Candelilla Wax as Natural Slow-Release Matrix for Fertilizers Encapsulated by Spray Chilling

Noé Navarro-Guajardo[1], Edgar Miguel García-Carrillo[2], Carlos Espinoza-González[2],*, Rubén Téllez-Zablah[1], Fátima Dávila-Hernández[2], Jorge Romero-García[2], Antonio Ledezma-Pérez[2], Jesús Alfonso Mercado-Silva[3], Claudia Anahí Pérez Torres[4] and Nicolaza Pariona[5]

[1]Technological Institute of Saltillo, Blvd. Venustiano Carranza 2400, C.P. 25280, Saltillo, Coahuila, México
[2]Research Center for Applied Chemistry, Sonomimetikos Research Group, Blvd. Enrique Reyna Hermosillo 140, C.P. 25294, Saltillo, Coahuila, México
[3]Research Center for Applied Chemistry, National Laboratory of Graphene, Blvd. Enrique Reyna Hermosillo 140, C.P. 25294, Saltillo, Coahuila, México
[4]CONACyT – Institute of Ecology A.C., Carretera antigua a Coatepec 351, El Haya, C.P. 91070, Xalapa, Veracruz, México
[5]Network for Advanced Molecular Studies, Institute of Ecology A.C., Carretera antigua a Coatepec 351, El Haya, C.P. 91070, Xalapa, Veracruz, México

ABSTRACT   Efforts to improve crop yields with efficient use of fertilizers are needed to guarantee global food security. Enhanced slow-release fertilizer systems (SRFs) encapsulated in biodegradable matrices are being developed to address this global concern. From a wide range of strategies for SRFs development, we explored a nature-inspired solution based on the plant cuticle model and its function as a membrane for water and nutrient transport control. Here, Candelilla wax, extracted from Candelilla wild plants (*Euphorbia antisyphilitica*), is studied as a renewable slow-release matrix for fertilizers encapsulated by a modified spray chilling process. From this process, microencapsulates containing 40 wt% of phosphorus fertilizer are obtained with distinctive sizes and chemical characteristics, presenting a slow-release behavior. Considering the abovementioned features, novel insights into fertilizer release mechanisms based on plant cuticle models are discussed.

KEYWORDS: Candelilla wax, encapsulation, phosphorus, slow-release fertilizers (SRFs), spray chilling

1   INTRODUCTION

Food security is a global concern that needs to be addressed with strategies that allow actual and future generations to satisfy an ever-growing demand. However, our current agricultural practices to improve crop productivity use an excess of fertilizers, which have a negative impact on the environment, human health and resource conservation [1]. In particular, phosphorus (P) is the second most important macronutrient for plants after nitrogen.

When P is applied as fertilizer, plant uptake is limited to 10–25% and the rest is lost to complexation reactions and leaching in soil [2]. Nutrient depletion in crops requires intensive fertilizer applications, which in turn contaminate water and soil through leaching [1]. In addition, global phosphorus reserves derived from phosphate rock are expected to reach peak global production by 2033; so, depletion in 50–100 years is expected [3].

One strategy to improve nutrient use efficiency is by implementing slow-release fertilizers (SRFs), which are classified as a) organic-N low-solubility compounds, b) encapsulated fertilizers, and c) inorganic low-solubility compounds [1]. In the second category, fertilizer particles are coated or encapsulated with polymer matrices, which enables nutrients to be released over an extended period [1]. In these systems, synthetic polymers have been predominantly used due to their availability and variety. However, the use of non-biodegradable synthetic matrices leads to serious environmental problems associated with the accumulation of plastic residues, due to their persistence and extremely slow decomposition in soil [1,4]. These concerns have motivated research towards the development of encapsulated fertilizers with biodegradable natural or synthetic

Corresponding author: carlos.espinoza@ciqa.edu.mx

polymers/blends derived from renewable natural resources. Some examples include matrices of chitosan [5], cellulose [6], starch [7], natural rubber [8], among others. Nevertheless, the limitations of these matrices pose new challenges in the development of lower-cost advanced technologies and novel concepts in the mechanisms of slow-release fertilizers [2].

Nowadays, biodegradable compounds derived from renewable resources are being used in artificial systems to replicate their functions in nature [9]. This novel approach, named *biokleptic*, is gaining a lot of attention in the development of new materials focused on solving global concerns through nature-based solutions. In nature, the outermost layer of plant surfaces is covered by epicuticular waxes, whose varied chemical composition, crystallinity, and hierarchical structures permit water and nutrient transport control, while retaining its protective functions against colonization or predation by animals and microorganisms [10]. Yeats and Rose described how waxes regulate up to 95% of the water diffusion in the plant cuticle through possible channels created by the amorphous domains containing more polar and cyclic waxes [11]. These hydrophilic channels display a high dependency on temperature and relative humidity [12]. Therefore, these features of natural waxes could be used as a novel idea for the development of slow-release microencapsulate fertilizers.

