Saudi Pharmaceutical Journal 26 (2018) 410–415

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Release and permeation profiles of spray-dried chitosan microparticles containing caffeic acid

Caroline Magnani Spagnol [a], Ana Melero Zaera [b,*], Vera Lucia Borges Isaac [a], Marcos Antonio Corrêa [a], Hérida Regina Nunes Salgado [c]

[a] Laboratory of Cosmetology, Department of Drugs and Medicines, São Paulo State University (UNESP), School of Pharmaceutical Sciences, Araraquara, SP, Brazil
[b] Department of Pharmacy, Pharmaceutical Technology and Parasitology, University of Valencia, Valencia, Spain
[c] Quality Control Laboratory, Department of Drugs and Medicines, São Paulo State University (UNESP), School of Pharmaceutical Sciences, Araraquara, SP, Brazil

ARTICLE INFO

Article history:
Received 13 September 2017
Accepted 31 December 2017
Available online 8 January 2018

Keywords:
Caffeic acid
Chitosan microparticles
Spray-dryer
Controlled release
Permeation

ABSTRACT

Caffeic acid (CA), a phenolic compound found in plants with antioxidant and antimicrobial activity, induces collagen production and prevents premature aging of the skin. The objective of this study was to develop two types of chitosan microparticles (MP) containing CA and to relate the morphology with the release and permeation profiles. One type of MP was prepared from a hydroalcoholic solution (MPI) and the other from an aqueous solution (MPII). Their morphology and size was evaluated by high-resolution scanning electron microscopy. The release profile of CA was evaluated using the cellulose membrane from the two MPs in Franz diffusion cells and the permeation profile was evaluated using human abdominal skin samples; the epidermal membranes were prepared by the heat-separation technique. MPII was spherical with a smooth surface, suitable for the controlled release of substances, whereas MPI was porous with non-internalized residual material. This result was consistent with their release and permeation profiles because MPII exhibited a slower and more controlled release than MPI. Thus, the method of preparation of MP and their composition influence the release profile of CA. Therefore, the production conditions must be closely controlled.

1. Introduction

Caffeic acid (CA), a phenylpropanoid, is widely distributed in plant tissue metabolites. This polyphenol is present in coffee, blueberries, apples, and ciders (Magnani et al., 2014; Clifford, 2000). The benefits for its application to the skin are promising due to its antimicrobial action that can help in the treatment of dermal infections, such as acne and rosacea. It can also act as a depigmenting agent, since it inhibits the action of the enzyme tyrosinase, responsible for the formation of melanin, reducing skin spots and melasmas. And finally, CA is capable of neutralizing the harmful effects of free radicals on cells, due to its high antioxidant properties, and thus retarding and preventing premature aging of the skin (Sato et al., 2011; Magnani et al., 2014a,b).

The challenge in dermal applications is to overcome the anatomical and physiological barriers and provide an effective concentration at the site of action. Often drugs cannot perform their functions in the most appropriate manner due to the properties of the active ingredients and characteristics of the skin (Corrêa, 2012). To overcome the skin barrier, micro and nanostructured systems, which are intended to supply the active substance to the tissue for a prolonged period of time without causing damage or toxicity, have been developed to facilitate the delivery of hydrophilic or lipophilic substances.

The microencapsulation of drugs is a technique that involves the encapsulation of small particles of the drug or drug solution in a polymer shell (Wang and Xia, 2014). Chitosan is a natural polymer and is biocompatible, biodegradable and non-toxic. Its characteristics have widened its scope of application, particularly in the pharmaceutical and cosmetic field as it can be observed in many scientific publications. Microencapsulation has also been used to improve survival of probiotic and prebiotic substances in simulated gastro-intestinal conditions (Chávarri et al., 2010). Chitosan has also been used associated to cellulose acetate for the fabrication of composite nanofibrous mats for cell culture (Li et al., 2012). Liu et al. (2013) developed alginate/quaternized carboxymethyl chitosan/clay nanocomposite microspheres in order to improve the drug-controlled release. More recently a chitosan

* Corresponding author at: Av. Vicent Andrés Estellès, s/n, 46100, Burjassot, Valencia, Spain.
E-mail address: ana.melero@uv.es (A.M. Zaera).

https://doi.org/10.1016/j.jsps.2017.12.021
1319-0164/

and silica hydrogel with ibuprofen as controlled drug release system was obtained to coat titanium implants surface (Zhao et al., 2014). Furthermore, chitosan is known for its antimicrobial activity against many microorganisms, in which the most probable antimicrobial mechanism is found to include the presence of charged groups in the polymer backbone and their ionic interactions with bacteria wall constituents. The antimicrobial own properties of chitosan can improve those of caffeic acid (Cruz-Romero et al., 2013; Goy et al., 2016).

