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Production of Carboxymethyl Cellulose Films Incorporating Rue (*Ruta graveolens*) Essential Oil

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Carboxymethyl cellulose (CMC) is considered a non-toxic, biodegradable and biocompatible polymer. CMC-based films are rated as flexible, clear, odorless, moderately tear resistant, resistant to oil and grease migration, and water soluble. Due to its hydrophilicity, CMC can serve as matrix to encapsulate hydrophobic substances, like essential oils, by emulsion dispersions. The essential oil of rue (*Ruta graveolens*) has antifungal, antimicrobial, insecticide and therapeutic properties already reported in the literature. For the incorporation of rue essential oil in the CMC films, the ultrasound-assisted emulsion technique was used. The films were produced by casting evaporation. The rue essential oil emulsion and CMC polymeric films with the incorporation of emulsified essential oil were evaluated according to the emulsion droplet size, chemical composition, morphology and thermogravimetric profile. The droplet size obtained for the emulsion was 229.1 nm. The films showed good transparency, regularity and chemical characteristics similar to those of the pure polymer.

Keywords: *Polymers, encapsulation, ultrasound, natural products, controlled releasing systems.*

1. Introduction

Cellulosic materials are widely used in the food, pharmaceutical, cosmetic and chemical industries in general because they are mostly non-toxic, biodegradable and biocompatible materials. Carboxymethyl cellulose (CMC) is one of these materials with numerous applications. It is a copolymer derived from the polysaccharide cellulose through the introduction of a carboxymethyl group through β-1,4-glycosidic bonds between β-D-glucose and the monosodium salt of β-D-glucopyranose and 2-o- (carboxymethyl)[1,2]. The preparation of CMC is carried out by reacting cellulose in an alkaline medium. The greater the degree of substitution (DS) of the hydroxyl group (-OH) of the polymeric chain by groups containing carboxyl groups (-COOH), the greater its solubility in water, and the more stable and viscous the formed solution will be[2].

Due to this solubility variation, CMC is capable of forming edible and coating films and other applications such as stabilizing and aggregating solid particles, forming gels and stabilizers for food, protective agents, film formers and in cosmetics[3]. Currently, polymeric systems are being a new strategy for the incorporation of active substances, and for that, several encapsulation methods are used. Encapsulation is defined as the imprisonment of materials of interest such as: active compounds, phytochemicals, living cells, flavor compounds, pigments, nutrients, enzymes, essential oils, vegetable extracts, vegetable oils, among others. And it aims to protect constituents from aggressive environments or release them in a controlled manner for a period or in targeted locations. The most applied encapsulation techniques are: spray drying, spray chilling, spray cooling, lyophilization, emulsification, ionic gelation, extrusion, coacervation, liposomes, fluidization, inclusion complexes, cocrystallization, interfacial polymerization, among other techniques. The choice of technique is determined by the substance in which you want to imprison[4].

Among the substances that are being used in polymer films are essential oils. Essential oils are natural substances that play a protective role in plants, being recognized as GRAS (Generally Recognized as Safe) for human consumption. They are also known as volatile oils with a complex structure of lipophilic and odoriferous substances[5]. They can be extracted from leaves, stems, roots, flowers, seeds, roots, among others, which varies according to the plant. Depending on the type of material to be extracted, the technique is chosen, the main industrial extraction techniques are: steam distillation, cold pressing, hydrodistillation, enfleurage, solvent extraction and supercritical fluids.

Many essential oils were successfully incorporated into CMC matrices. Ginger (*Zingiber officinale*) essential oil was incorporated in CMC/polyvinyl alcohol films using high-speed homogenizer. The results as bread packaging increased shelf life from 4 to 30 days[6]. Ajowan (*Trachyspermum ammi*) essential oil was loaded into CMC/gelatin films and the potential use as package was evaluated using an experimental design. The optimized films containing ajowan essential oil presented improved antibacterial and mechanical properties[7]. CMC films with walnut and lemon peels essential oil increased the shelf life of mushrooms[8].

Rue essential oil (*Ruta graveolens*) has antifungal, antimicrobial, insecticide, therapeutic properties, among others, already reported in the literature. *Ruta graveolens* is the scientific name of the popularly known rue or rue.

