stirring process. No statistically significant differences on PDs were found regarding the ME regions of the three production processes. This occurs, probably, due to spontaneous formation of such systems, in which the potential energy involved in the production process does not determine the structural formation. It can be also inferred that the diagrams with a Smix ratio of 2:8 were similar in the absence of ME regions' formation. The vortex, magnetic stirring and sonic probe methods showed about 45.56%, 10% and 58.89% of regions of emulsion, respectively. The high percentage of emulsion is justified, for the third method, due to the high quantity of energy involved in the production process. In diagrams with Smix ratio of 5:5 the vortex method of production showed bigger region of emulsion (63.33%) than the magnetic stirring method, which produced the greatest region of separation of phases. In diagrams at Smix ratio of 8:2 large regions of emulsions, when produced by vortex and magnetic stirring (50.5%), were found. Additionally, the vortex method showed the greatest formation of gel (11.11%). In diagram at Smix ratio of 9:1, there was no significant difference between the emulsion regions (30%) produced by the three methods. The sonic probe method generates bigger nanoemulsion region (10%) than the others, probably due to the high input of energy involved in the production process in which small droplets are formed. In diagrams at Smix ratio of 10:0 no significant difference between the emulsion regions was found. Also, the nanoemulsion regions were similar to the previous diagrams and the separation of phases area was smaller for the sonic probe method than to the others.

CONCLUSIONS

The PD reveals how useful could be this tool to predicting formation of colloidal systems. The formation of colloidal structures depends also on the method of production, and the behavior of the PDs were dependent on the input of energy supplied to the system. In fact the stirring process induces the formation of different structures by the magnitude of agitation that improves the degree of interaction between the components.

As a conclusion, it can be inferred, however, that the regions of MEs produced on the PDs were not dependent on the level of energy supplied to the system, probably due to the thermodynamic stability of such systems. Moreover, due to inherent instability of the systems, each agitation process showed considerable variations in percentage of different regions.

REFERENCES

- Cunha, K. M. D. et al (2003) Smooth muscle relaxant effect of kaurenoic acid, a diterpene from Copaifera langsdorffii on rat uterus in vitro. Phytother Res 17 320-24.
- Fernandes, R. M. et al (1992) Anti-inflammatory activity of copaiba balsam (copaifera cearensis, Huber). Rev. Bras. Farm 73 53–56.
- Hoar, T. P. et al (1943) Transparent water in oil dispersions: the oleopathic hydromicelle. Nature 152 102 - 03.
- Lawrence, M. J. et al (2000) *Microemulsion-based media as novel drug delivery systems.* Adv Drug Delivery Reviews 45 89-121.
- Paiva, L. A. F. et al (2004) Protective effect of Copaifera langsdorffii oleo-resin against acetic acid-induced colitis in rats. J. Ethnopharmacology 93 51-56.
- Tenjarla, S. et al (1999) *Microemulsions: An overview and pharmaceutical applications.* Crit Reviews Ther Drug Carrier Syst 16 461-521.
- Trotta, M. et al (1997) Effect of oil phase composition on the skin permeation of felodipine from O/W microemulsions. Pharmazie 52 50-53.