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# Preparation of pellets containing *Pothomorphe umbellata* extracts by extrusion-spheronization: improvement of 4-nerolidylcatechol photostability

***César A. de Araújo-Júnior,[1] Fernanda S. de Oliveira Costa,[1] Stephânia F. Taveira,[1] Ricardo N. Marreto,[1] Marize C. Valadares,[2] Eliana M. Lima*,[1]***

*[1]Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade Federal de Goiás, Brazil,*
*[2]Laboratório de Farmacologia e Toxicologia Celular, Faculdade de Farmácia, Universidade Federal de Goiás, Brazil.*

**Abstract:** *Pothomorphe umbellata* (L.) Miq., Piperaceae, has been extensively used in Brazilian folk medicine and it is well known for its strong antioxidant properties. However, its main active constituent, 4-nerolydilcatechol (4-NC), is sensitive to ultraviolet and visible light, which can limit the use of intermediate and final herbal preparations of this species. In the present work, coated multiparticulate solid dosage forms of *P. umbellata* were obtained with the purpose of increasing the stability of 4-NC. *P. umbellata* extract was used as a wetting liquid for the preparation of pellets by extrusion-spheronization. Pellets were coated in a fluidized bed by three different polymers (hydroxypropylmethylcellulose (HPMC), polyvynilpirrolidone K-30 (PVP-K30), and polyvinyl alcohol-polyethylene glycol *graft*-copolymer (PVA-PEG)). 4-NC photostability was evaluated by an accelerated photostability protocol. Pellets showed a narrow size distribution and low friability. 4-NC photodegradation followed a second order degradation kinetics with similar *k* values for the percolate, uncoated pellets and HPMC coated pellets. Photoprotection was higher in pellets coated with PVP-K30 and PVA-PEG. PVA-PEG coated pellets with 6 and 9% weight gain resulted in a final concentration of 4-NC approximately cinco times higher than uncoated pellets or liquid extracts, suggesting the potential of this formulation as a multiparticulate solid dosage form for *P. umbellata* extracts.

*Article*

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## Introduction

*Pothomorphe umbellata* (L.) Miq., Piperaceae [syn. *Heckeria umbellata* (L.) Kunth., *Piper umbellata* L., *Piper hilarianum* Stend.] is known in Brazil as "pariparoba" and is widely employed in folk medicine as an analgesic, diuretic and anti-spasmodic agent, as well as for the treatment of inflammatory disorders, malaria, asthma, liver diseases and gastrointestinal disorders (Perazzo et al., 2005; Ropke et al., 2005).

*Pothomorphe umbellata* was included in the first edition of the Brazilian Pharmacopoeia (Silva, 1926) and its antioxidant activity has been reported in different experimental models (Barros et al., 1996; Desmarchelier et al., 1997; Ropke et al., 2005). The biological activity of *P. umbellata* is mainly attributed to its most abundant metabolite, 4-nerolidylcatechol (4-NC), a lipophilic compound found in the roots and leaves (Kijjoa et al., 1980).

Previous results from our research group demonstrated that the *P. umbellata* root extract and the isolated 4-NC did not have mutagenic effect on mouse bone marrow cells, but conversely, a protective effect against cyclophosphamide induced genotoxicity was observed (Valadares et al., 2007). In addition, the root extract was proven more effective than the isolated compound. In spite of their potential, 4-NC and *P. umbellata* extracts presented high photosensitivity to UVA and visible light radiation (Costa et al., 2011), which can limit their use. Costa et al. (2011) also reported that *P. umbellata* extracts exhibited a more potent antioxidant activity, but were more photosensitive compared to isolated 4-NC. To improve the usefulness of *P. umbellata* preparations it is necessary to overcome these photoinstability issues. A viable technological strategy towards improving

photostability of compounds in solution is their incorporation into solid substrates, since the rates of photolytic reactions are lower in solid state. Therefore, the incorporation of *P. umbellata* liquid extracts into solid dosage forms might be an interesting approach in order to stabilize the photolabile 4-NC.