Chitosan belongs to the hydrocolloid class of biopolymers. It is positively charged at biological pH or presents itself as a polycationic polymer, while most hydrocolloids are negatively charged under the same conditions. The positively charged chitosan interacts with negatively charged tissues such as skin. Furthermore, the bioadhesiveness of chitosan is the main factor underlying its use in cosmetics (Ito et al., 2000; Sinha et al., 2004).

There are several microencapsulation methods. In this study, microparticles were obtained by atomization drying (spray-drying) in which the activecomponent and the polymer were dissolved in the same solvent to obtain a solution. The production of microparticles by spray-drying is simple, rapid, and reproducible. The process of forming microparticles by spray dryer dispenses washing with organic solvents to isolate the microparticles and it is enable the use of various lipophilic and hydrophilic polymers and drugs (Silva et al., 2003).

In order to incorporate the actives, whether they are in their free or encapsulated forms, the classic emulsions are widely used by consumers owing to their pleasant and refreshing sensory characteristics. However, preparations developed in the form of films or dried pellicles present an alternative technology owing to their ease of transportation (Spagnol et al., 2015, 2017).

Previous studies on skin permeation performed by our research group demonstrated that CA is retained in the stratum corneum, with a small concentration reaching the dermis. Thus, nanoencapsulation is intended to control the release of the active component, increasing molecule interaction/preventing skin irritation, as well as increase residence time of the molecules and the concentration and activity of CA in the dermis. The evaluation of these characteristics is only possible through liberation and permeation studies. Currently, there are no formulations with CA present in polymeric microparticles in the market; therefore, we explored new delivery systems for CA.

This work aims to develop conditions for the quality control of these products. Research on quality control of cosmetic products for active content identification and study of the physical and chemical characteristics are of fundamental importance to ensure the quality of the final product. Studies on identification of *in vitro* methods that do not use animal models for evaluating the effectiveness of cosmetics are increasing due to growing awareness among people. In this context, this project is cruelty-free as it was conducted in accordance with the current legislation in most countries that prohibit the use of animals to determine the efficacy and safety of cosmetic products. The objective of this study was to obtain the *in vitro* release and permeation profiles of two types of microparticles containing CA from an emulsion and a film.

2. Methods

2.1. Preparation of microparticles by spray-drying

For MPI, a 0.5% (w/v) chitosan solution with medium molecular weight (Sigma-Aldrich®) in 1% (v/v) acetic acid was prepared. This dispersion was diluted in ethanol to obtain a final concentration of 0.1%. To this dispersion, a mass of CA equivalent to 30% of the dry weight of chitosan used to prepare the dispersion and 0.01% of stearylamine, was added. The 0.1% (w/v) chitosan dispersion containing CA was atomized in BUCHI-191 Mini Spray Dryer. The following parameters were programmed into the equipment: air inlet temperature, 85 °C; pump flow, 30%; atomizing air flow, 450 NL/h; aspirator efficiency, 90%; and air outlet temperature, 65 °C (Ventura et al., 2008; Desai and Park, 2005).

To obtain MPII, a dispersion of chitosan with medium molecular weight (Sigma- Aldrich®) was prepared at 0.5% (w/v) in 1% (v/v) acetic acid. To this dispersion, a mass of CA equivalent to 30% of the chitosan dry weight and 1% of polysorbate 80 was added for CA solubilization in the dispersion. The dispersion of chitosan [0.5% (w/v)] with CA was atomized in BUCHI-290 Mini Spray Dryer. The following parameters were programmed into the equipment: air inlet temperature, 180 °C; pump flow, 10%; atomizing air flow, 450 NL/h; aspirator efficiency, 90%; and air outlet temperature, 80 °C.

