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Original Article

Development and Characterization of Kojic Acid and *Carnaúba* Wax-Based Solid Lipid Microparticles

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ABSTRACT

Background: The treatment of dyschromia can be conducted using dermocosmetics containing depigmenting agents, such as kojic acid. **Objectives:** This study aimed the development and characterization of *Carnaúba* wax-based micro particles, in order to disseminate kojic acid in cosmetic and dermatological products. **Methods:** These elements were developed and characterized by electron microscopy and spectrophotometry, in addition to the evaluation of the encapsulation effectiveness and the release profile. **Results:** The different formulations showed that the variation of the concentrations of the active compound did not interfere with the Solid Lipid Micro particles (SLM) morphology, showing spherical shape and variation in their sizes. The formulation that showed the best yield was M1. All formulations showed low effective encapsulation effectiveness, varying approximately between 5 and 15%. **Conclusion:** The stirring speed and the stirring time are critical factors in the obtainment process and in the morphology of solid lipid micro particles.

Key words: Kojic acid, Carnaúba, Lipid Microparticles.

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INTRODUCTION

Changes in skin color, named dyschromias, are the result of quantitative differences in the melanin pigment and may arise due to solar radiation and skin aging.[1] The treatment against dyschromia can be conducted by means of dermocosmetics containing depigmenting agents, such as kojic acid, which, in addition to inhibiting melanin synthesis, acts as an antioxidant due to its anti-free radicals activity.[1-3]

Since the last decade, several strategies for developing drug delivery systems have contributed to the improvement of topical formulas, thereby improving the therapeutic index of drugs, increasing their effectiveness and reducing their toxicity.[4,5] Micro particulate delivery systems are modern and relevant tools in the delivery of drugs, as they are capable of compartmentalizing or encapsulating the active compound, thereby directing it to specific targets and regulating its release speed without changing the transported chemical molecule.[4,5]

Solid Lipid Microparticles (SLM) represent the most modern concept of lipid carriers. They are derived from oil-in-water emulsions, changing the liquid lipid by a lipid or mixture of solid lipids at room temperature and stabilized by surfactants.[6-10] The lipids used can be triglycerides, glycerides and waxes. In them, the active compound is dispersed and encapsulated in the form of particles that control its release by diffusion through pores or erosion.[7,10]

Waxes are interesting materials for the production of SLM, as they have the advantage of being well-known raw materials and dispense with the use of organic solvents.[7-10] Among the natural waxes, we can find beeswax, *Carnaúba* wax and *cupuaçu* wax and many others like them.[7] Due to its lipophilic and non-polar nature, *Carnaúba* wax stands out for its antioxidant, photoprotective and stable properties, being widely used in the chemical, pharmaceutical, food, cosmetic, computer and automobile industries.[7-12] In addition, its extraction is an important economic activity in the Brazilian Northeast, where the state of Ceará is one of the main exporters.[12-15]

In the U.S.A., *Carnaúba* wax is classified as Generally Recognized as Safe (GRAS) and its use takes place in the amounts that are required to adhere to the good manufacturing practices (GMP) in several foods.[14]

In light of the foregoing, this study had the objective of developing and characterizing *Carnaúba* wax-based microparticles, in order to disseminate kojic acid in cosmetic and dermatological products intended for skin care, with exfoliating and depigmenting action. The lack of scientific articles and patents constitutes this invention as a novelty of an inventive character.

We should highlight that, through this study, it was possible to make a patent deposit of the product formulated under the protocol BR102017009172-4 in the patent bank of the National Institute of Intellectual Property of Brazil. This work was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) with registration number ABF9765.

MATERIALS AND METHODS

Materials

Kojic acid and *Carnaúba* wax were purchased from All Chemistry, Brazil. Some filters with 0.45 µm pores and a Chromafil® P-45/25 syringe, 25 mm, was obtained from Macherey-Nagel, Düren, Germany. C42 blue band quantitative filter paper 125 mm, JP41 black band quantitative filter paper 12.5 cm and JP42 blue band quantitative filter paper 9 cm were purchased from J Prolab, São José dos Pinhais, Paraná, Brazil. Tween 80 (Pharmaceutical grade) was purchased from Via Farma, Brazil.

