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# Phase equilibrium data of guaçatonga (*Casearia sylvestris*) extract + ethanol + $CO_2$ system and encapsulation using a supercritical anti-solvent process

Patrícia Benelli[a], Sibele R. Rosso Comim[a], J. Vladimir Oliveira[a], Rozangela C. Pedrosa[b], Sandra R.S. Ferreira[a,*]

[a] EQA-CTC/UFSC, Chemical and Food Engineering Department, Federal University of Santa Catarina, C.P. 476, CEP 88040-900, Florianópolis, SC, Brazil
[b] BQA-CCB/UFSC, Biochemistry Department, Federal University of Santa Catarina, C.P. 476, CEP 88040-900, Florianópolis, SC, Brazil

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ABSTRACT

The aim of this research was to investigate the phase equilibrium behavior of a system containing guaçatonga extract + ethanol + $CO_2$ in order to help define the adequate conditions of temperature and pressure for the co-precipitation process, performed by means of supercritical anti-solvent (SAS) technique. Guaçatonga (*Casearia sylvestris*) is a native medicinal plant from Brazil, rich in valuable components such as β-caryophyllene, α-humulene and bicyclogermacrene. Phase equilibrium data were obtained by the static method using guaçatonga extract dissolved in ethanol (1:100, wt/wt), at temperatures ranging from 35 to 75 °C and $CO_2$ mass content from 60 to 90 wt%. It was noticed that the system exhibited solid–vapor–liquid, solid–liquid–liquid and solid–vapor–liquid–liquid transition types and a lower critical solution temperature behavior. Phase behavior study was considered for the definition of the SAS conditions applied for the encapsulation of guaçatonga extract in the biopolymer Pluronic F127. The conditions tested ranged from 80 to 140 bar at 45 °C. At 80 bar only segregated particles of extract and the biopolymer were detected, while at 110 and 140 bar an extract encapsulation was achieved.

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## 1. Introduction

*Casearia sylvestris* is a medicinal plant native from Brazil and popularly known as "guaçatonga", "cafezinho-do-mato", "café-silvestre" and "erva-de-bugre". In folk medicine its leaves are used for skin and oral wound healing. It is also applied as an anti-snake poison, topical anesthetic and antiseptic and anti-ulceration agent [1–3].

The major components present in guaçatonga extracts, mainly in the essential oil, are β-caryophyllene, α-humulene, bicyclogermacrene, germacrene B and D [4], which among other substances confer several biological potency such as antitumor, cytotoxic, antifungal and anti-inflammatory activities [5–7]. Phytochemical investigations revealed that some compounds isolated from guaçatonga exhibit both cytotoxic and antifungal activities [8,9], and these biological activities are attributed to the combined effect of the compounds present in the natural extract [10]. Due to the complexity of multicomponent systems usually found in natural products, the synergetic effect of the components are still under investigation in order to provide a complete elucidation of the mechanisms involved in biological actions of complex extract [11].

The coating or encapsulation of a natural extract into a polymer, in a micrometer range, is interesting for food and pharmaceutical industries because it preserves the bioactive properties of natural extracts [12–14].

The phase behavior of complex systems formed by natural product extracts and solvent plays a crucial role to elucidate the precipitation mechanism involved in the particle formation (micronization) like nucleation, particle growth kinetics, and mass transfer. The phase behavior also aids the determination of the most satisfactory operating conditions for the precipitation and encapsulation processes [15,16].

The particle formation and encapsulation using traditional techniques such as spray-drying, coacervation, freeze-drying, interfacial polymerization, and others, may present some disadvantages such as reduced control of particle size and morphology, thermal degradation of sensitive substances, and low encapsulation efficiency [17].

The use of supercritical anti-solvent (SAS) for micronization and encapsulation processes have been proposed in order to obtain

* Corresponding author. Tel.: +55 48 37214069/+55 48 37212537; fax: +55 48 37219687.
*E-mail address:* sandra@enq.ufsc.br (S.R.S. Ferreira).

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solid particles with better control in particle size, size distribution, morphology and crystalline structure, difficult to obtain in traditional methods [15,16,18]. These characteristics provided by SAS methods are due to the fast mixture of solvent, anti-solvent and extract which leads to higher super saturation of the solvent and induces the formation of smaller particles [19–21]. Also, the efficient separation of the solvent and anti-solvent from the precipitated particles avoids solvent residues in the final product and permits the reuse of solvent and anti-solvent [17], preserving the particulate materials quality, aspects difficult to obtain in traditional methods due to the presence of organic solvent residues and relatively high processing temperatures [22–25].

For the encapsulation of natural products, for food and pharmaceutical uses, the coating film must be biodegradable and non-toxic, such as the co-polymers of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO), commercially known by the trade name Pluronics or the generic name poloxamers. Certain classes of poloxamers, like Pluronic F127, have been approved by Food and Drug Administration (FDA) for use in the human body. Pluronic F127 presents molecular weight of 12,600 g/mol, is water-soluble, biocompatible, bioabsorbable, exhibits low toxicity, especially in low concentrations (less than 10,000 mg/$kg_{rats}$.day), and can be used as an injectable biomaterial [26,27]. Its medical uses include controlled delivery drugs to the eye, nasal passage and parenteral and subcutaneous administration. Furthermore, Pluronic F127 presents physical properties such as water permeability and hydrophilicity that empowers the coating layer with the characteristic of slowly releasing the encapsulated material [21,28].