2.2. Characterization of microparticles by scanning electron microscopy

The morphology of the microparticles was analyzed using photomicrographs obtained by scanning electron microscopy (Kulkarni et al., 2005). The samples were covered with a thin layer of gold photomicrographed at 10.000× magnification with an electron beam of 2.0 kV using the high-resolution scanning electron microscope S-4800 (Serial Number: HI-9269-0009).

2.3. Preparation of formulations

Three types of formulations were prepared: an aqueous dispersion, a film, and an emulsion containing MPI or MPII with 5 mg/mL CA. The film was composed of 6% pullulan, 1% hydroxypropyl methylcellulose, 1.5% propylene glycol, 1% PEG-12 dimethicone, and water. The emulsion was composed of 6% cetearylalcohol, 2% ceteareth-20, 3% ethylhexylstearate, 3% glycerylstearate, 3% propylene glycol, 0.05% disodium EDTA, 0.18% methylparaben, 0.02% propylparaben, and water.

2.4. Stability of CA in the receptor solution

The stability of CA was evaluated in three types of receptor solutions [buffer solution pH 5.5 + ethanol (50:50); buffer solution pH 7.4 + ethanol (50:50); water + ethanol (50:50)] over 336h to choose the most appropriate receptor solution for the *in vitro* release tests.

The concentration of CA in the solutions was evaluated by high-performance liquid chromatography (HPLC) mobile phase ethanol: purified water (40:60 v/v), flow rate 0.7 mL/min; pH 2.5; column RP18 (XDB, 4.6 × 250 mm, 5 μm, Waters), detection wavelength 325 nm (Spagnol et al., 2016).

2.5. Evaluation of the in vitro release profile

The release assays were developed using Franz cells with a diffusion area of 1.76 cm^2 and cellulose membrane (Sigma-Aldrich) (Marquele et al., 2006).

The modified Franz cell receptor compartment was filled with 12 mL of pH 5.5 phosphate buffer with ethanol (50:50). The formulations tested were dispersion, film, and emulsion with MPI or MPII containing CA. Aliquots of 1 mL of each formulation were placed on the membrane. The solubility of the active component in the receptor solution was tested to assure sink conditions.

Aliquots of 200 μL were collected over time. They were replaced with the same volume of fresh buffer.

The receptor solution was continuously stirred at 300 rpm with a magnetic stirrer in Franz cells and maintained at 32 ± 1°C in a

water bath. The experiments were repeated six times for each time point and concentration of CA. The quantification was performed in triplicate by HPLC using a previously described method (item 2.4).

2.6. Evaluation of the in vitro permeation of CA

The CA permeation was evaluated with samples from the abdominal skin of females of European-descent (from donor females aged 38–48 years, randomly assigned). After the removal of connective tissues and excess fatty tissue, the samples can be stored in a freezer at −26 °C until three months. The heat-separation technique was used to separate the epidermis from the dermis. This technique is based on the immersion of the skin in water at 60 °C for 90 s (Melero et al., 2008).

In vitro permeation studies were conducted using glass Franz-type cells with an available diffusion area of 1.76 cm^2 and 12 mL of receptor cell volume. The receptor phase was composed of a phosphate buffer solution pH 5.5 + ethanol (50:50) (Melero et al., 2008).

Subsequently, 1 g of each formulation was added to each compartment on the stratum corneum side of the skin. Samples (200 μL) were withdrawn at specified time intervals from the receptor compartment, followed by replacement with fresh receptor solution (Melero et al., 2008).

Three controls were also applied to the stratum corneum side of each donor compartment: a buffer solution pH 5.5 + ethanol (50:50) with CA; a solution of CA and chitosan; and a solution of CA, chitosan, and polysorbate 80. All of them contained 5 mg/mL CA. The content of CA was analyzed by HPLC as described in item 2.4.