Quantification of kojic acid

We used the spectrophotometric method developed and validated by Sato (2003).[16] A standard solution of kojic acid at 40 µm/mL was prepared at room temperature 25°C and, from that, diluted solutions of concentrations 4, 8, 12, 16, 20 and 24 µm/mL were obtained. We used distilled water as White, where the wavelength was 269 nm, using the spectrophotometer (Genesys 10S UV-Vis model, Thermo Scientific®). Moreover, we constructed a standard curve with six points.

Production of Solid Lipid Microparticles (SLM)

It was used the hot homogenization method adapted from Gugu (2015).[17] An aqueous solution containing 1% Tween 80 and kojic acid was heated to 90°C using a heater (Q 261 A model, Quimis®). The *Carnaúba* wax, at a concentration of 10%, was melted at the same temperature and then the aqueous solution was dispersed in the lipid material using an Ultra Turrax® homogenizer (T 25 D S32 model, Ika®) with stirring. After stirring, the material was placed in an ice bath where it remained until the temperature of 20°C was reached. Subsequently, the material was filtered through a vacuum pump (P-730 BN model, Pall Life Science®), using JP42 blue band quantitative filter paper. The solid lipid microparticles disposed on the surface of the filter paper were recovered by oven drying (EB-100 model, Lab Trade®), at 40°C for 24 hr.

Evaluation of the influence of the active proportion/ lipid matrix, stirring speed and time in the production of SLM

This evaluation was performed in 3 steps. In Step 1, we produced 3 SLM (M1, M2 and M3), with a concentration of kojic acid at 10, 20 and 50% in relation to the lipid mass, respectively. In this step, we used a stirring speed of 15000 rpm and 5 min of stirring. In Step 2, we produced 3 SLM (M4, M5 and M6), with 50% kojic acid in relation to the total mass of the lipid matrix. In this step, the variables were stirring speed and stirring time. In order to accomplish the production of M4, we used a stirring speed of 5000 rpm and a stirring time of 5 min; M5 was prepared at 3400 rpm for 5 min and M6 was prepared at 5000 rpm for 2 min. In Step 3, we produced a SLM (M7) with 10% kojic acid with a stirring speed of 5000 rpm in a stirring time of 5 min. The objective of this step was to compare M1 and M7, in order to observe the influence of the stirring speed on the production process.

Characterization of SLM

This procedure was performed through the analysis of the SLM morphology, the process yield, the encapsulation effectiveness and the release of the active compound.

Scanning Electron Microscopy (SEM): The samples were characterized by Scanning Electron Microscopy (SEM) (Quanta 450 FEG, FEI Company, USA) at a 30 kV voltage, after the previous coating with the silver under vacuum spraying, using a QT150 ES device (Quorum Technologies, USA).

Analysis of the process yield: It was obtained by dividing the actual yield (amount of formulation obtained at the end of the process) by the theoretical yield (amount of formulation expected to be obtained considering all material used) multiplied by 100.

Evaluation of the encapsulation effectiveness: The EE% encapsulation effectiveness of kojic acid in the prepared SLM was determined using the following equation: (1)

$$EE\% = (A/B) * 100$$

Here, A is the actual amount of kojic acid encapsulated in SLM and B is the theoretical amount of kojic acid in SLM.[17,18] The A values were determined as follows: In order to melt the encapsulating lipid matrix and release the active compound, we prepared a mixture with 25 ml of deionized water and 0.1 g of the M1 SLM formulation and then this mixture was heated at 90°C with magnetic stirring (mini magnetic stirrer with heating, Q-261A model, Quimis®) for 10 min. The mixture was removed from heating, left to stand at room temperature 25°C for 5 min and taken to an ice bath until the wax was solidified. Subsequently, we performed vacuum filtration (vacuum pump, P730-BN model, Pall Life ScienceS®) using JP42 blue band quantitative filter paper. The amount of kojic acid extracted was determined using a UV-visible spectrophotometer (Genesys 10S model, Thermo Scientific®) at a wavelength of 269 nm. The testing was performed in triplicate.

