

Microemulsion of babassu oil as a natural product to improve human immune system function

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Background: The aim of this study was to develop and characterize a babassu oil microemulsion system and determine the effect of this microemulsion on the functional activity of phagocytes.

Methods: The microemulsion was formulated using distilled water, babassu as the oil phase component, Sorbitan monooleate-Span 80[®] (SP), Polysorbate 80-Tween 80[®] (TW), and 1-butanol (BT). Pseudoternary diagrams were prepared, and microemulsion diagram regions were preselected. Rheological characterization and preliminary and accelerated stability tests were performed. The effect of the microemulsion on the interactions between leukocytes and bacteria was determined by superoxide release, phagocytosis, and microbicidal activity.

Results: The developed formulation SP/TW/BT (4.2/4.8/1.0) was classified as oil/water, showed a Newtonian profile, and had linear viscosity. When we assessed the interaction of the microemulsion or babassu oil with phagocytes, we observed an increase in superoxide, phagocytosis, and microbicidal activity.

Conclusion: The babassu oil microemulsion system is an option for future applications, including for vaccine delivery systems. Babassu oil is a natural product, so is an alternative for future immunotherapy strategies, in particular for infectious diseases.

Keywords: vegetable oil, babassu, microemulsion, phagocytes, delivery systems

Introduction

The development of drug and vaccine delivery systems has increased in recent years. Many such products improve the treatment of disease or stimulate the immune system.¹ The literature has demonstrated that microemulsions (MEs) are an interesting strategy for delivery of therapeutic molecules.² MEs are homogeneous dispersions of oil and water; they are translucent and thermodynamically stable, are stabilized by an interfacial film composed of conjugated surfactant and cosurfactant,³ and exist as droplets with a diameter in the order of 100 nm.⁴ The controlled-release characteristics of an emulsion are determined by factors such as oil phase viscosity, the oil-to-water phase ratio, and emulsion droplet size.

Interest in identifying the relationships between MEs as delivery systems, their adjuvant activity, and their regulation of immune responses is growing, but study results remain controversial. Many investigators are in agreement that the particles are crucial to their adjuvant activity.⁵ One factor that may affect the system is the material used to form the particles. To clarify the influence of the ME on adjuvant activity, the ME-induced immune responses must be compared. Conversely, medicinal plants and plant extracts still have great relevance because of the use of active substances, such as pharmaceutical materials, to obtain both drugs and adjuvants.

In Brazilian ethnobotany, babassu, which is a generic name given to palm oil belonging to the family Arecaceae (Palmae) and members of the genera *Orbignya* and *Attalea*.⁶

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Studies demonstrate that babassu has analgesic and anti-inflammatory biological properties.⁷ Studies performed with a plant-derived compound in which the highest concentration was of babassu extract, confirmed that this herb activates the functional mechanisms of human phagocytes^{8,9} and can activate the immune system when combined with a modified-release system.¹⁰ Several studies have also demonstrated that babassu oil has anti-inflammatory activity^{11,12} and healing properties.^{6,11,13}

The main product used by the population is the oil of the babassu fruit. Babassu oil has a white to slightly yellowish color and corresponds to 4% of the total fruit weight. Babassu oil contains a wide variety of fatty acids, including high lauric and myristic acid concentrations,¹⁴ but lauric acid is the main component responsible for the therapeutic properties of the plant.¹⁵ Despite several studies of the properties of the plant and its products, the activity of babassu oil as a possible delivery system has not yet been elucidated. The aim of this study was to develop and characterize a babassu oil ME system and verify the effects of an ME on the functional activity of phagocytes.

Materials and methods

System composition

The MEs were formulated using distilled water; babassu oil, with a hydrophilic–lipophilic balance (HLB) of 10.0 (Mundo dos Óleos, Brasília DF, Brazil); Sorbitan monooleate, Span[®] 80 (SP), with an HLB of 4.3 (Emfal, Betim, Brazil); Polysorbate 80, Tween 80[®] (TW) with an HLB of 15.0 (Vetec, Rio de Janeiro, Brazil), and 1-butanol (BT) (Vetec). The system is abbreviated as SP/TW/BT.

Determination of surfactant mixing ratio

To act as a cosurfactant, BT was used in a ratio equivalent to 10% ($F_c = 1$) of the surfactant mixture. The HLB of the surfactant mixture and the composition required to achieve an HLB corresponding to the oily phase was calculated. The HLB of the SP and TW surfactants were taken into account, according to Ribeiro et al.¹⁶

Development of the ME system

Development of formulations utilized pre-established quantities of the components in which each component ranged from 20% to 80%. The samples were visually rated after 72 hours at 25°C in the following regions: liquid, gelling, emulsion liquid, gel emulsion, and phase separation.

Titration was performed on aqueous mixtures with surfactant/oil phase mass ratios of 1:9 and 9:1 under agitation to obtain the delimiting points, area and classification of different systems to form the diagram regions. The titration was performed by adding distilled water in quantities between 0.05 mL and 0.20 mL. During this process, the mixture was agitated both manually and mechanically. After homogenization of each titrant, the formulation volumes were visually classified.

Pseudoternary phase diagram construction

Pseudoternary diagrams were constructed from the sample and titration data using SigmaPlot version 8.0 software. In the diagram, the superior vertex represents 100% surfactant/cosurfactant, the lower right represents 100% oil phase, and the lower left represents 100% aqueous phase.