3. Results

3.1. Characterization of microparticles by scanning electron microscopy

Fig. 1 shows the photomicrographs of MPI obtained from hydroalcoholic dispersion and MPII obtained from an aqueous dispersion. The amount of caffeic acid was 30% (w/w) of the microparticles. The diameter of MPI and MPII ranged from 1 to 5 μm and positively charged (MPI = 41.6 ± 1.1 mV and MPII = 32.4 ± 0.7). MPII was spherical and had a smooth surface, which is ideal for controlled release, whereas MPI was porous with non-internalized residual material.

3.2. Stability of CA in the receptor solution

In this study, pH 5.5 and 7.4 were chosen as the physiological pH of the dermis and the skin surface, respectively. The Fig. 2 shows that buffer solution pH 7.4 + ethanol (50:50) caused the degradation of caffeic acid after 8 h of study.

3.3. Evaluation of the in vitro release profile of CA

MPII exhibited a slower and controlled release than that by MPI (Fig. 3). In the first 10 h of release, MPI demonstrated zero order release profile, and in the next few hours, saturation of the receptor phase was observed. The power release profile did not fit well for MPI; however, this model perfectly described the controlled release profile of MPII. These results were consistent with the release profile of CA in microparticles when incorporated into films and emulsions.

3.4. Evaluation of the in vitro permeation of CA

The flow values of CA through the epidermis (J), the permeability coefficient (Kp), and the latency time (Tl) under different conditions are shown in Table 1.

There was no statistically significant difference in the flow, permeability coefficient, and latency time between the controls, which indicated that chitosan and polysorbate 80 did not influence these kinetic parameters.

The results presented in Table 1 do not indicate a relationship between the permeability coefficient and the relative thermodynamic activity of CA in these vehicles. Table 2 shows the kinetic parameters of the epidermal permeation of CA-containing microparticles incorporated into dispersion, film, or emulsion. As the permeability coefficient is evaluated only for immediate release solutions, it was not calculated for formulations with CA-containing microparticles.

4. Discussion

MPI presented evident deformations, which may have been caused by the rapid evaporation of the hydroalcoholic solution at elevated temperatures or the shearing of the droplets during spray-drying. Plasticizing agents, such as polysorbate 80, added to MPII reduce the contact surface tension and intercalate between the chitosan molecules in the matrix, modifying its structure and inducing spheronization, which leads to the formation of microparticles with fewer deformations (Alpar et al., 2005; Ventura et al., 2008; Reynaud et al., 2011).

The pKa of CA was calculated using ACD/Labs 6.0 software, which showed values of 12.79 and 9.97 for the hydroxyl group and 4.04 for the carboxyl group present in CA. Thus, at higher pH, the hydrogen in the carboxylic group is lost and the molecule is oxidized, which explains the greater degradation of CA at pH 7.4 (Magnani et al., 2014). In addition, ethanol was selected for facili-

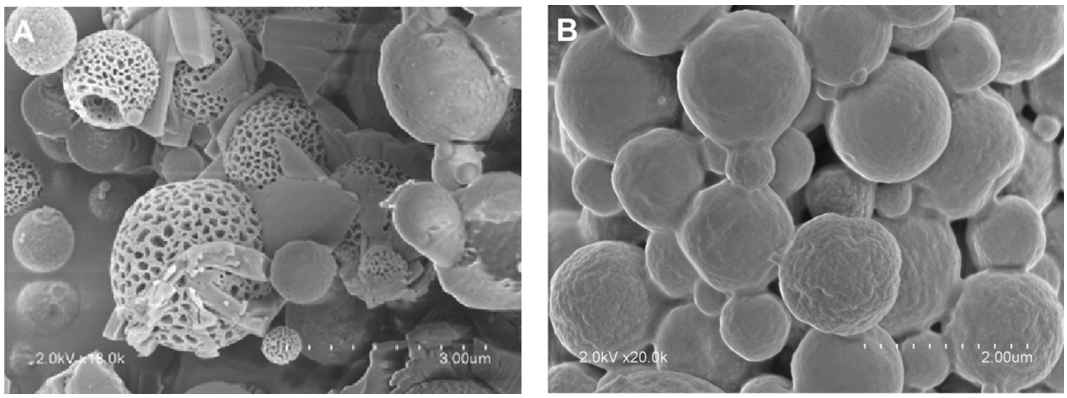

Fig. 1. Photomicrographs of (A) MPI and (B) MPII.