In the present wok, microcrystalline cellulose pellets containing *P. umbellata* extracts were prepared by extrusion-spheronization technique and subsequently coated with different types of polymers. Pellets characteristics and photostability profiles of 4-NC were determined and compared.

## Materials and Methods

### *Materials*

The roots of *Pothomorphe umbellata* (L.) Miq., Piperaceae, were purchased from Centroflora (Botucatu, SP, Brazil, batch number 204090080). The material was ground in an industrial mill to obtain an average particle diameter of 250 µm. A sample of the dried and ground commercial sample has been deposited in the Herboteca da Faculdade de Farmácia da Universidade Federal de Goiás (registry number FF001). Ethanol used in the extraction procedure and glacial acetic acid used in the HPLC mobile phase were of analytical reagent grade. Acetonitrile and methanol were HPLC grade and were purchased from JT Baker (USA). Isolated 4-NC used as reference standard was obtained from the hydroethanolic root extract according to Gustalfson et al., (1992) by Prof. Kennia R. Resende, from the School of Pharmacy, Federal University of Goiás, Brazil.

For the preparation and coating of the pellets materials used were microcrystalline cellulose PH 101 (Blanver, São Paulo, Brazil), lactose monohydrate (Milkaut, Santa Fé, Argentina), polyvinylpyrrolidone K30 (ISP, São Paulo, Brazil), Kollicoat® (Basf, Ludwigshafen, Germany), Opadry YS 1-7007 (Colorcon, São Paulo, Brazil), polyethylene glycol 6,000 (Polioles, Ciudade de Mexico, Mexico), titanium dioxide (Química Brasil LTDA, São Paulo, Brazil).

### *Methods*

#### Percolation procedure

The percolation was performed according to Brazilian Pharmacopoeia (Silva, 1926) with 1000 g of *P. umbellata* roots. The ethanol/water mixture (3:1, v/v) was added to the percolator until the plant material was entirely exhausted. The fractions obtained were combined and the solvent was evaporated under reduced pressure in a rotary vacuum evaporator (IKA, RV 10, Germany) at 40 °C to a final volume of 4000 mL. The ethanol and total solid content of the percolate were determined by standard pharmacopeial procedures (Brazilian Pharmacopeia, 1988).

Colloidal silicon dioxide and polysorbate 80 were added to the percolate (0.25 and 0.75% w/v, respectively) and the mixture was submitted to an additional concentration step in a rotary evaporator (IKA, RV 10, Germany) at 40 °C. The concentrated extract was used as the wetting liquid in the preparation of *P. umbellat*a pellets.

#### Preparation of pellets by extrusion-spheronization

A dry powder mixture containing 80 g of microcrystalline cellulose, 16 g of lactose monohydrate and 4 g of polyvinylpyrrolidone K-30 was homogenized in a Stinfer® planetary mixer (São Paulo, Brazil) and then wetted with 100 g of *Pothomorphe umbellata* concentrated extract. A Caleva Extruder 20® (Caleva Process Solutions Limited, United Kingdom) fitted with a 1 mm screen was used to obtain extrudates from the wet mass. The extruder operated at a speed of 110 rpm. The extrudates were then spheronized in a Caleva Multi Bowl Spheronizer MBS 250 (Caleva Process Solutions Limited, United Kingdom) at rotating speed of 1,800 rpm for 2 min. Pellets were then dried in a ventilated oven (Lawes, São Paulo, Brazil) at 40 °C for 24 h.

#### Pellet characterization

Pellet size was determined by sieve analysis using a Sonic Sifter Separator® L3P-26 (Advantech MFG, Wisconsin, USA) equipped with a series of sieves with apertures ranging between 300-2,000 µm. The mean diameter of pellets was calculated by weighted mean. For friability evaluation, an aliquot of uncoated pellets was put into a Nova Etica friabilator (Nova Etica, Brazil) working at 20 rpm for 5 min. Friability and size analyses were performed in triplicate.