Epicuticular waxes of desert plants are of interest since they must allow, under extreme insolation conditions, a controlled water release while optimizing absorption and retention of this scarce resource. The Candelilla plant (*Euphorbia antisyphilitica*) is a shrubby spurge native to the Chihuahuan Desert region, an area encompassing northeastern Mexico and southeastern United States (Figure 1a). To survive the harsh environmental conditions, this succulent secretes a thick wax layer as protection, whose multiple uses in the food, cosmetic and pharmaceutical industries have led to its being exploited ever since 1910 [13]. Processes for harvesting and extracting Candelilla wax (CWax) nowadays remain rustic, as approximately 3500 rural households in northeastern Mexico cut the uncultivated plants from the wild lands and then prepare a raw isolate called "cerote" by boiling them in hot water with citric acid (Figure 1b) [14]. CWax has a melting point in the range of 69–73 °C, with a chemical composition mainly formed by odd-numbered n-alkanes (C_{29} to C_{33}), wax esters, acid esters, secondary alcohols in even-numbered carbon chains (C_{28} to C_{34}), free acids, free alcohols, sterols, resins and minerals [15]. To guarantee the sustainability of this renewable source, several efforts towards plant reforestation, wax yield improvements, and the development of advanced materials for new applications must be fulfilled.
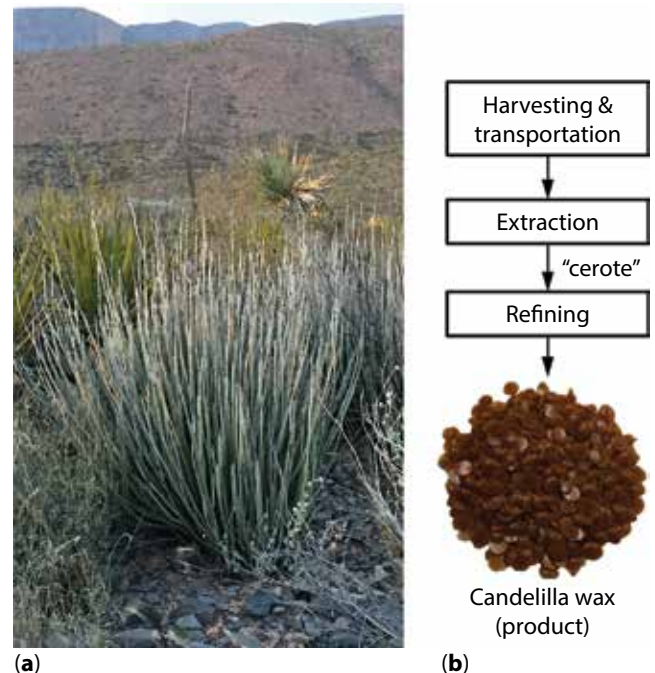
Figure 1 Source and extraction process of Candelilla wax. (**a**) Candelilla wild plant (specimen) (*Euphorbia antisiphylitica*) grown in the northern Chihuahuan Desert. Location: 24° 57′ 23.8″N and 102° 02′ 43.9″W, Salitrillos, Zacatecas, México. (**b**) General process for the extraction of epicuticular waxes in Candelilla plants.

Non-biodegradable synthetic waxes, such as paraffin, have been used for the development of SRF encapsulating matrices [16,17]. Nonetheless, the implemented encapsulation technologies are time and energy consuming, and require the use of organic solvents. Therefore, more sustainable and low-cost advanced technologies are needed for the use of natural waxes and their features in novel encapsulating matrices. A potential solution lies in the spray chilling process, as it is one of the most used encapsulation technologies in the pharmaceutical, cosmetics and food additives fields [18]. This technology consists of the atomization of a melt matrix, containing a suspension or emulsion of an active ingredient, into an environment maintained at temperatures below the melting point of the matrix. The atomization leads to the formation of small droplets, which then solidify on cooling, generally resulting in microencapsulates with spherical shapes and smooth surfaces [18]. This technology is reasonably simple to apply and scale-up, as it does not require the use of organic solvents and the application of high temperatures in the process. Despite its numerous economic and industrial scale-up advantages, this technology has not been widely used for agricultural applications. In this context, low molecular weight matrices such as vegetable oils, with melting points around 70 °C, are

being used as microencapsulating matrices of fertilizers by spray chilling [19].

In the present work, the use of biodegradable matrices derived from renewable resources and sustainable encapsulation technologies were considered to address one of the main concerns in agriculture associated with the excessive use of fertilizers. Herein, we studied the performance of Candelilla wax as a biodegradable matrix for microencapsulation of a P fertilizer using a modified spray chilling process. The choice of a cuticular wax as encapsulating matrix should confer a slow-release behavior that replicates the regulated water and nutrient transport features of this material in plants. The stability of microencapsulates produced by spray chilling and the release profiles were evaluated and the results discussed.

2 EXPERIMENTAL SECTION

2.1 Materials

Refined Candelilla wax in the form of flakes (7833 – Candelilla Real® Refinada) was purchased from Multiceras S.A. de C.V. (Monterrey, México). CWax has a melting point in the range of 69–73 °C. Commercial-grade monoammonium phosphate (MAP) granules purchased from GreenHow S.A de C.V. (Jalisco, México) were used as P fertilizer source (61 wt% P_2O_5). MAP has the highest P content of any common solid fertilizer. All reagents were used as received.