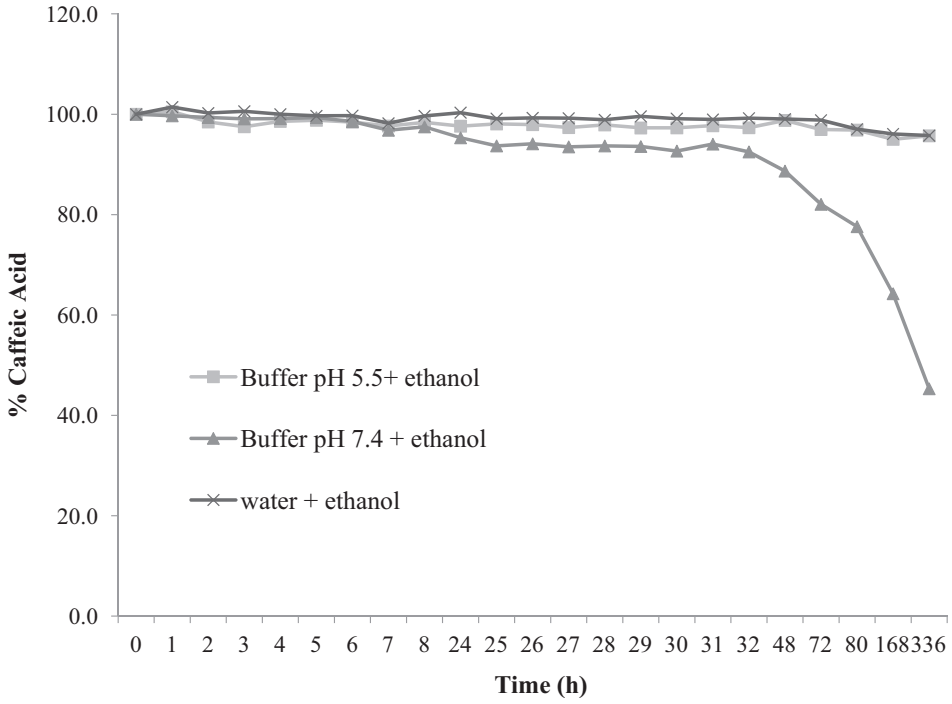

Fig. 2. Stability of caffeic acid in three different types of receptor solutions, buffer pH 5.5 + ethanol (50:50); Buffer pH 7.4 + ethanol (50:50); Water + ethanol (50:50) over 336 h.

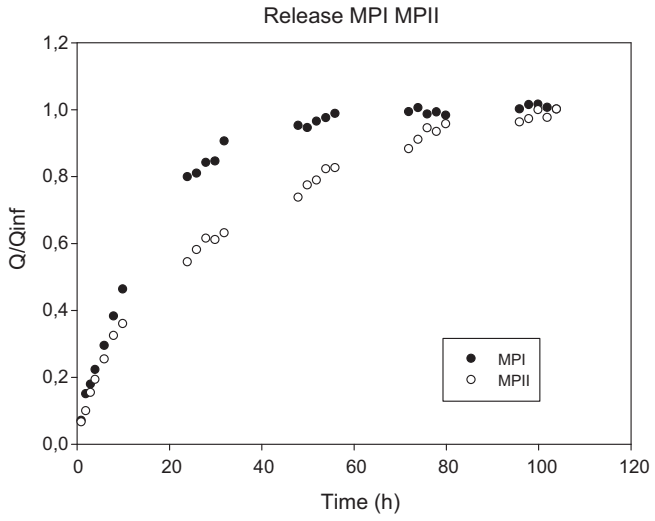

Fig. 3. Release profile of CA from MPI and MPII.

Table 1
Kinetic parameters of CA epidermal permeation in the controls.