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Around the world it is known as: Ruda in Spanish; Common Rue or Herb of grace-in English; grace-herbe in French and Henruda in Japanese[9]. It is a plant of the rutaceae family, originating in the Mediterranean region and has grown well in tropical, subtropical and temperate climates[10].

The chemical composition of rue may vary according to environmental conditions and genetic factors of the plant. Bitter elements, resins, gums, tannins, rutin, psoralen, quercetin, alkaloids, organic acids, allantoin, triterpene saponins and mucilage are located. The constituents of the plant can be analyzed by the high-resolution gas chromatography method, which indicates the predominance of long-chain aliphatic esters such as 2-undecanone[11], which can be confirmed by means of the Table 1, which demonstrates the percentage of the chemical composition of rue essential oil commercially purchased from the company Laszlo Aromaterapia Ltda and chromatographic analysis carried out in the Chromatography Laboratory of the chemistry department of UFMG, the product of origin in Spain.

To incorporate essential oils into polymeric materials, the most common encapsulation technique is based on emulsification technologies. Emulsion is a mixture of two immiscible liquids, one of which is dispersed in globules (dispersed phase) in the other liquid (continuous phase). In addition to these two components, there is a third component called emulsifying agent, which contributes to making the emulsion more stable, because it is interposed between the dispersed and dispersing phase, thus delaying its separation, and which constitutes the interface. In this way, these are thermodynamically unstable systems, requiring a considerable input of energy to obtain them, generally mechanical energy[12].

Thus, this work proposes the production of carboxymethyl cellulose films by the casting method with the incorporation of essential oil through the emulsion technique with an ultrasound probe. This work was motivated by the lack of studies using the essential oil of rue (*Ruta graveolens*) in polymeric matrices. As presented before, the rue essential oil is an interesting candidate in many applications. The use of emulsion can cope with the difficulty to incorporate the oily phase in the CMC hydrophilic matrix.

Table 1. Chemical composition of rue essential oil sold by Laszlo.

Constituent	%
α-Pinene	0.2
Camphene	0.2
Sabinene	0.2
β-Pinene	0.1
Limonene	0.4
2-Nonanone	1.6
Camphor	0.1
2-Decanone	0.9
2-Undecanone	93.2
2-Undecane	0.6
Trans-Anethole	0.1
2-Dodecanone	0.3
β-Caryophyllene	0.9
2-Tridecanone	0.3

Source: Laszlo Aromatherapy Ltda

2. Experimental

2.1. Materials and instrumentation

All commercially available reagents and chemicals were of high purity and without further purification: sodium salt carboxymethyl cellulose (CMC, viscosity: 2000–3000 cps) from Labsynth LTDA (Brazil), Polysorbate 80 (Tween 80 P.A) from LS Chemical (India) and rue essential oil (*Ruta graveolens* 100% pure, GT Spain) from the company Laszlo Aromaterapia Ltda (Brazil).

2.2. Preparation and characterization of rue essential oil emulsion

For the preparation of the emulsion essential oil of rue used the technique of emulsion assisted by ultrasound (55W, model Q55, brand Qsonica Sonicators), prepared in aqueous medium with a surfactant agent the polysorbate 80 (Tween 80) and essential oil of rue. The preparation methodology used was developed by Vieira et al.[13], in a proportion of 85% water, 10% surfactant and 5% rue essential oil (density of 0.83 $g.cm^{-3}$). The standard suspension contains 1.0 g of surfactant; 0.5 g of essential oil and 8.5 g of deionized water are stirred in an ultrasound probe at an amplitude of 50% for 120 seconds. It can later be stored in an amber bottle in a refrigerated environment for a few days, but it is recommended to use it immediately after preparation. Thus, the analysis of the distribution of the drops was carried out using the laser particle meter equipment (model Zeta Nano ZS, brand Malven Panalytical). The study reports the size of the drops and the polydispersity index (PDI).

2.3. Preparation and characterization of CMC films with rue essential oil incorporation

The carboxymethyl cellulose solution is prepared in aqueous medium, with 2 g (2% w/w) of polymer and 98 g of deionized water, this value was based on preliminary tests. Then it is taken to the magnetic stirrer for complete homogeneity for 24 hours to contain a minimum number of bubbles, and it can be stored at room temperature for up to 2 months. To prepare the filmogenic solution, 25 g of solution is prepared, consisting of 5 g of emulsion and 20 g of CMC solution. Soon after, they were homogenized in a magnetic stirrer for 3 hours at room temperature and spread in a Petri dish of 20 cm diameter, containing approximately 20 g of the filmogenic solution and taken to an air circulation oven, at a temperature of 25°C overnight.