2.2 Preparation of MAP-Fertilizer Microparticles by Spray Drying

The general scheme for preparation of MAP-fertilizer microparticles encapsulated in Candelilla wax is presented in Figure 2. First, MAP microparticles were obtained by spray drying. In brief, 100 g of pre-dried MAP was dissolved in 200 ml of deionized water and heated to 60 °C under magnetic stirring. The MAP solution was spray dried with a lab scale mini spray dryer (Büchi Mini Spray Dryer B-290, BÜCHI Labortechnik, Switzerland). The processing conditions of spray drying (tested previously) were: liquid feed of 5 ml/min, inlet drying air temperature of 140 °C, outlet air temperature of 70 °C, aspiration rate set at 100% (38 m^3/h), atomizing air flow of 473 L/h and nozzle air pressure of 500 kPa. At these operating conditions, dried microparticles of MAP-fertilizer were produced and collected in the drying chamber and collector glass (Figure 2). To prevent rehydration, the dried powder obtained from the collector glass (smaller particles) was stored with silica gel in an oven at 80 °C until used.

2.3 Preparation of a Melt Dispersion of MAP-Fertilizer in Candelilla Wax

A dispersion of MAP microparticles (40 wt%) in CWax was produced by melt mixing. First, 60 g of CWax was melted at 90 °C in a 600-mL beaker on a stirring hot plate provided with a temperature controller. Then, 40 g
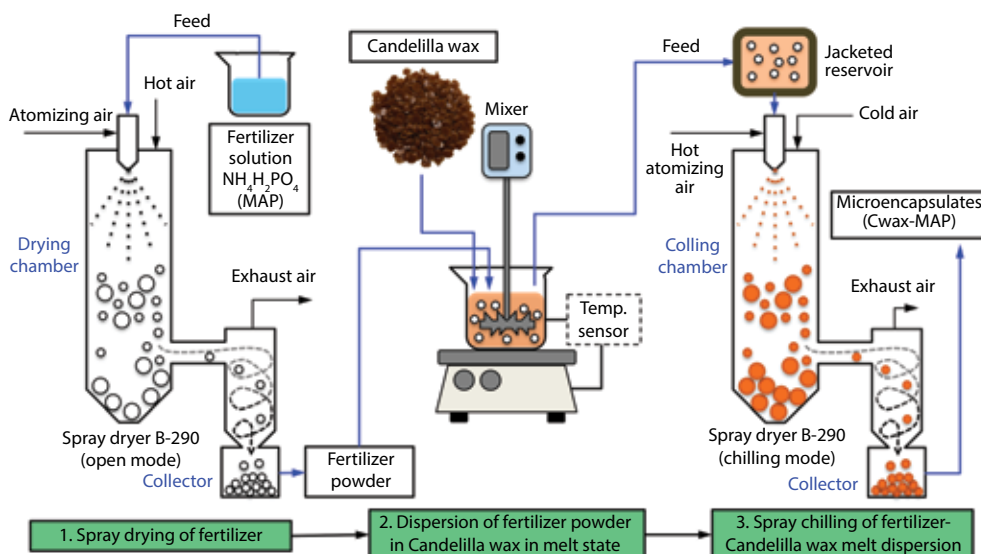
Figure 2 Schematic representation of the production of microencapsulates of MAP in Candelilla wax by spray chilling.

of MAP microparticles (from the collector fraction) was added to the CWax melt. The mixture was vigorously mixed for 4 h at 800 rpm, using an IKA Eurostar mixer (Staufen, Germany) with a propeller stirrer.

2.4 Encapsulation of MAP-Fertilizer in Candelilla Wax by Spray Chilling

In order to obtain microencapsulates of MAP in the CWax matrix, the previous melt dispersion was atomized by spray chilling. The spray-chilling configuration of the Mini Spray Dryer B-290 was used for this encapsulation process. For the preparation of microencapsulates, 100 g of the melt dispersion was placed into a jacketed reservoir of the spray-chilling accessory. This jacketed reservoir contains ethylene glycol (PEG-400) at 120 °C in order to preserve the dispersion in melt state during the atomization process. Then, the needle valve was completely opened for dosage of the melt dispersion to the pneumatic two-fluid atomizer. The microencapsulates were formed and solidified upon contact with cooling air. The processing conditions of spray chilling (tested previously) were: inlet cooling air temperature of 13 °C, aspiration rate set at 100% (38 m³/h), atomizing air flow of 600 L/h, and nozzle air pressure of 500 kPa. The atomizing air was set at 140 °C using an auxiliary heating unit adapted to equipment. At these processing conditions, microparticles of encapsulated systems (CWax-MAP) were produced and collected in the cooling chamber and collector glass. In addition, CWax microparticles without fertilizer were prepared for comparative analysis. All systems obtained from spray chilling were collected in sealed bags and stored in a desiccator.