	J (μg/cm²/h)	Kp (cm²/s)	Tl (h)
CA	5.50 ± 0.46[a]	$3.13 \times 10^{-7} \pm 2.66 \times 10^{-8}$[b]	1.78 ± 0.34[c]
CA + Q	5.03 ± 0.74[a]	$2.79 \times 10^{-7} \pm 4.13 \times 10^{-8}$[b]	1.17 ± 1.80[c]
CA + Q + T	6.17 ± 0.48[a]	$3.40 \times 10^{-7} \pm 2.68 \times 10^{-8}$[b]	1.05 ± 1.11[c]

J: Flow through the epidermis; Kp: permeability coefficient; Tl: latency time; CA: Caffeic Acid solution; CA + Q: solution of caffeic acid and chitosan; CA + Q + T: solution of caffeic acid, chitosan and Tween 80. a, b, c: equal letters indicate statistically equal values.

tating the solubilization of CA in the medium, which guarantees the sink conditions and results in fewer deformations in the biological membranes. Phosphate buffer solution pH 5.5 + ethanol (50:50) was selected for further *in vitro* release studies.

Table 2
CA epidermis permeation kinetic parameters from the formulations (dispersion, film and emulsion).

	J (μg/cm²/h)	Tl (h)
Dispersion MPI	4.28 ± 0.73	2.33 ± 0.50
Dispersion MPII	3.37 ± 0.53	2.46 ± 0.81
Film MPI	3.16 ± 0.75	3.29 ± 0.59
Film MPII	2.15 ± 0.63	3.59 ± 0.22
Emulsion MPI	2.52 ± 0.53	2.88 ± 0.92
Emulsion MPII	0.92 ± 0.24	4.04 ± 0.28

J: Flow through the epidermis; Tl: lag time; MP: Microparticles.

In the first 10 h of release, MPI presented zero order release profile, and in the next few hours, the saturation of the receptor phase occurred. The zero-order model, describing the dissolution of the active component from the matrices that do not disaggregate and release the drug slowly (Kalpana et al., 2015), can be represented by the equation:

$$Q_0 - Q_t = K_0 t \quad (1)$$

The rearrangement of Eq. (1) yields

$$Q_t = Q_0 + K_0 t \quad (2)$$

where Q_t is an amount of drug dissolved at time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$), K_0 is the zero order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from *in vitro* CA release studies were plotted as the cumulative amount of drug released *versus* time (Hadjiioannou et al., 1993; Narasimhan and Peppas, 1997). This relationship can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low-soluble drugs in coated forms or osmotic systems (Freitas and Marchetti, 2005, Dash et al., 2010; Rosenzweig et al., 2013).

The power release profile did not fit well for MPI, but the model perfectly described the controlled release profile of MPII.

The power law equation, represented by Eq. (3), was used as it is versatile.

$$\frac{Qt}{Qinf} = k * t^n \quad (3)$$

Q_t and Q_{inf} are the absolute cumulative amount of drug released at time t and infinite time, respectively. K is a constant that includes structural and geometric characteristics of the device and *n* is the release exponent, indicative of the mechanism of drug release. A special case of *n* value use is at the Higuchi relationship ($n = 0.5$), which can be used to facilitate comparisons.

Through the power release profile curve of CA from MPII, it was possible to calculate some release parameters. The value of Kd (0.1249 μg/cm^2/h) indicates the release constant value of the process; it was obtained from the *a* value of the curve. The value of n (0.4570), which is the diffusional release exponent, was derived from the *b* value of the curve. According to Berrozpe et al. (2013), values of $0.43 < n < 0.5$ indicate that the release process is governed mainly by swelling or erosion of the matrix.

The coefficient of determination (R^2) is a measure of adjustment of a statistical model related to the observed values. R^2 varies between 0 and 1, indicating in percentage, how much the model can explain the observed values. The higher the R^2, the more explanatory the model is, and the better it fits the sample. In this case, the R^2 of the power model is 0.9927, which means that 99.27% of the dependent variable, can be explained by the regressors present in the model.

Non-linear regression was performed using the software Sigmaplot®10.0. The quality of fit was evaluated by the correlation between experimental values and values predicted by the model. The confidence interval ($\alpha = 0.05$) was considered for the parameters to draw conclusions about the release mechanisms.

The study of the *in vitro* release profile of substances is an important step in the characterization of release systems as it allows the evaluation of the technological parameters in relation to the conventional formulations. The release profile is directly related to the distribution of the drug in the polymer matrix and thus depends directly on the physicochemical characteristics of the active substance and the components of the polymer matrix.