Release testing for kojic acid

We performed this procedure by adding 1 g of the M1 SLM formulation to 250 ml of 0.1% aqueous Tween 80 solution. The paddle was rotated at 100 rpm and maintained at a temperature of 37 ± 1°C. The USP paddle method was adopted in this study. The total testing time was 6 hr. In the first hour, 5 mL aliquots were removed every 15 min. After the first hour, aliquots were removed every 30 min. The aliquots were removed using a syringe attached to a filter. With each aliquot removed, the volume was reconstituted with 0.1% Tween 80 aqueous solution so that it remained constant throughout the testing procedure. The filtered solutions were taken to an uv-vis spectrophotometer (Genesys 10S model, Thermo Scientific®) and readings were performed in triplicate at a wavelength of 269 nm.[17]

RESULTS

Quantification of kojic acid

The calibration curve for quantification of kojic acid showed a linear equation (y) = 0.0326x + 0.0049 and correlation coefficient (r) = 0.9999, thereby demonstrating a linear response in the concentration range between 4 and 24 µm/mL.

Evaluation of the influence of the active proportion/ lipid matrix, stirring speed and time in the production of SLM

The results were obtained from the microscopic visualization of the morphology of the formulations (Figures 1-3).

The M1, M2 and M3 formulations demonstrated that the variation of the active concentrations did not interfere with the SLM morphology; however, when the micro particles are loaded with 20% (M2) and 50% (M3) kojic acid, some sort of surface irregularity was observed in the spheres. When comparing the M4, M5 and M6 formulations, it was observed that the stirring speed and the stirring time are critical factors of the process. Among the three mentioned formulations, M4 obtained greater homogeneity in relation to the presence of non-emulsified wax crystals in the production process of SLM. This result may be associated with higher stirring speed and time applied during the production process. When relating the M4 and M5 formulations, whose only variable was the stirring speed, it was found that M5 showed a slight presence of non-emulsified wax crystals in the process, besides a larger size of the microspheres. This is due to the low stirring speed applied in the production process of M5. When comparing M4 and M6, whose only variable was the stirring time, it was observed that M6 showed an expressive presence of non-emulsified wax crystals in spite of the same stirring speed. Regarding the M5 and M6 formulations, it was found that M5 showed a smaller amount of non-emulsified wax crystals, but a larger size of spheres. This result proved that the stirring speed and the stirring time are critical factors in the production process of SLM.

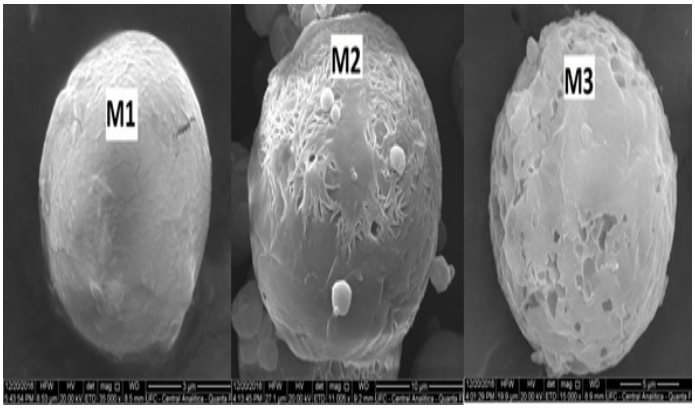

Figure 1: Scanning Electron Microscopy (SEM) images of the M1, M2 and M3.

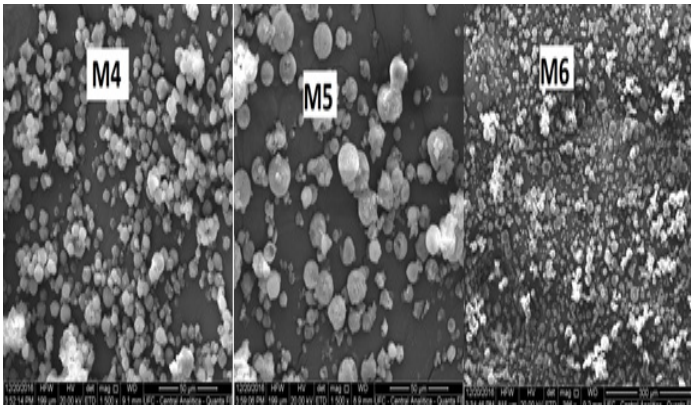

Figure 2: Scanning Electron Microscopy (SEM) images of the M4, M5 and M6.