2.5 Characterization of CWax-MAP Microencapsulates

The morphology of microencapsulates was analyzed by scanning electron microscopy (SEM) using a JCM 6000 NeoScope benchtop SEM (JEOL, USA). The samples were placed on a carbon tape and analyzed with an accelerated voltage of 15 kV at low vacuum conditions. A particle size characterization was realized using imaging software (ImageJ). X-ray powder diffraction (XRD) analysis was performed to determine the crystalline structure of microencapsulates using a Bruker D8 Advance diffractometer (Bruker AXS, Germany). X-rays of λ = 1.54056 were generated with a CuKα source. The diffraction was measured in 2-theta (2θ) range from 10 to 60° with a step of 0.02 °/s. Thermal stability of microencapsulates was determined by means of derivative thermogravimetric analysis (DTGA) using a TA Instruments Q500 TGA.

A heating scan from 30 to 600 °C at 10 °C/min under nitrogen atmosphere was performed for each sample.

For comparative purposes, XRD analysis of MAP-fertilizer and Candelilla wax microparticles, as well as DTGA measurements for Candelilla wax microparticles were also performed. Additionally, molecular weight analysis of the Candelilla wax microparticles was conducted by size exclusion chromatography (SEC) using an Agilent 1100 GPC (Agilent, USA) apparatus equipped with a refractive index detector. The samples were dissolved in chloroform (CHCl$_3$).

2.6 Determination of the Release Profile of Fertilizer from Microencapsulates

The release profile of MAP from microencapsulates (CWax-MAP) was determined using an electrical conductivity test. Microencapsulate samples (2.5 g) were placed into sealed pouches made of unbleached natural coffee filters, and then incubated in 200 mL of deionized water at 25 °C without stirring. The electrical conductivity of the samples was measured after 4, 8 and 24 h during the first day and then every 24 h until the end of the experiment (7 days). The released nutrient concentration was determined directly from a standard calibration curve for MAP. The fertilizer release profile was estimated as the cumulative release percentage versus the incubation time. All tests were conducted in duplicate.

3 RESULTS AND DISCUSSION

3.1 Morphology of Microencapsulates

Generally, the preparation of slow-release fertilizers using waxes consists of the pulverization of fertilizer granules (0.5–2 mm) in a hammer mill to obtain smaller particles (300–100 μm), which are then coated by a thin layer of wax melt [16, 17]. This preparation method produces reservoir-type microencapsulates in which the nutrient is covered by a layer or multiple layers of the encapsulating matrix. A major disadvantage of the reservoir-type microencapsulates is the nonhomogeneous length of nutrient travel paths, i.e., nutrients at the core follow longer paths compared with those at the surface. Therefore, the release rate generally decreases over time [20]. On the other hand, matrix-type microencapsulates are microparticles containing solid microdomains (less than 100 μm) or nanodomains (at nanoscale) of nutrients dispersed in the encapsulating matrix throughout the volume of the microparticle. This type of microencapsulates, which can be produced by atomization processes, provides a more uniform nutrient path.

In order to produce matrix-type microencapsulates by spray chilling, we first produced microparticles of MAP by spray drying. Fertilizer microparticles displayed a mostly spherical morphology with a particle size distribution in the range of 2–10 µm (see Supplementary Data, Figure S1). The MAP microparticles were added to the CWax melt, obtaining a stable suspension, since solid particles remained suspended for a relatively long time. A stable dispersion melt is attributed to good interactions between the fertilizer and the CWax matrix. The hydrophilic components in CWax would facilitate the breakdown of agglomerates and aid dispersion.
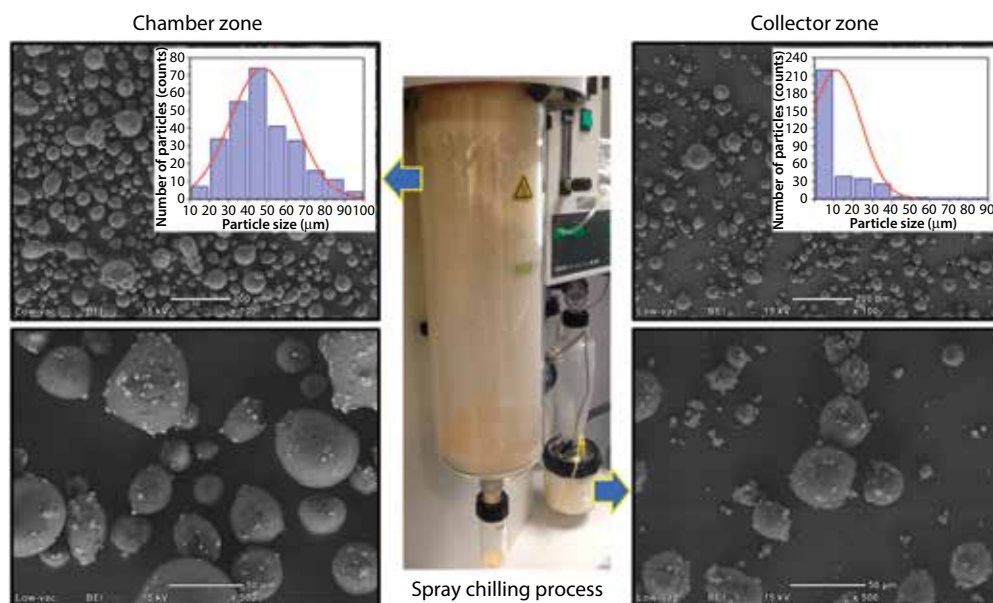
The SEM images of CWax-MAP microencapsulates are shown in Figure 3. Microencapsulates were distributed in an approximate mass ratio of 50:50 between the chamber and collector zones, and displayed a mostly spherical morphology. The microencapsulates present significant differences in particle size, which are attributed to complex interactions between processing conditions with the chemical and physical characteristics of the encapsulating matrix [21]. All available pneumatic-type two-fluid atomizers have a processing limitation for melt dispersions containing solid particles in a mass fraction exceeding 30 wt%. Different modifications to the atomizer design have been realized to increase the feeding limit up to 50 wt% [22]. Herein we demonstrated that by increasing the temperature of the atomizing air by means of an auxiliary heating unit adapted to equipment, it is possible to fragment melt dispersions with higher content of solids into sub-millimetric particles.