Preselection of systems

After acquisition of the pseudoternary diagram, composition of the systems that fall into the ME liquid region can be determined. This region contained preselected points that were distributed in lines dividing the region to achieve representative samples of the studied systems.

Rheological characterization

Rheological parameters were measured using a modular compact rheometer (MCR 102, Anton Paar Germany GmbH, Ostfildern, Germany) according to França et al.¹⁷ In all of the experiments, 600 µL of ME was added to the surface of the reading plate, and the excess sample was removed. Readings were taken with continuous control of the gap measurement with the supported TruGap[™] in 0.099 mm. The measuring cell was a Toolmaster[™] CP 50, and precise temperature control was achieved with T-Ready[™]. The data were compiled using Rheoplus V3.61 software. Graphics were also obtained using Rheoplus software. The flow and viscosity curves were based on established parameters for the control of shear stress (τ) to 0–5 Pa for the upsweep and 5–0 Pa for the curve downward. These tests were conducted under isothermal conditions at 25°C, and 75 readings were taken for analysis.

The viscosity curve was taken using established parameters that were relative to temperature and based on fixed control shear stress (τ) to 1 Pa with variation in temperature from 5°C±0.1°C to 45°C±0.1°C and a heating rate of 1°C per minute. Parameters were recorded every 0.5°C±0.1°C, and 41 readings were taken for analysis.

Physicochemical characterization

A method to verify the physicochemical parameters was designed to test the suitability of the formulations to act as delivery vehicles and to perform the initial characterization of each sample. This method verified the physical and chemical parameters of the formulations 24 hours after preparation and at the end of the stability study; the tests were performed in triplicate ($n=3$).

Aliquots of some preselected vehicles were centrifuged (Baby I, Fanem, São Paulo, Brazil) at $1,570\times g$ for 30 minutes at room temperature. After centrifugation, the samples with visual heterogeneity were excluded.

The pH of the vehicles was determined with a pH meter (Del Lab, Campinas, Brazil) that was calibrated with standard pH 7 and pH 4 solutions. The electrical conductivity was measured using a conductivity meter (Lida, São Paulo, Brazil) calibrated with a 0.1 mol/L KCl solution to identify the system type (water in oil or oil in water) and any tendency toward phase inversion.

Stability studies

Samples were divided into two groups according to temperature: those that were refrigerated at $5^{\circ}\text{C}\pm 1^{\circ}\text{C}$ and those that were heated to $40^{\circ}\text{C}\pm 1^{\circ}\text{C}$. These systems were preliminarily subjected to alternating cycles of $5^{\circ}\text{C}\pm 1^{\circ}\text{C}$ and $40^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 24 hours each, with the cycles completed on the 14th day. After the cycle, it was possible to identify the most thermodynamically stable formulations.

The formulations were subjected to extreme conditions to determine their stability over a long period. The systems were divided into three groups according to temperature: $5^{\circ}\text{C}\pm 1^{\circ}\text{C}$, $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$, and $40^{\circ}\text{C}\pm 1^{\circ}\text{C}$. The groups were assessed in triplicate for a period of 90 days. Every 30 days, the formulations were maintained at room temperature for 24 hours to determine the physicochemical properties and to reassess the rheological profiles.

Dynamic light scattering

Colloidal suspensions of formulation 2B were prepared for analysis by the dynamic light scattering technique. It was then possible to investigate the hydrodynamic diameter of the dispersed solid. The samples were prepared from a 1:1,000 dilution of the formulation in deionized water in quartz cuvettes using deionized water as the reference. Dynamic light scattering analyses were performed using a Zetasizer Nano Z90 (Malvern Instruments, Malvern, UK) with excitation at 632.8 nm.

Modulation of blood phagocyte functional activity by babassu oil ME

Blood sampling and blood cell separation

Blood samples (10 mL) were collected from 20 volunteer donors in tubes with anticoagulant. This study was approved by the institutional research ethics committee, and all of the subjects gave their written informed consent before entering the experimental protocol. The samples were centrifuged at $160\times g$ for 15 minutes to separate plasma from the cells. Cells were separated over a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden), producing preparations with 95% pure mononuclear cells as analyzed by light microscopy. Purified monocytes were resuspended independently in serum-free Medium 199 (Sigma-Aldrich Co., St Louis, MO, USA) at a final concentration of 2×10^6 cells/mL. The cells were used immediately for superoxide release, phagocytosis, and microbicidal activity assays.

Phagocyte treatment with babassu oil and ME

To assess the effect of babassu oil (Mundo dos Óleos) and ME on superoxide anion release, phagocytosis, and microbicidal activity, mononuclear phagocytes (2×10^6 cells/mL) were incubated with 20 μL (final concentration 100 ng/mL) and immediately used in the assays. A control was performed with Medium 199 only.