Analysis of the morphology of the films by SEM (model TM3000, Hitachi) provides relevant information about the homogeneity and interaction between the components, allowing a better discussion of results on the impact of different essential oil concentrations on the structure of the films. The images were taken at 1,000; 5,000 and 10,000 x magnification with 5 kV and 15 kV electron beam capture, seeking the best resolution of the images.

The film samples were cut into small squares, after being dried and kept in plastic packaging without contact with air, the analyzes were carried out in a Spectrometer in the Infrared Region (FTIR) (model 640IR, Varian) equipped with attenuated total reflectance mode, in the region from 650 to 4500 cm^{-1}, with a resolution of 4 cm^{-1}.

The thickness analysis of the films was performed with the aid of a digital micrometer (model Digimatic Micrometer Series 293 MDC-Lite, Mitutoyo Corporation, Japan) with a precision of ±0.0001 mm, in eleven random points, the average being considered as the response to the test. Film density (ρ) was obtained by cutting the film samples into squares (25 x 25 mm, approximately) to calculate the area (A). They were weighted (W) and from the average measured thickness (t), density was calculated using Equation 1.

$$\rho = W / A * t \quad (1)$$

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were carried out on samples of films of pure CMC, CMC with polysorbate 80, CMC with incorporation of the emulsion of essential oil of rue and pure essential oil of rue. Sample analysis were carried out using a thermogravimetric analyzer (model DTG-60, Shimadzu) and subjected to a heating rate of 10ºC.min^{-1} and a synthetic air atmosphere of 50 mL.min^{-1}.

3. Results and Discussion

3.1. Emulsion droplet size distribution

The stability of an emulsion depends on the droplet size distribution, nature and proportion of surfactant and preparation method used. The PDI value is a measure of heterogeneity in the distribution of droplet sizes, in which PDI values close to 1 indicate heterogeneous size distribution and values close to 0 homogeneous size distribution[14]. In this study, the emulsion presented a PDI value of 0.390 resulting in an emulsion with homogeneous distribution.

The Z-average droplet size of the prepared emulsion was 229.1 nm. To be considered a nanoemulsion, the droplet size should be between 10 and 100 nanometers[15]. This size of the drops confers more stability. Still using the laser particle meter, it is possible to obtain the result of the particle size distribution as can be seen in Figure 1, a statistical analysis showed that the drops were concentrated in the region of 100 to 900 nm and high peaks between 160 to 230 nm, in general, a uniform and homogeneous distribution was obtained, as confirmed by the PDI value, close to 0.

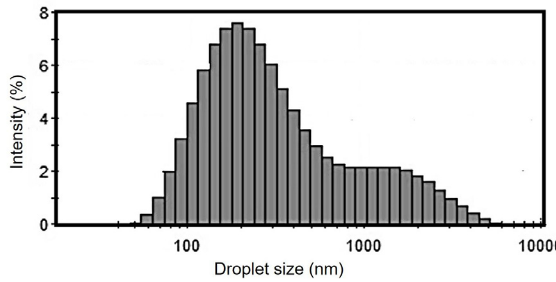

Figure 1. Rue essential oil emulsion droplet size distribution. Source: Authors.

Figure 2. CMC film with EO rue for transparency and opacity evaluation. Source: Authors.

3.2. CMC films morphological characterization

The films produced had a homogeneous surface, a continuous matrix with dispersed particles, without bubbles and were easily detached from the Petri dishes without breaking, not being sticky or too fragile. The films showed transparency on different surfaces and against the light, as shown in Figure 2. When producing films, the objective is to obtain materials with a smooth surface and without roughness, as they may harm the contact surface with the material to which it is to be applied. In addition, depending on the color and transparency of the film, the type of application will be predicted. Thus, through visual analysis, it can be confirmed that homogeneous films were produced as expected, which will later be reaffirmed by scanning electron microscopy analysis. Same behavior was observed by incorporating different essential oils (rosemary, coriander, and nutmeg oil)[16].