Porous microspheres, such as MPI, have a high surface area, allowing greater contact with the medium and leading to a considerable increase in the release rate in all the release systems (Yang et al., 2000). The drug released within the first hours may be located on the surface or in the outer layers of the particle and thus the small diffusional distance between its location and the receptor solution may have led to the faster release (Freiberg and Zhu, 2004). MPII, which is less porous, has a smoother and more uniform surface and was able to better control the release of CA in all the formulations (aqueous dispersion, film, and emulsion).

The permeation studies demonstrated increased lag time and a reduction in the CA flux in the dispersion > film > and emulsion. This can be explained by the viscosity of the systems as the emulsion was the most viscous system, followed by the film, and the dispersion, proving that viscosity is one of the factors that affect the flow of the permeant (Berrozpe et al., 2013).

As it can be observed in the Table 2, MPI provided a caffeic acid flow of 2.52 ± 0.53 μg/cm^2/h, whereas MPII showed a flow of J = 0.92 ± 0.24 μg/cm^2/h. This means that MPI allowed a faster CA permeation than MPII. The opposite phenomenon was observed in the lag time (Tl), because MPI showed a lag time of Tl = 2.88 ± 0.92 h and MPII of Tl = 4.04 ± 0.28. Thus, the emulsion containing MPII, in addition to promoting a slower flow of AC, also took longer to begin to permeate when compared to MPI. These results can be easily explained through the release kinetics of both microparticle types, as MPII showed a more controlled release than MPI. Therefore, as CA is retained in the MPII formulation in a more efficient manner, it can also be expected that the permeation profile is also more controlled. The drug in MPII will therefore stay in contact with the skin, for a longer time, thus improving its antimicrobial, depigmenting and antioxidant activity directly on the target site, as desired when designing the formulation.

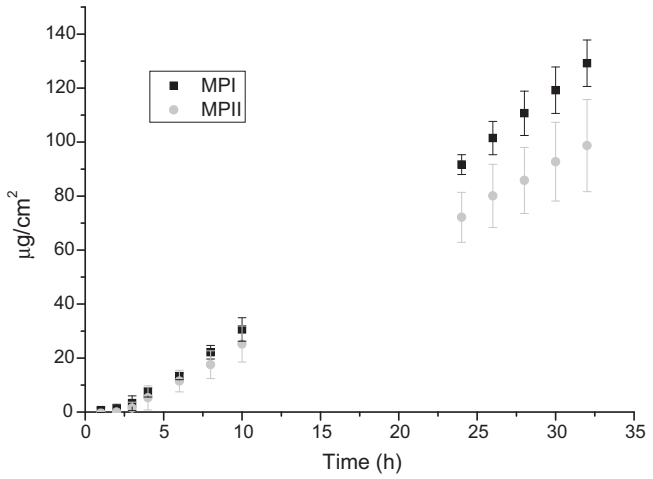

Fig. 4. Permeation profile (mean ± standard deviation) of the CA through the epidermis from the MPI and MPII.

In all formulations (dispersion, film, and emulsion), the flow was lower and the latency time was higher for MPII than for MPI, indicating a similarity between the permeation and release profiles, wherein MPII showed a slower and more controlled release of CA than MPI (Fig. 4).

5. Conclusion

The results demonstrated that spray-drying is an efficient method to obtain chitosan MP (1–5 μm in diameter) containing CA. The MP obtained from aqueous solution was spherical with a smooth surface, suitable for controlled release of substances. Conversely, MP obtained from hydroalcoholic solution was porous with non-internalized residual material. This result was consistent with their release and permeation profiles as the MP obtained from the aqueous solution exhibited a slower and more controlled release than that obtained from the hydroalcoholic solution.

It can be concluded that the method of preparation and composition of microparticles influence the release profile of CA; therefore, all production conditions must be closely controlled.

Acknowledgments

Authors are thankful to Allcrom by donation of chromatographic columns and to Ana Melero Zaera from University of Valence for receiving the doctoral student to carry out the research. This work was financially supported by supported by Fapesp (2015/02619-3) and BEPE Fapesp (2016/06742-7).

Disclosure of interest

The authors report no conflicts of interest.

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