Escherichia coli strain

The enteropathogenic *E. coli* (EPEC) used was isolated from stools of an infant with acute diarrhea (serotype 0111: H⁻ AL⁻, eae⁺, eaf⁺, bfp⁺). This material was prepared and adjusted to 10^7 bacteria/mL, as previously described by Honorio-França et al.¹⁸

Superoxide anion release

Superoxide release was determined by reduction of cytochrome C (Sigma-Aldrich, St Louis, MO, USA).¹⁸ Briefly, mononuclear phagocytes and bacteria were mixed and incubated for 30 minutes to assess phagocytosis. Cells were then resuspended in phosphate-buffered saline containing 2.6 mM CaCl₂, 2 mM MgCl₂, after which cytochrome C (2 mg/mL) and babassu oil (20 μL) or babassu ME (20 μL) was added. The suspensions (100 μL) were incubated for 1 hour at 37°C on culture plates. A control was performed using only the spontaneous release of cells. The reaction rates were measured by absorbance at 550 nm, and the results were expressed as nmol/O₂⁻. All of the experiments were performed in duplicate.

Cellular viability and bactericidal assay

Cellular viability, phagocytosis, and microbicidal activity were evaluated using the acridine orange method.¹⁹ Equal volumes of bacteria and cell suspensions were mixed and incubated at 37°C for 30 minutes under continuous shaking. Phagocytosis was stopped by incubation on ice. To eliminate extracellular bacteria, the suspensions were centrifuged twice (160× *g*, 10 minutes, 4°C). Cells were resuspended in serum-free Medium 199 and centrifuged. The supernatant was discarded, and the sediment was stained with 200 µL acridine orange (14.4 g/L; Sigma-Aldrich) for 1 minute. The sediment was resuspended in cold Medium 199, washed twice, and observed under immunofluorescence microscopy at 400× and 1,000× magnification.

The viability index was calculated by counting the number of orange-stained (dead) and green-stained (alive) cells out of 100. The phagocytosis index was calculated by counting the number of cells that ingested at least three bacteria in a pool of 100 cells. To determine the bactericidal index, we stained the slides with acridine orange and counted 100 cells with phagocytosed bacteria. The bactericidal index was calculated as the ratio between orange-stained (dead) and green-stained (alive) bacteria ×100. All of the experiments were performed in duplicate.

Statistical analysis

Data were expressed as the mean ± standard deviation. Statistically significant differences in superoxide anion release, phagocytosis, and bactericidal index in the presence or absence of ME were evaluated using analysis of variance; statistical significance was considered for a *P*-value <0.05.

Results

Determination of surfactant mixing ratio

The concentration of cosurfactant BT was defined as 10%, and the percentage of surfactant SP and TW were calculated to achieve the required babassu oil HLB. The surfactant mixture (SP/TW/BT) was 4.2:4.8:1.0 to achieve the required HLB, which was the same as the oily phase.

ME system development

Pseudoternary phase diagrams were assembled to classify the points, and domains of each region are demonstrated in Figure 1. The SP/TW/BT (4.2/4.8/1.0) surfactant mixture was compared with babassu oil and distilled water in proportions that were established by the diagram; these data resulted in 36 points with divergent equilibrium characteristics. It was observed that with over 40% of surfactants, the thermodynamic equilibrium became efficient because homogeneous and translucent systems predominated (Figure 1A).

Figure 1B shows the pseudoternary diagram with delimited domains from the diagram points and aqueous titrations. The formulations were preselected from the ME liquid region to study the SP/TW/BT. Analysis of the phase behavior indicated that large range surfactant compositions produced stable systems. When the domain surfactant concentration was below 55%, some instability and phase separation were also observed, indicating that these surfactant concentrations could not balance high proportions of aqueous and oily phases (Figure 1B). Formulations 1–16 exhibited characteristics of ME liquid (Figure 2A), whereas the formulations of 17–36 exhibited characteristics of ME gel or phase separation (Figure 2B).