For this, pure CMC films and those incorporated with rue essential oil were analyzed. As Figure 3 demonstrates the surface of pure CMC, homogeneous films are observed, without cracks and with the presence of minimal roughness, due to the high viscosity of the CMC. Figure 4 shows CMC film with the incorporation of 5 g of emulsion. Films with spherical globules were produced using ginger essential oil[6], what was not observed in the films produced with rue essential oil. The small droplet sized produced a better distribution on the polymeric matrix. Changing in the microstructure was reported when a higher content of clover essential oil is included in the CMC films[17].

3.3. CMC films chemical composition

Figure 5 presents the results for pure CMC films, films with the incorporation of rue essential oil and pure rue essential oil.

The pure CMC film showed vibration marks of 3370-3350 cm^{-1} related to the O−H stretching of the water molecules and the CMC hydroxyl groups, at 1596 cm^{-1} presence of the C=O stretching of the carboxyl group, at 1418 cm^{-1} of the CH_2 group, in 1324 cm^{1} absorption of the CH_2 group and in 1063 cm^{-1} of the C−O stretch. The bands below 1000 cm^{-1} are complex in nature and encompass polysaccharide fingerprints.

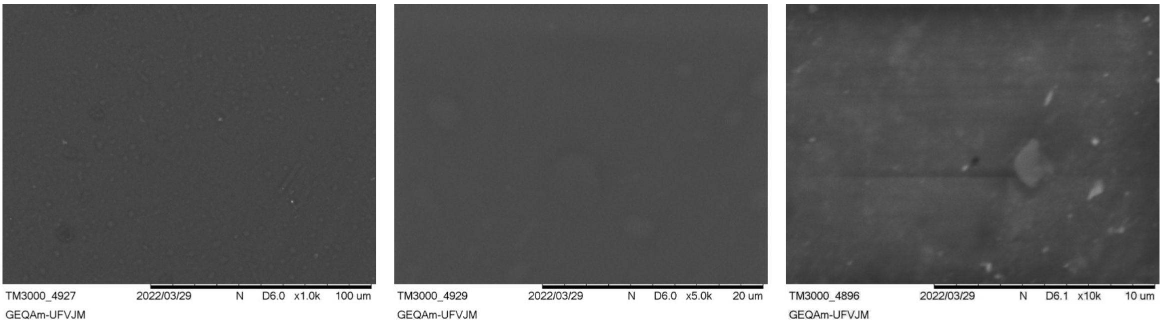

Figure 3. Pure CMC film with 1,000; 5,000 and 10,000 x resolution, respectively. Source: Authors.

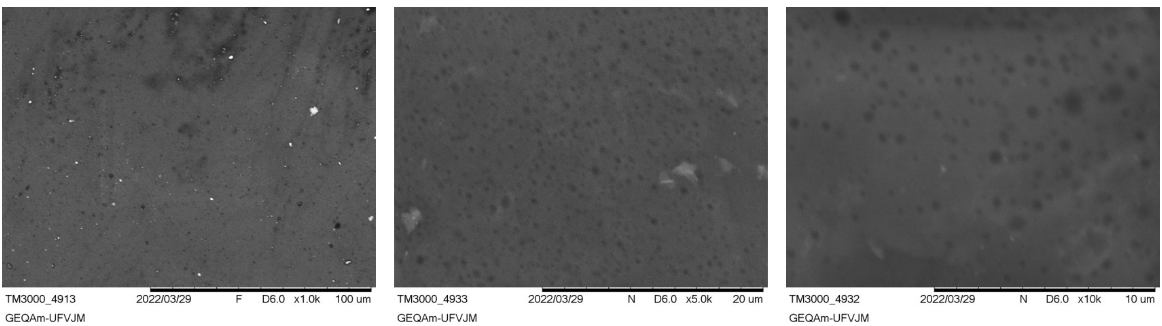

Figure 4. CMC film incorporated with rue essential oil with 1,000; 5,000 and 10,000 x resolution, respectively. Source: Authors.

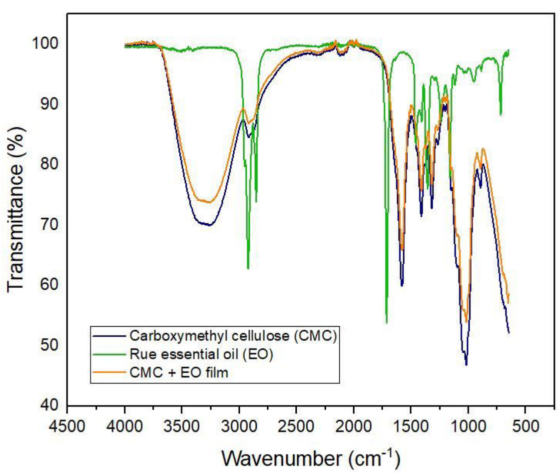

Figure 5. FTIR spectra from CMC film (blue), pure rue essential oil (green) and CMC film incorporated with rue essential oil (orange). Source: Authors.