On the other hand, microencapsulation by spray chilling has presented some technological drawbacks associated with a low encapsulating efficiency when processing crystalline encapsulating matrices. During the solidification stage, fast cooling of the melt particles induces unstable arrangements of the chains (polymorphic form or imperfect crystallization), which tend to achieve more stable arrangements during storage, resulting in the expulsion of the active ingredient from the encapsulating matrix [23]. From SEM images in Figure 3, it can be noticed that fertilizer microparticles are embedded in the encapsulating matrix. Even after 2 months of storage at room temperature, fertilizer microparticles remained embedded within the microencapsulate, demonstrating a good encapsulate stability. Therefore, the stabilization of fertilizer microparticles in the CWax-encapsulating matrix could be influenced by the crystalline behavior of the wax.

3.2 Crystalline Microstructure of Microencapsulates

X-ray diffraction (XRD) analysis provides information on crystalline characteristics of materials, such as crystalline structure and preferred crystal orientation, which can be useful for studying structure-property relationships. For controlled and slow-release systems, it has been reported that the


Figure 3 SEM images of the CWax-MAP microencapsulates obtained by spray chilling. The encapsulated systems obtained from the chamber (left-hand side) and collector zone (right-hand side) are shown. Inset graph: corresponding particle size histogram with Gaussian fit.

behavior of nutrient release depends on the crystalline microstructure of the encapsulating matrix [24, 25]. On the other hand, the stability of micro- and nanoparticle dispersions in polymer matrices is crucial for high performance in functional materials, which is influenced by the crystalline behavior of the matrix [26].

The XRD patterns in Figure 4a show the crystalline structure of microencapsulates and their separate components. The microencapsulates from chamber and collector zones present crystalline planes corresponding to those presented by their components (MAP and CWax). From Figure 4a, the XRD pattern for MAP-fertilizer microparticles present prominent peaks corresponding to the (101), (200) and (112) lattice planes, which are characteristic of its tetragonal phase according to a MAP spectrum from the International Centre for Diffraction Data database (ICDD card no. 00-089-7401). The differences in the peak intensity can be attributed to the different sizes and orientation of the fertilizer powder. On the other hand, Candelilla wax microparticles present two high-intensity X-ray peaks corresponding to the (110) and (200) lattice planes, which are characteristic of the orthorhombic phase presented for the majority of cuticular waxes [27,28]. The predominant constituent or compound class often defines the crystalline structure of the epicuticular wax crystals. In this context, the peaks corresponding to (110) and (200) lattice planes were found to be characteristic of long-chain odd n-alkanes crystals [27]. Recently, it was found that the orthorhombic phase of CWax is defined by its main component, n-hentriacontane (C_{31}) [29]. These reports are in good agreement with the XRD patterns obtained for CWax (Figure 4a).