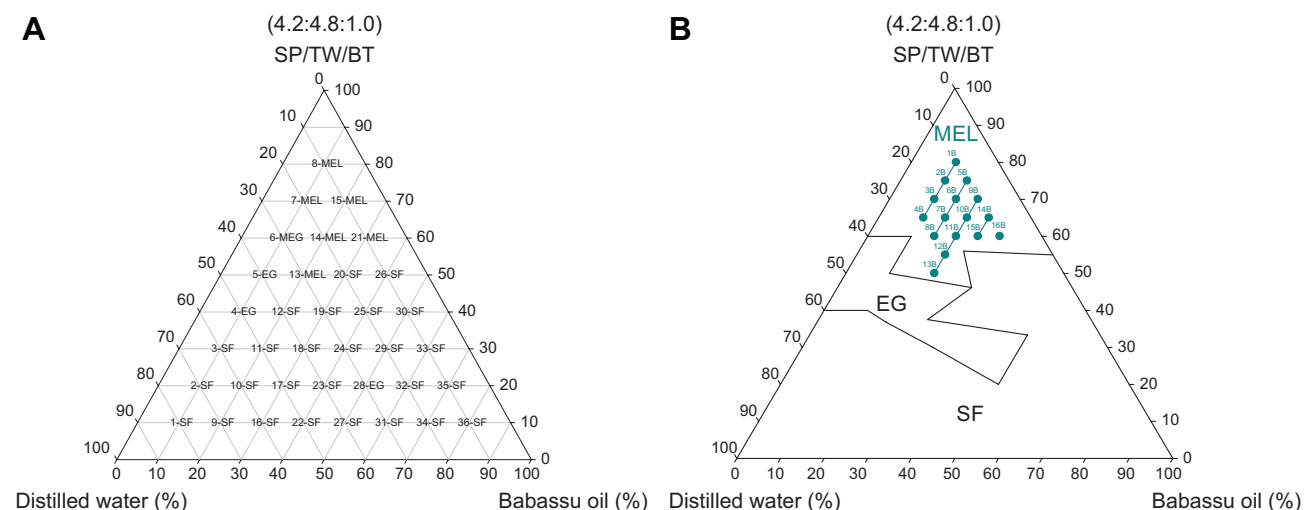


Figure 1 Diagram of the pseudoternary classification of all 36 points in pre-established proportions (A) and domains with points selected for the study system SP/TW/BT (B). **Abbreviations:** MEL, microemulsion liquid; MEG, microemulsion gel; EG, emulsion gel; SF, phase separation; SP, Sorbitan monooleate-Span 80®; TW, Polysorbate 80-Tween 80®; BT, I-butanol.

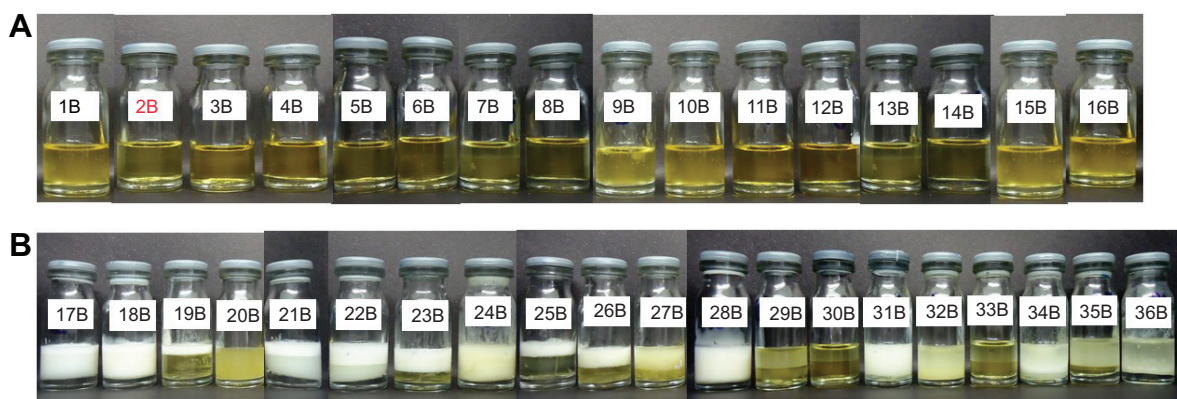


Figure 2 Formulations of study system SP/TW/BT [4.2/4.8/1.0] of 36 points with divergent equilibrium characteristics. Microemulsion liquid (A), microemulsion gel or phase separation (B).

Abbreviations: SP, Sorbitan monooleate-Span 80[®]; TW, Polysorbate 80-Tween 80[®]; BT, 1-butanol.

Rheological characterization

Formulations 12B, 13B, 14B, 15B, and 16B were observed to show nonlinear flow and viscosity (Figure 3A and B), and these formulations were discarded. The stable formulations (1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B, 9B, 10B, and 11B) present in the flow curve began at the origin and then exhibited linear ascending and descending behavior (Figure 3A). This behavior indicates that the formulations are Newtonian fluids.

Viscosity was similar between the formulations and ranged from 0.20 to 0.40 Pa. Formulation 5B demonstrated increased viscosity (Figure 3B).

Figure 4 demonstrates data for stable formulation viscosity variation versus temperature scanning. The viscosity changed with temperature. All of the formulations displayed a gradual reduction in viscosity with increased temperature. For temperatures below 20°C, the MEs demonstrated

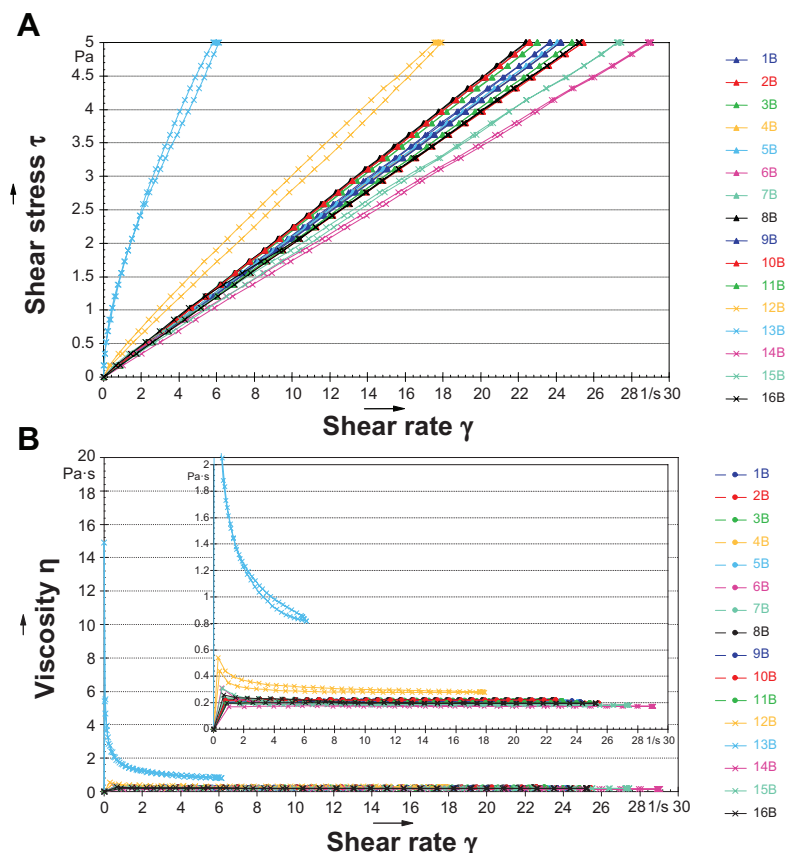


Figure 3 Flow curves (A) and formulation viscosity (B) during the preliminary stability period (25°C).