In this context, the aim of present study was to investigate the phase equilibrium behavior by means of the static method for the multicomponent system composed by guaçatonga (*C. sylvestris*) extract, ethanol and supercritical carbon dioxide ($CO_2$). This relevant thermodynamic data aid the establishment of appropriate operating conditions for SAS process for the encapsulation of guaçatonga extract in Pluronic F127. The morphology of particles obtained by SAS method and the particle size were characterized by scanning electronic microscopy (SEM) and the interaction between the polymer and the encapsulated extract was verified by differential scanning calorimetry (DSC).

## 2. Materials and methods

### 2.1. Sample preparation and obtention of guaçatonga extract

Leaves of guaçatonga, provided by Brazervas Laboratório Fitoterápico Ltda., Osório/RS, Brazil, were air-dried at room temperature up to 13.72 ± 0.05% (w/w) of moisture content. The dried material was ground in a knife mill (De Leo, Porto Alegre/RS, Brazil) and characterized by size classification in a vertical vibratory sieve shaker (Bertel Metalurgic Ind. Ltda., Caieiras/SP, Brazil). The mean particle diameter was calculated based on mean size distribution as described by Gomide [29], resulting in 372 ± 37 μm. The sample was stored at −18 °C until the extractions were performed.

The guaçatonga extract was obtained by supercritical fluid extraction (SFE), performed in a dynamic extraction unit previously described by Zetzl et al. [30], with the extraction procedure presented by Michielin et al. [31]. Briefly, the extraction consisted of placing 15 g of dried and milled material inside the column to form the particles fixed bed, followed by the control of temperature, pressure and solvent flow rate. The extraction was performed and the solute collected in amber flasks and weighed in an analytical balance (OHAUS, Model AS200S, NJ, USA). The SFE assays were performed with $CO_2$ added with ethanol (ETOH) as a co-solvent. The extraction was done at 50 °C, 300 bar and $CO_2$ flow rate of 8.3 ± 2 g/min for 3.5 h of extraction and using 5% (wt/wt) of ethanol. The $CO_2$ used for the SFE was 99.9% pure (White Martins, Brazil). The remaining co-solvent present in the guaçatonga extract (ethanol) was separated by reduced pressure in a rotary evaporator (Fisatom, 802, Brazil). The supercritical guaçatonga extract was then dissolved in ethanol 99.5% pure (1:100, wt/wt) and used for phase equilibrium experiments.

### 2.2. Phase equilibrium apparatus and experimental procedure

Phase equilibrium experiments were accomplished through the static method in a high-pressure variable-volume view cell. The experimental apparatus and the procedure adopted were based in a variety of studies [16,32]. Briefly, the equipment consists of an equilibrium cell, with maximum internal volume of 27 mL and two sapphire windows (for light entrance and for visual observation), an absolute pressure transducer (Model 511, Huba Control, Würenlos/Denmark) and a syringe pump (260HP Teledyne Isco, Lincoln/NE/EUA) with pressure range from 0.7 to 655.2 ± 0.5 bar. The phase transitions as a consequence of the pressure manipulation (syringe pump) were visually observed (sapphire windows). Initially, a precise amount of the guaçatonga extract dissolved in ethanol (1:100, wt/wt) was weighed in an analytical balance (Ohaus, Model AS200S, NJ, USA) with ± 0.0001 g of precision and loaded into the equilibrium cell. A known amount of solvent at 5 °C and 100 bar was loaded into the equilibrium cell using the syringe pump, resulting in an accuracy of ± 0.005 g in $CO_2$ loadings until a desired mass fraction was achieved (from 60 to 95% of $CO_2$). The cell content was kept at continuous agitation with a magnetic stirrer and a Teflon-coated stirring bar. At the desired temperature the pressure system was increased up to the formation of one-phase system. At this point, the pressure was slowly decreased (at a rate of 3.5 bar/min) until incipient formation of a new phase. This procedure was repeated at least two times for each temperature (35–75 °C) and global composition (guaçatonga extract and $CO_2$) tested.

### 2.3. Encapsulation using supercritical anti-solvent (SAS) process

The supercritical encapsulation was performed in a SFE unit adapted to the SAS process as described by Mezzomo and Ferreira [33]. Briefly, the SAS system requires the use of a pump (M111, Maximator, Germany) to obtain the desired high pressures. The $CO_2$ was cooled at −5 °C (C10-K10, Termo Haake, Germany) to guarantee operation in the liquid state. The air-driven piston pump compresses the liquid $CO_2$, with a gear ratio of the booster piston of 1:130. The oscillation frequency of the piston is controlled by the throttle valve, controlling the system pressure. An HPLC pump (Constametric 3200 P/F, Milton Roy Company, USA) was used to feed the organic solution (extract + polymer + organic solvent) into the precipitator chamber, assembled in AISI 316 stainless steel (329 mm length × 20.42 mm inner diameter, and internal volume of 107.75 mL). These two streams ($CO_2$ and organic solution) were mixed by means of a concentric tube nozzle placed at the top of the precipitation vessel. The contact between $CO_2$ and organic solution allow the organic solvent (ethanol) solubilization by the $CO_2$, and therefore precipitating the extract/polymer mixture (1 μm porous filter was placed at the cell bottom to hold the precipitated particles). After the system depressurization the organic solvent was collected and the $CO_2$ flow was measured by a flow meter (10A61, ABB, Switzerland). The process temperature was controlled by a thermostatically water bath (DC30-B30, Thermo Haake, Germany). The conditions of temperature and pressure were measured with instruments directly connected to the precipitation vessel, with accuracies of ± 0.5 °C and ± 2 bar, respectively.

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