When comparing M1 and M7, it was observed that the increase in speed is inversely proportional to the size of the particles.

Characterization of SLM

Determination of SLM morphology: The SLM morphology can be observed in Figures 1-3. SLM showed a spherical shape and variation in their sizes in all formulations.

Analysis of the process yield: The result of the calculation of the yield of the formulations is described in Table 1.

The formulation that showed the best yield was M1. When comparing M1, M2 and M3, it was observed that M1 showed the best yeld. In view of this, it was observed that there is an inverse relationship between the process yield and the concentration of the active compound. When comparing the M1 and M7 formulations to the M3 and M4 formulations, whose only variable was the stirring speed, it was observed that the formulations produced with higher stirring speed showed a better result in the yield.

Evaluation of the encapsulation effectiveness: The result of the evaluation of the encapsulation effectiveness is described in Table 2.

All formulations showed low effective encapsulation effectiveness, varying approximately between 5 and 15%. These results signalize the difficulty of encapsulating an active compound with a hydrophilic nature in a lipid matrix by the chosen method. When comparing the M1 and M3 formulations, it was observed that the result shown for M1, even using a lower concentration of kojic acid, was very close to that shown by M3. When relating the M1 and M7 formulations with the M3 with M4 formulations, whose only variable was the stirring speed, it was observed that the formulations produced with a higher stirring speed demonstrated better results of encapsulation effectiveness. The characterization results selected the M1 formulation, with concentration of 10% kojic acid in relation to the lipid mass, developed with a stirring speed of 15000 rpm and 5 min of stirring, in order to proceed with the release testing for kojic acid.

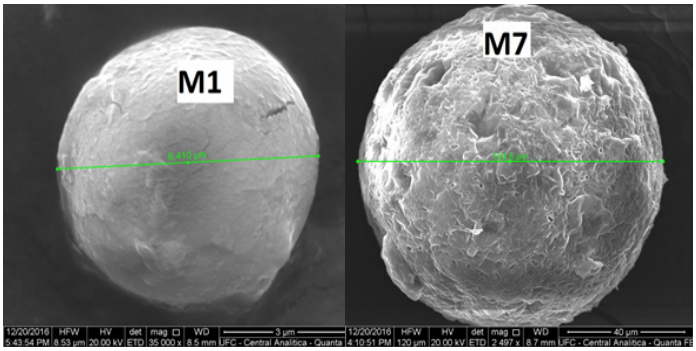

Figure 3: Scanning Electron Microscopy (SEM) images of the M1 and M7.

Release testing for kojic acid

The release profile of kojic acid can be observed in Figure 4 and Table 3.

We performed the release testing for kojic acid with the M1 formulation because it was the one that showed the best result in the characterization of SLM. The release value of kojic acid at the end of the analysis was 41%. The results reveal that the release of kojic acid took place slowly and progressively, thereby proving the modified release character of SLM.

DISCUSSION

Microscopic analysis is the simplest direct method for the morphological determination of particles. In the present work, SLM showed a spherical shape and variation in their sizes. Chambi *et al.* (2006) developed SLM produced by spray cooling, containing water-soluble compounds of different molecular masses and found similar results in obtaining

Table 1: Fomulations outputs results.

	M1	M2	M3	M4	M5	M6	M7
Output (%)	87,7± 0,21	81,4± 0,87	71,2± 1,24	64,54± 1,7	64,2± 0,92	64,8± 1,67	83,7± 1,1

Table 2: Results of the encapsulation efficiency test of the formulations.

	M1	M2	M3	M4	M5	M6	M7
Encapsulation efficiency (%)	11,68± 0,65	5,26± 2,3	15± 1,1	5,42± 2,8	6,04± 0,71	4,55± 0,39	7,04± 1,7

Table 3: Values (time and average of%) to build the graphic and standard deviation values to add error bars.