fluctuations in viscosity, indicating less uniformity and a greater tendency to destabilize, whereas at temperature between 20°C and 45°C, the formulations demonstrated linear and stable flow curves.

Evaluation of formulation rheological behavior and maintenance over time during the accelerated stability study is presented in Figure 5. All of the formulations that were submitted to the accelerated stability study demonstrated a similar profile both in flow curve and viscosity. Formulations 5B and 6B exhibited a rheological profile during 30 days, 10B for 45 days, and 1B, 2B, and 3B were stable for 90 days (Figure 5A and B).

Physicochemical analysis

The results of a physicochemical analysis after temperature cycling for the stable formulations are demonstrated in Table 1. Formulations 4B, 8B, and 11B displayed macroscopic instability after centrifugation, and formulations 7B and 9B displayed changes in translucence under refrigeration; further stability tests for these samples could not be pursued.

All of the formulations were classified as oil in water because they had electrical conductivity values above that of distilled water ($>1.3 \mu\text{S}/\text{cm}$). The pH values obtained before and after the test remained between 6.3 and 6.8.

The physicochemical analyses of the formulations in accelerated stability conditions are presented in Table 2. Formulations 5B, 6B, 9B, and 10B exhibited macroscopic instability after 30 days. Only formulations 1B, 2B, and 3B remained with MEs during the entire test. The pH

values obtained revealed little variation from pH 6.6 to 7.1. Formulations demonstrated minor changes in conductivity and retained oil in water characteristics over the entire trial period.

According to rheological parameters and physicochemical analysis, the 2B formulation (10% oil, 15% water, and 75% surfactants) remained more stable (Figure 2A). Thus, the results were conducted with this formulation.

Hydrodynamic diameter

The hydrodynamic diameter values that were obtained in the developed formulation are shown in Figure 6. The results show the histogram distribution of the hydrodynamic diameter of formulation that resulted in a mean diameter of 277.40 nm.

Superoxide release

Superoxide release increased when the mononuclear phagocytes were treated with oil or ME in the presence or absence of bacteria compared with spontaneous release. The highest superoxide concentrations regardless of bacterial presence were observed when phagocytes were incubated with the ME (Table 3).

Phagocyte viability index

The viability index was higher when the cells were incubated with the babassu oil ME. Cells incubated only with oil demonstrated a similar viability index to that obtained when cells were incubated with culture medium (Table 4).

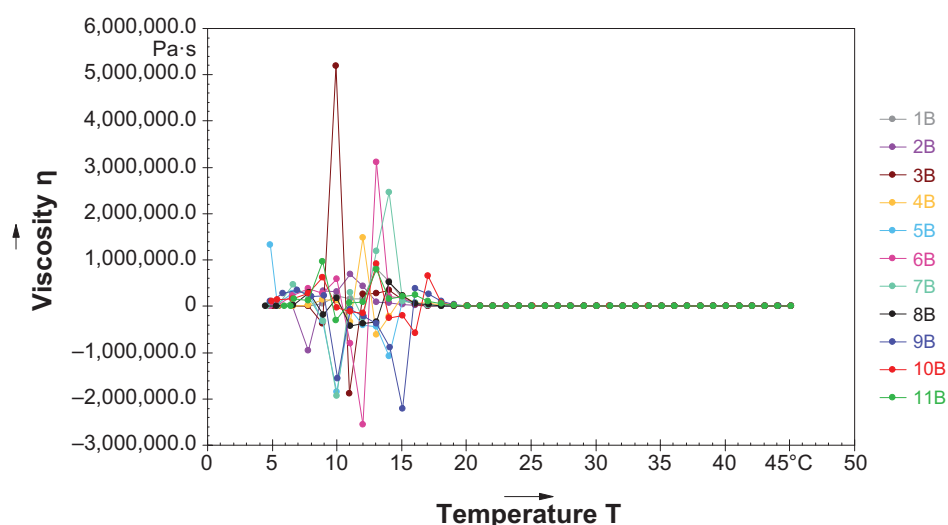


Figure 4 Viscosity curves with respect to temperature (5°C–45°C) for stable formulations with the SP/TW/BT system.
Abbreviations: SP, Sorbitan monooleate-Span 80®; TW, Polysorbate 80-Tween 80®; BT, 1-butanol.