Rue essential oil presents vibrational marks at 2950 cm^{-1}, representing the carbonyl group of ketones, possibly representing the 2-undecanone compound, which represents the highest percentage of essential oils and, according to the literature, has vibrations in this region. At approximately 1680-1700 cm^{-1} C=O stretch, possibly the presence of compounds such as 2-nonanone, 2-decanone, 2-dodecane, 2-tridecanone which are in smaller percentage. At 1300-1000 cm^{-1} C−O stretch of esters and more properly at 1250-1060 cm^{-1} of aliphatic esters. It is not possible to predict the exact location of these compounds in lower concentrations because essential oils are composed substances with numerous substances together.

The spectrum of the rue film presents similar spectra to the pure CMC film, which indicates that there was no interaction between the active groups of the oil with the functional groups of the CMC, therefore these compounds were free and could be inhibitors of several microorganisms and repellency. Another possible perspective presented for the non-change of functional groups is the low contraction of essential oil in the film[18].

3.4. CMC films thickness and thermal characterization

The film had a thickness of 0.038 mm and exhibited a density of 1.52 $g.cm^{-3}$. Even the film presenting a fixed deposition mass on the Petri dish and the spreading being carried out manually, presented a low thickness and density.

Thermal analysis of CMC films provides relevant information about the thermal degradation process and quantitatively presents the mass loss of the materials. The results of TGA and DTG for the CMC film incorporated with rue essential oil are presented in the Figure 6. According to the results, it is possible to observe the main weight loss occurring around 290ºC. It represents a slightly decrease on thermal stability when comparing with the film without essential oil.

This signal is related to the CMC degradation, in which polymeric chains are degraded under the oxidizing atmosphere. This same behavior was observed for all the analyzed films (pure CMC, CMC with Tween and CMC with Tween and essential oil). The analysis for the pure oil indicated a complete weight loss at 100ºC, indicating that the oil evaporated at low temperatures. Below 200ºC the weight loss is related to the humidity releasing and the most volatile components, such

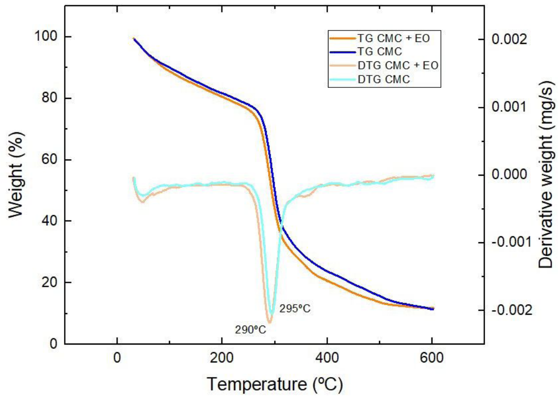

Figure 6. TGA and DTA analysis for the CMC film (blue) and CMC film incorporated with rue essential oil (orange). Source: Authors.

as the rue essential oil. Comparing the weight loss profile between the CMC films prepared with essential oil, it was possible to quantify a difference of 1.2% in weight from the sample with tween only. The film containing essential oil, at 200ºC, presented a lower weight. It may indicate the amount of released essential oil during the analysis. Similar result is reported[6], in which the increment of essential oil decreases the thermal resistance. On contrary, an increase on thermal stability up to 5% in the weight of CMC films when chickpea hull polysaccharides are incorporated is reported[19]. It may represent an alternative on CMC films production.

4. Conclusions

From this study, it can be concluded that the production of the essential oil emulsion of rue was successfully obtained, producing droplet sizes in the order of 229 nm. It was possible to incorporate the emulsion into carboxymethyl cellulose films using the casting technique. The films showed chemical identity close to those of pure CMC, with a homogeneous appearance. The material has good prospects for use in intelligent packaging for the controlled release of active compounds, which still will be analyzed.

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