Time (Minutes)	% Released	Standard Deviation
15	4,92	1,24
30	10,76	0,89
45	16,96	2,75
60	21,57	4,71
90	26,14	4,55
120	29,46	3,99
150	32,46	1,98
180	35,06	2,78
210	37,46	4,23
240	39,76	3,12
270	41,16	4,31
300	42,46	3,11
330	43,48	2,73
360	44,5	1,98

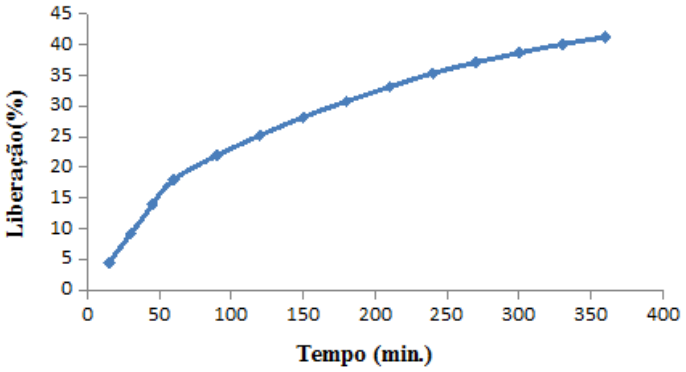

Figure 4: Kojic acid release profile.

spherical SLM of varying sizes.[19] When comparing the production methods of SLM and their yield results, the method of this work revealed results superior to those of Lira (2007), who found a 45% yield result by the ionotropic gelation method in the production of SLM of retinoic acid and chitosan.[20] Nosari (2012) obtained yield results varying from 21 to 32% using the spray congealing method in the production of SLM from green coffee oil and beeswax.[12] All formulations showed low effective encapsulation effectiveness, varying approximately between 5 and 15%. The result may have been due to the fact that the lipid matrix is composed only of *Carnaúba* wax. When the lipid matrix is composed of a single type of lipid, crystallization leads to the formation of a perfectly ordered crystal with minimal defects, thereby resulting in less space for the filling to be accommodated. Accordingly, the lipid matrix will hardly be able to effectively incorporate significant amounts of the active compound.[6-8] It was possible to observe that formulations with higher stirring speed demonstrate better results of encapsulation effectiveness. This result suggests that the high shearing speed may favor a greater contact among the hydrophilic active compound, the surfactant and the lipid matrix, thereby increasing the encapsulation effectiveness of the process. Another critical factor that can influence encapsulation effectiveness is the type of method used in the production process of SLM. The results of the encapsulation effectiveness of SLM produced by spray congealing found by Nosari (2012) varied between 20 and 40%.[10-12] Fernandez (2014) developed SLM by spray cooling with results of effective encapsulation effectiveness between 51 and 65%.[19] Lira (2007) found an encapsulation effectiveness value of less than 5% in SLM produced by the multiple emulsion method, thereby concluding the inappropriateness of the method. When he switched to the ionotropic gelation method, he found encapsulation effectiveness values varying between 54 and 63%.[20] The study verified the modified release character of the produced SLM. The lipid matrix composed of *Carnaúba* wax makes the particle wall more rigid and stable, thereby delaying the release of the active compound. The release of water-soluble substances encapsulated with *Carnaúba* wax in an aqueous medium may be due to the characteristic of the polarity of the filling that tends to migrate to the solution, associated or not with the erosion of the lipid matrix.[18-20] The study developed by Fernandez (2014) found results varying from 22 to 72% in the release profile of glucose in aqueous solution for 120 min. An analogous result was found in the present study, as the release profile of kojic acid in aqueous solution for 120 min was 25%.[19]

CONCLUSION

The stirring speed and the stirring time are critical factors in the obtainment process and in the morphology of solid lipid micro particles. Accordingly, the higher the stirring speed, the smaller the micro particles obtained and the higher the process yield. The use of a single surfactant at a concentration of 1% was not sufficient to obtain a high encapsulation effectiveness, since the proposed system is the microencapsulation of a hydrophilic active compound using a single lipid as a carrier matrix. Conversely, we have successfully obtained the slow and progressive release of kojic acid, thereby proving the modified release characteristic of solid lipid micro particles.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SLM: Solid Lipid Microparticles; **GRAS:** Generally Recognized as Safe.

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