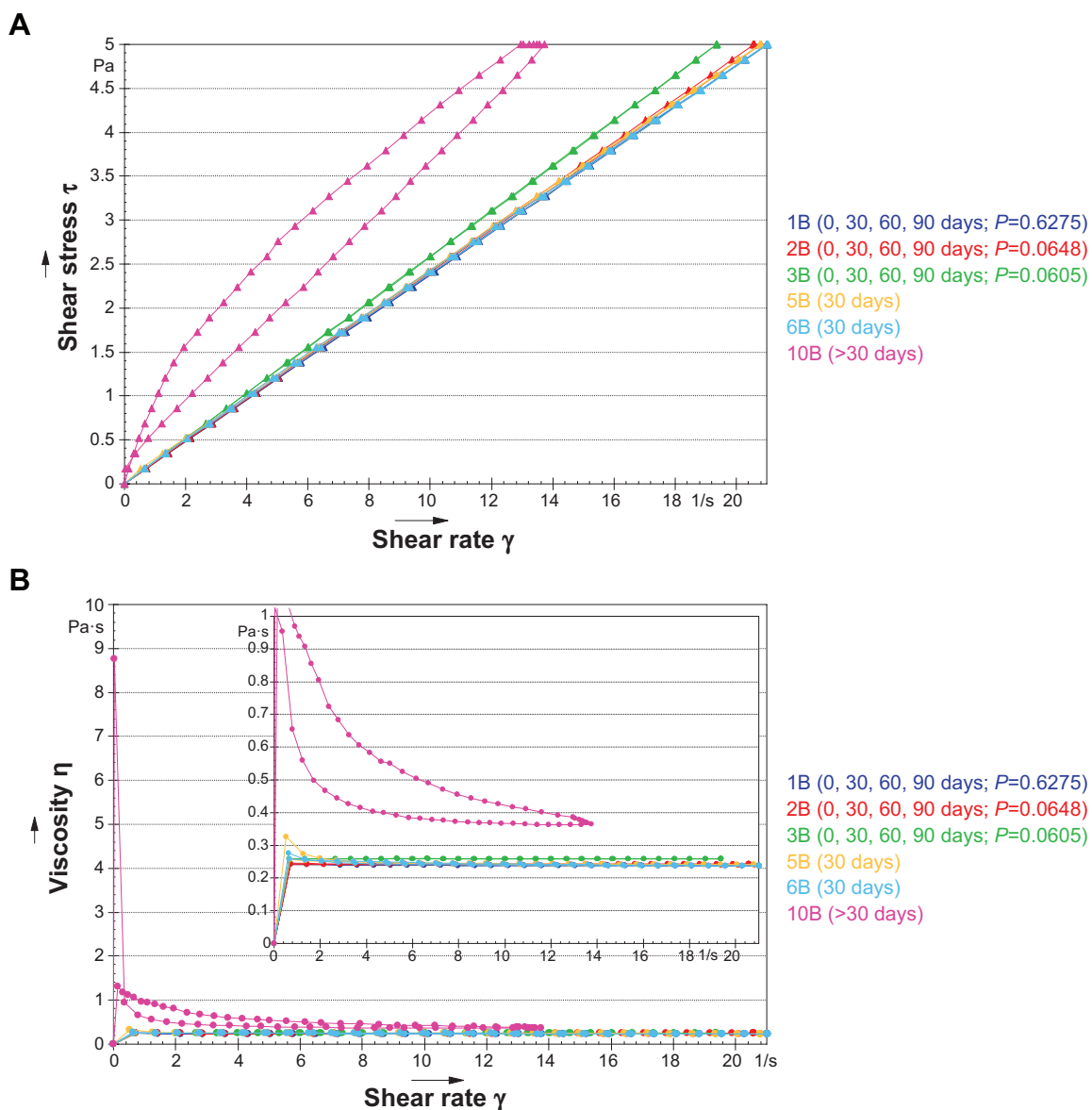


Figure 5 Flow curves (A) and viscosity (B) of formulations during the accelerated stability period (25°C).

Table I Physicochemical analysis for preliminary formulation

Formulation	Centrifugation analysis	Conductivity ($\mu\text{S/cm}$)		pH	
		Before	After	Before	After
1B	N	8.95	7.915	6.33	7.51
2B	N	13.33	22.45	6.33	6.92
3B	N	47.25	41.9	6.33	6.88
4B	A	92.35	57.15	6.33	6.60
5B	N	11.06	11.86	6.33	6.92
6B	N	15.02	12.35	6.33	6.58
7B	N	56.35	46.75	6.33	6.59
8B	A	110.5	77.15	6.33	6.55
9B	N	11.4	9.17	6.33	6.88
10B	N	33.15	24.52	6.33	6.87
11B	A	65.65	55.55	6.33	6.40

Abbreviations: N, normal; A, altered.

Table 2 Physicochemical analysis for accelerated formulation

Formulation	Time (days)							
	0		30		60		90	
	pH	Conductivity (μS/cm)	pH	Conductivity (μS/cm)	pH	Conductivity (μS/cm)	pH	Conductivity (μS/cm)
1B*	6.94	12.13	6.94	9.63	6.60	8.17	7.07	10.04
2B*	6.88	24.00	6.77	19.35	6.89	22.07	6.98	20.28
3B*	6.76	31.80	6.74	36.20	6.92	41.83	6.84	36.73
5B	6.66	11.20	6.94	8.67	Deleted by alteration			
6B	6.83	24.20	6.83	18.92	Deleted by alteration			
9B	7.60	10.25	7.01	8.13	Deleted by alteration			
10B	6.94	27.30	6.88	21.53	Deleted by alteration			

Note: *Stable formulations during all periods.