From Figure 4 it can also be noticed that MAP and Candelilla wax microparticles have the (200) lattice plane in common. The presence of this lattice plane in common opens up a discussion on the CWax-MAP interactions and its implications in fertilizer encapsulation. In this regard, it is known that the presence of inorganic particles affects both crystal nucleation and growth of semicrystalline polymer matrices. Due to a high affinity between the crystalline structure of a particle and the crystal phase of polymer, the particles act as nucleation sites promoting heterogeneous crystal nucleation of the polymer matrix [30]. A condition of high crystalline affinity occurs when the periodic distances of the molecules conforming to a lattice plane of the matrix are an integer multiple of an identically oriented lattice plane of the substrate (inorganic particle), i.e., the periodicities that conform to their lattice dimensions are phase coherent or coincident [31]. In our case, the fertilizer and wax
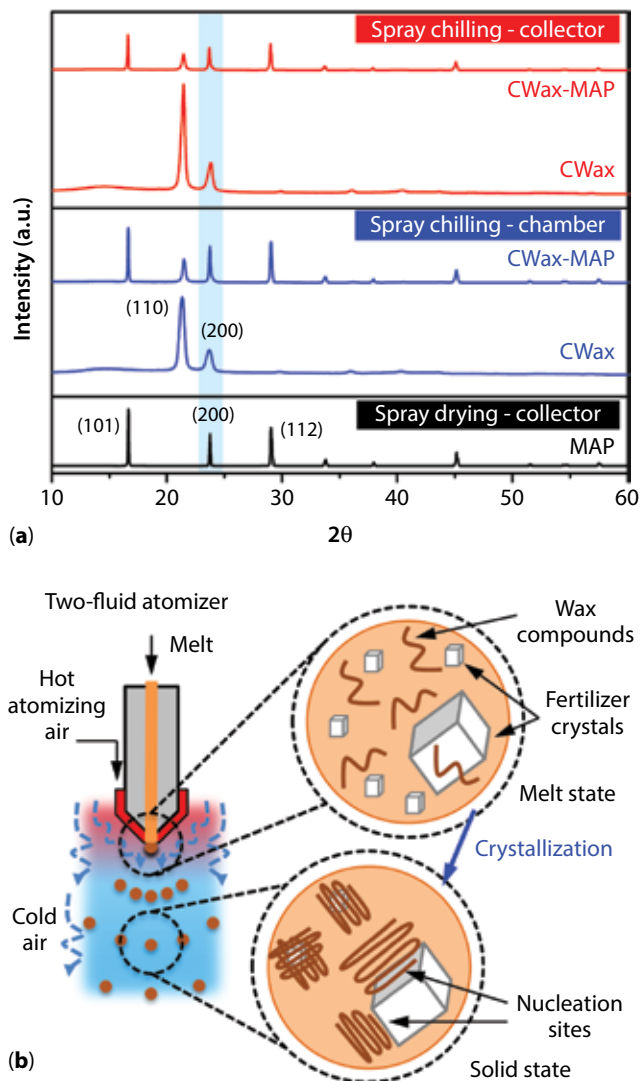
Figure 4. X-ray analysis reveals lattice match conditions between fertilizer-CWax crystals with effects on the encapsulating process. (**a**) XRD patterns of microencapsulates and their separate components. (**b**) Schematic representation of the encapsulating process of the fertilizer microparticles by crystallization of the wax matrix.

crystals match at the (200) lattice plane (Figure 4a); therefore, this coherent interface seems to satisfy conditions that drive crystal nucleation of CWax from the surface of the fertilizer microparticle. As a result, during cooling of sub-millimetric particles in spray chilling, some wax compounds could nucleate around the surface of a fertilizer crystal, using it as a substrate for crystal formation. In fact, the lattice-matching condition is used as a strategy to improve physicochemical properties of drugs, such as aqueous solubility and stability, through formation of co-crystals by spray chilling [32].

Accordingly, it is possible that crystallization of CWax compounds allows the "capture" of fertilizer microparticles, embedding them in the encapsulating matrix (Figure 4b). These observations could support the outstanding encapsulation stability after 2 months of storage at room temperature mentioned in the previous section [23]. As a plausible explanation, the unique chemical composition of CWax and its resulting crystalline structures might play an important role in the stability of microencapsulates.

3.3 Thermal and Chemical Characterization of Microencapsulates

Thermogravimetric analysis (TGA) was performed to evaluate the thermal stability of the microencapsulates. Figure 5 shows the differential weight loss (DTGA) curves for CWax-MAP microencapsulates derived from the weight loss (TGA) curves (inset graph). The TGA curves for microencapsulates display a multistage thermal decomposition, where the overall onset temperature of decomposition is detected above 180 °C from DTGA curves. Thus, these materials can withstand an encapsulation process at 140 °C (temperature of the atomizing air used in this work) without any thermal decomposition.

From DTGA curves, four characteristic mass losses are observed. The first mass loss is near 180 °C, which is attributed to tightly bound water in fertilizer microparticles [33]. The second mass loss at DTGA peak comprised in 200–320 °C range corresponds mainly to n-alkanes, since Candelilla wax contains approximately 50 wt% of these compounds [15]. The third
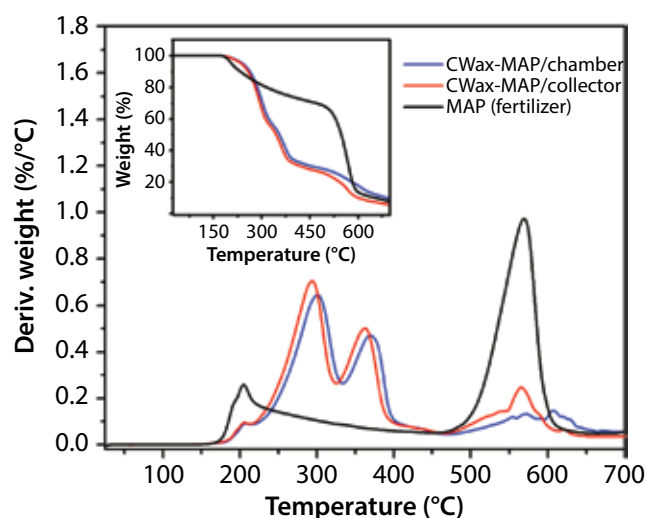
mass loss has a DTGA peak at 320–400 °C, attributed to long-chain n-alkanes and high-chain fatty acid esters and alcohols [34]. The fourth mass loss at DTGA peak comprised in 470–640 °C range corresponds to MAP-fertilizer degradation.