Phagocytosis and microbicidal activity

Phagocytosis of cells in the presence of oil or ME is demonstrated in Table 4. Blood cells, irrespective of treatment with babassu oil or ME, increased phagocytosis. Babassu oil increased the microbicidal activity of mononuclear blood phagocytes compared with untreated phagocytes. ME in the presence of phagocytes showed increased microbicidal rates compared with untreated cells, but lower microbicidal activity when compared with cells treated with babassu oil (Table 4) ME babassu oil-mediated bacterial killing by mononuclear phagocytes is shown in Figure 7.

Discussion

In this study, we developed a liquid ME formulation from babassu oil with diagrams that demonstrated linear oil phase viscosity and a Newtonian profile. It was classified as oil-to-water, had a biocompatible pH, and was nontoxic, stable, and capable of interacting with blood phagocytes.

The stability of emulsion systems depends intrinsically on interphase interactions established by the emulsifier agent and between the immiscible phases that constitute them. The selection and characteristic ratio of surfactant to be used in the preparation are determined by verifying the HLB of substances, which allows for evaluation of the expected behavior of the compound across polar and non-polar compounds.³

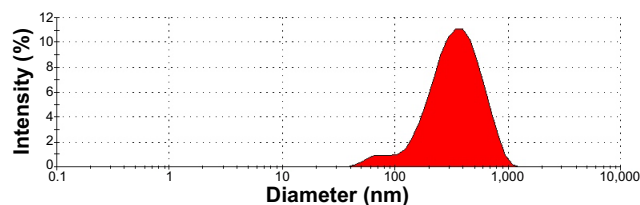


Figure 6 Hydrodynamic diameter of the formulations.

The HLB values for a mixture of surfactants possibly indicates that the systems have a certain classification phase, ie, oil in water or water in oil, during preparation of stable systems. The combination of surfactants also provides HLB values closest to the required HLB in the oily phase.^{2,16} In this study, the mixture of surfactants used for development of the system was SP/TW/BT in a ratio of 4.2:4.8:1.0, which provided an HLB value equal to what was required by babassu oil, which is 10.0. The literature has reported that substances with an HLB between 9 and 16 exhibit hydrophilic characteristics and produce oil in water emulsions.²⁰

Studies have reported that a high HLB indicates more hydrophilic properties and provides lower core growth and a smaller final particle size.²⁰ These observations are theoretically expected because correct HLB adjustment can produce systems with a small particle diameter, thereby generating systems with higher thermodynamic stability.²¹

The phase diagrams indicate that formation of ME regions and emulsified liquid crystalline varies with the proportions of the constituents, and these regions may be conducted in accordance with the applicability to delivery system development.²²

Table 3 Superoxide release by blood mononuclear phagocytes

Mononuclear phagocytes	Superoxide release (nmol)
Spontaneous	3.6±0.4
Oil (babassu)	4.3±0.7*
Microemulsion (babassu)	22.9±2.4*
Bacteria	4.6±0.4*
Bacteria + oil (babassu)	5.6±0.5*
Bacteria + microemulsion (babassu)	22.0±4.0*

Notes: The results are shown as the mean ± standard deviation (n=8 in each treatment). Blood mononuclear cells were treated or not treated with babassu oil or microemulsion in the presence or absence of enteropathogenic *Escherichia coli*. *Indicates differences between phagocytes treated (babassu oil and microemulsion) and incubated with bacteria and the control (without bacteria).

Table 4 Viability of cells (%), phagocytosis and bactericidal indices by blood mononuclear phagocytes as determined by the acridine orange method

Mononuclear phagocytes	Viability (%)	Phagocytosis index (%)	Bactericidal index (%)
Medium 199	93.2±2.4	47.0±13.9	21.6±9.5
Oil (babassu)	94.3±3.1	63.5±9.4*	47.9±15.4*
Microemulsion (babassu)	98.0±0.8*	69.1±12.3*	31.8±5.2*

Notes: The results are shown as the mean ± standard deviation (n=8 in each treatment). Phagocytes were incubated with enteropathogenic *Escherichia coli* in the presence of babassu oil and microemulsion. *Indicates differences from the Medium 199 and treatment (babassu oil and microemulsion).

In this study, phase diagram construction resulted in systems with various characteristics and organization types from a wide range of component combinations. The regions defined in the diagram were dependent on the surfactant concentration. Low surfactant concentrations did not stabilize component mixtures, leading to phase separation, while at higher concentrations, emulsion systems were formed, indicating that higher concentrations of surfactant may maintain the stability of the system.

Parameters indicating physical and chemical stability of emulsions and MEs can be measured by rheological analysis; this technique provides data that are relevant to product structure and allow changes that result in destabilization.²³ Of the 16 formulations that were submitted to rheological tests, only formulation 11 demonstrated a Newtonian profile.

According to the literature, viscosity is largely influenced by controlled-release systems associated with change of temperature.^{2,17} In this study, the rheological behavior of the formulations at different temperatures was characteristic of babassu oil, which is rich in lauric acid, demonstrating altered viscosity and flow curves at temperatures below 25°C.²⁴

In addition to rheological characteristics, other stability studies were performed by physical and chemical analyses.²⁵ Several factors are involved in this process, including physical (temperature, agitation, freezing, centrifugation), chemical (pH, presence of electrolytes, lipid peroxidation),

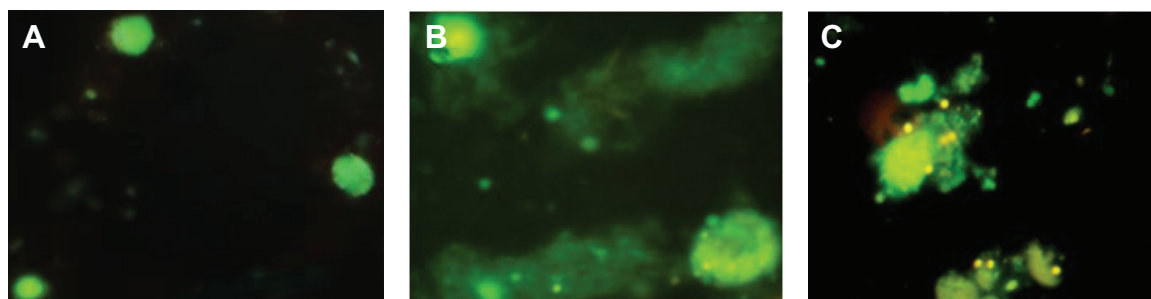
contamination by micro-organisms, and composition of the system.²⁶

In this study, formulations 4B, 8B, 12B, and 13B demonstrated macroscopic instability after centrifugation at a pH of 6.3–6.8 and 6.9–7.1 in preliminary and accelerated stability, respectively. All points and formulations that were studied maintained the oil in water profile after thermal stress. Thus, on the basis of rheological and physicochemical characteristics, the 2B formulation remained more stable.

Light scattering is a routine technique to determination the diameter of the internal phase of MEs.^{3,4} In this study the 2B microemulsion showed diameters with a mean of 277.40 nm. The literature showed the diameters of the structures to be dependent on the ratio of surfactant and oil phase of ME.²² Occurs the formation of nanoscale structures and hydrodynamic diameter decrease when the surfactant proportion predominate over the oil phase.^{3,21–23} These results reinforce the biocompatibility of the system developed.

In contrast, studies have demonstrated that ME systems can be valuable for release of plants with medicinal characteristics^{2,13} as well as for use as vaccine adjuvants.¹ The biological effects of medicinal plants have been validated by research demonstrating microbicidal,^{8,17} anthelmintic,²⁷ antiseptic,²⁸ anti-inflammatory,¹² and immunomodulatory effects.⁹

In this study, the viability of mononuclear blood phagocytes did not change in the presence of the

**Figure 7** Bacterial killing by mononuclear phagocytes in the presence of microemulsion babassu oil.

Notes: Mononuclear phagocytes viability (A). The mononuclear phagocytes was incubated with bacteria and stained with acridine orange and analyzed by fluorescent microscopy. Orange-stained bacteria (dead) and green-stained bacteria (alive). Phagocytosis of bacteria (B) and bacterial killing (C).

babassu oil ME. Several other groups have also related cell viability to functional activity, demonstrating that stimuli such as medicinal plants^{10,29,30} and hormones³¹ can increase release of superoxide anions from mononuclear phagocytes.

Generation of free radicals is reportedly an important organismal defense mechanism during infectious processes.^{20,32–34} In this work, we found that both ME and babassu oil increased release of superoxides from human blood phagocytes. These data are in agreement with the literature,^{9,10,18} in which babassu oil had an immunostimulatory action on superoxide release by phagocytes.

The literature reports that increased superoxide release by phagocytic cells is associated with increased phagocytic and bactericidal activity.^{19,35} However, the use of EPEC is an adequate model in which to analyze the functional activity of phagocytes in the presence of a modified-delivery system.^{10,29–31} Phagocytosis and the microbicidal activity of phagocytes, with production of active oxygen metabolites such as free radicals, comprise an important defense mechanism against a number of bacterial,^{18,19,35} fungal,³⁶ and protozoal infections.³⁷ Here, the babassu oil and ME system increased the phagocytosis of EPEC, which was reflected in microbicidal mechanisms. Conversely, the babassu oil-induced stimulation of microbicidal activity determined pro-oxidative effects and subsequent EPEC killing, suggesting an important immunomodulatory role for this oil. Interestingly, nutraceutical preparations can contain expressive amounts of bioactive compounds which can decrease diseases,^{38,39} and increase immunostimulatory activity. Some bioactive compounds from nutraceuticals can modulate and improve immune function.⁴⁰

Phagocytes produce superoxide-generating oxidants that act as microbicides, which are produced to combat invading micro-organisms; however, they can cause damage and participate in a large number of diseases. Maintenance of antioxidant defenses related to formation of reactive oxygen species is necessary for the survival of organisms and preventing inflammatory processes.⁴¹

Research on natural antioxidants has been intensified.^{30,34} In this study, the babassu ME formulation modulated the functional phagocytic activity. More studies should be conducted to verify the potential of this formulation as an adjuvant for delivery of vaccines.

In conclusion, babassu oil is a natural product, is easy to obtain, and can be added to the diet, so may be an alternative for future immunotherapy applications, in particular for infectious diseases. The babassu oil ME system is an

alternative for possible use as an adjuvant or in modified drug delivery systems.

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Disclosure

The authors report no conflicts of interest in this work.

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