Surprisingly, the microencapsulates obtained from the chamber zone present a higher thermal stability evidenced by a shift of the second and third peaks towards higher temperatures. Since CWax is a complex mixture of compounds, these results suggested that the encapsulating matrix of both microencapsulates could be made up of different wax compounds. To answer this question, we isolated the effect of the fertilizer by preparing only CWax microparticles using the same spray chilling processing conditions. Candelilla wax microparticles from chamber and collector zones were obtained with a similar particle size distribution as microencapsulates (see Suplementary Data, Figure S2). Then, a DTG analysis was developed for these wax microparticles, finding a similar thermal behavior as compared to microencapsulates. The TGA curves for wax microparticles confirmed a multistage thermal decomposition (see Suplementary Data, Figure S3). The CWax microparticles obtained from the chamber zone also presented a higher thermal stability evidenced by a significant shift of the second and third DTGA peaks towards higher temperatures. It has been found that high-chain esters enable a significant increase in the onset of decomposition temperature up to 30 °C, when the number of carbon atoms in the chain increases from C_{14} to C_{20} [34,35]. Therefore, our results suggest that the encapsulating wax matrix from the chamber zone could have a higher content of long-chain compounds including n-alkanes, esters and alcohols. The separation of wax compounds into short- and long-chain compounds seems to occur in melts during atomization.

To confirm this effect, we subsequently developed a GPC analysis for CWax microparticles obtained from the chamber and collector zones (see Suplementary Data, Figure S4). From this analysis, both microparticles presented a multimodal distribution curve with notable differences in the broadening and shifting. These results evidence that the chemical composition of the encapsulating matrix is different for both microencapsulate systems.

It should also be mentioned that, despite these differences in chemical composition of the encapsulating matrix, no major differences were found in the crystalline structure of microencapsulates (Figure 4a). The differences in chemical composition do not interfere with the encapsulation stability through "capture" of the fertilizer microparticles by wax crystals. It is possible that secondary compounds, different from n-hentriacontane, also play an important role in the

Figure 5. DTGA and TGA curves of microencapsulates reveal that the encapsulating matrix of both microencapsulates could be made up of different wax compounds.

stability of microencapsulates. However, the search for answers to these questions is beyond the scope of this study.

We would like to highlight that, to the best of our knowledge, there have been no other reports on the segregation of compounds during the spray chilling processing of natural waxes. Furthermore, this is the first report regarding microencapsulate fabrication with matrices of different chemical compositions in one-pot processing by spray chilling.

From these results, it is evident that the unique chemical composition of CWax has a distinct effect on the crystalline structure, encapsulation stability and thermal behavior of the resulting microencapsulates.

3.4. Fertilizer Release Profile of the Microencapsulates

Figure 6a shows a photograph of the release test developed for microencapsulates, whilst Figure 6b shows the MAP release profile from the CWax matrix. Within 7 days, microencapsulates from the chamber and collector zones released 40 and 36% of the total nutrients respectively, whereas the conventionally used granular fertilizer dissolved completely in 5 min. Both microencapsulated systems presented a similar release behavior.

The fertilizer release profiles obtained for microencapsulates are comparable to some synthetic encapsulating matrices using similar test conditions. For example, Liang *et al.* [36] evaluated the release profile of phosphates coated with poly(acrylic acid-co-acryl-amide), obtaining a 36% cumulative release of the nutrient after 7 days. Du *et al.* evaluated the release behavior of phosphates coated with polyurethane-like coating, resulting in a 30% cumulative release of the nutrient in 70 days [37]. The latter system presented a lower release percentage within a longer period of time in comparison with our system. However, the polyurethane system showed a lag period in the range of 35–40 days, which was associated with a low diffusion coefficient of the nutrient in the synthetic matrix. A prolonged lag period could lead to a plant nutrient deficit. On the other hand, similar results were obtained for a non-coated controlled-release fertilizer developed by Zhao *et al.* [38], where ~35% of a phosphate fertilizer was released after 7 days of testing. The obtained microencapsulates using Candelilla wax as a natural encapsulating matrix demonstrated a slow-release behavior, which competes in performance with synthetic ones.

The theorized property of fertilizer's slow release in CWax matrices can be attributed to its properties of water and nutrient transport control. How epicuticular waxes enable water absorption and nutrient
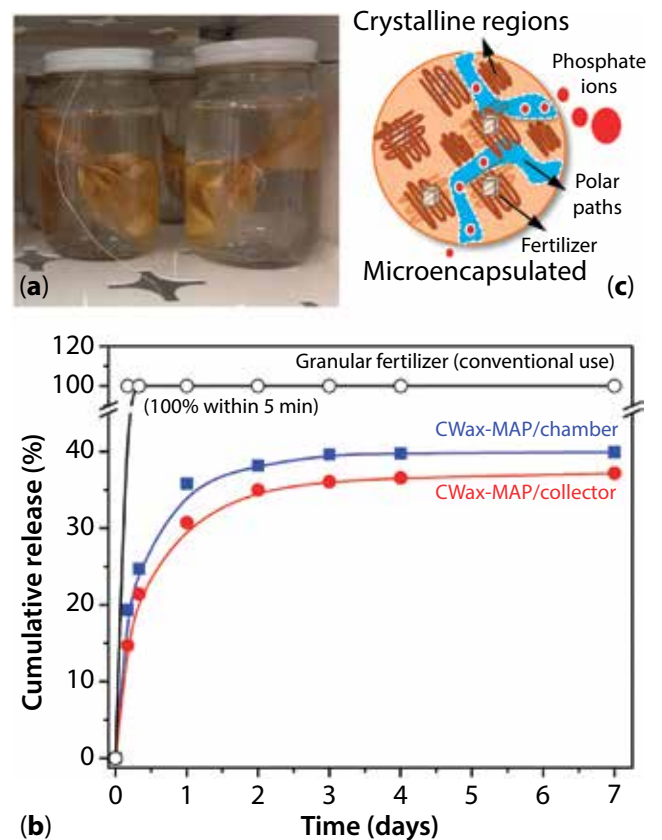
Figure 6 Candelilla wax acts as a natural slow-release matrix for MAP. (**a**) Photograph of the fertilizer release test. (**b**) Cumulative fertilizer release profiles from microencapsulates over the incubation time at 25 °C. (**c**) Schematic representation of the fertilizer release mechanism through polar paths in the Candelilla wax matrix.

transport through its crystalline structure is still a matter under discussion [39]. A plausible mechanism for slow release of MAP could be proposed based on two transport mechanisms for cuticular waxes under debate: (1) The number of crystals and its spatial arrangement determine the water and nutrient transport paths, which are located in the amorphous phase (non-crystalline region) [40]; (2) lipophilic pathways (composed of hydrophobic compounds) transport nonionic and lipophilic molecules, whereas polar pathways (composed of hydrophilic compounds) transport polar and ionic solutes [41,42]. Therefore, since the orthorhombic crystalline phase in CWax is mainly defined by *n*-hentriacontane (lipophilic compound) [29], the amorphous region could principally contain hydrophilic compounds acting as polar pathways for water absorption and fertilizer release (Figure 6c).

Evidently, the chemical composition and the crystalline structure of the cuticular waxes are critical

features determining the transport properties. In nutrient slow-release systems, highly ordered crystalline regions could significantly delay the absorption of water, hindering the dissolution and diffusion of nutrients. The release profiles for both microencapsulate systems (Figure 6b) did not differ significantly despite their differences in particle size (Figure 3) and chemical composition of the encapsulating matrix (Section 3.3). However, the influence of factors such as microencapsulate processing conditions, the pH and temperature of the release medium, could reveal hidden effects on release profiles, which is a topic that deserves further research. Once the fertilizer is released from the Candelilla wax microparticle, it is expected that wax compounds will be degraded in soil, since epicuticular waxes become one of the primary sources for organic matter in soils after plants die [43].

4. CONCLUSIONS

In this work, we have demonstrated that Candelilla wax, an epicuticular wax extracted from the Candelilla plant (*Euphorbia antisyphilitica*), can be used as natural encapsulating matrix for slow release of a fertilizer by a modified spray chilling process. The microencapsulates have been demonstrated to provide a slow-release behavior comparable in performance with synthetic ones. The encapsulation process of monoammonium phosphate with Candelilla wax and the study of the microencapsulate properties revealed two important features that have implications for encapsulation stability and nutrient release performance: (1) A possible phenomenon of segregation of wax compounds may occur during spray chilling of a melt dispersion, resulting in one-pot preparation of two microencapsulate systems with different particle size distributions and chemical compositions of the encapsulating matrix. (2) A good lattice match found between the MAP crystals and the encapsulating matrix suggests that wax compounds could nucleate around the surface of a fertilizer crystal, using it as a substrate for wax crystal formation.

From these features, we propose that *i*) the crystallization of CWax compounds allows the "capture" of fertilizer microparticles, embedding them in the encapsulating matrix, and further contributes to the good encapsulation stability observed during storage; *ii*) a specific mixture of hydrophilic wax compounds enables the formation of enough amorphous regions in both microencapsulate systems, creating polar pathways for water absorption and fertilizer release.

The nutrient release mechanism of these microencapsulates could replicate the functions of water and nutrient transport mediated by cuticular waxes, where the amorphous regions transport water and ionic solutes and the spatial arrangement of crystalline regions determine the water and nutrient transport paths responsible for the slow-release behavior. Future works should focus on the role of physical factors in the spray chilling of natural waxes, including the study of encapsulation and nutrient release mechanisms influenced by external factors such as pH and temperature.

In perspective, the results of this work provide new insights into the development of fertilizers encapsulated in biodegradable matrices from renewable sources to address one of the main concerns in agriculture. Also, this work contributes to the conservation efforts of Candelilla as an endemic plant and the sustainability of rural communities by means of technology development for the creation of novel added-value products.

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