Scanning electron micrographs were obtained in a Phenom® Scanning Electron Microscope (FEI Company,) following the deposition of a 20 nm gold coating using a sputter coater EM SCD 050 (Leica, Germany).

#### Pellet coating

Thirty five grams of pellets (800-1400 µm in diameter) were fluidized in a fluid bed unit (Mycrolab, Hüttlin®, Germany) and then coated with a polymer dispersion (Table 1) under the following conditions: atomization air pressure of 11 psi; fluidization air

**Table 1.** Composition of the polymer dispersions used for pellet coating.

| Formulation | Hydroxypropyl methylcellulose | Polyvinyl alcohol-polyethylene glycol graft-copolymer | Polyvinyl pyrrolidone | Polyethylene glycol 6000 | $TiO_2$ |
|---|---|---|---|---|---|
| HPMC coated pellet[a] | 3% | -- | -- | 3% | 3% |
| PVP coated pellet[a] | -- | -- | 3% | 3% | 3% |
| PVA-PEG coated pellet[b] | -- | 3% | -- | 3% | 3% |

[a]weight gain 3%; [b]weight gain 3, 6 and 9%.

flow of 1.8 $m^3$/h; spray rate 1.0 g/min and inlet air temperature of 40 °C. Polyethylene glycol 6,000 was added as plasticizer and titanium dioxide was used as opacifier in all formulations (Table 1). Following the application of the polymer dispersion, pellets were maintained in the fluidized bed unit for an additional drying time of 10 min at 45 °C. The amount of polymer dispersion used for the coating of the pellets allowed for a weight gain of either 3, 6 or 9%, as presented in Table 1.

Quantitative determination of 4-nerolidylcatechol by high performance liquid chromatography (HPLC)

4-Nerolidylcatechol content in the percolate, concentrated percolate and pellets was determined using a Varian HPLC system (PS410, Varian, USA) equipped with an UV-visible absorbance detector (PS325) and a quaternary pump (PS240). Separations were performed on a Varian ChromSpher 5 C18 reverse phase column (15 cm, 4.6 mm, 5 µm). The mobile phase consisted of acidified methanol (0.1% glacial acetic acid), water (0.1% glacial acetic acid) and acetonitrile (90:9:1). The flow rate was 1 mL/min and the injection volume was 10 µL. The detection wavelength was 282 nm, and the column temperature was 25 °C. All samples were filtered through a 0.45 µm filter prior to injection into the chromatographer. Dilutions of isolated 4-NC (used as external standard) in ethanol were used to obtain the concentration analytical curve of the 4-NC.

Photostability assay

Percolate extract and pellets containing *P. umbellata* (coated and uncoated) were submitted to an accelerated photostability assay in a 424 CF Photostability Chamber (Nova Etica, Brazil) equipped with a near-UV fluorescent lamp (15 W) with a spectral distribution from 320 to 400 nm and several cool white fluorescent lamps (15 W). Samples were exposed to radiation, which provided integrated UVA energies of 98.4, 196.8, 295.2 and 393.6 W/m2 corresponding to exposure periods of 12, 24, 36 and 48 h, respectively. The experiment was performed according to ICH (1996). After light exposure, samples were covered with aluminum foil and immediately analyzed by HPLC. A parallel experiment was run in the dark as a negative control for the effects of light on the degradation. For this assay, percolated samples were diluted with ethanol (1:1 ratio) and put into a Petri dish. Pellet samples (1 g) were spread across the Petri dish to give a unique solid layer. After light exposure, pellets were triturated with a mortar and pestle and 200 mg were appropriately extracted and diluted with ethanol and analyzed by HPLC for the quantitative determination of 4-NC content.

## Results and Discussion

The therapeutic usefulness of *Pothomorphe umbellata* (L.) Miq., Piperaceae, extracts may be compromised by the high photosensitivity of its main active constituent, the 4-NC (Soares et al., 2009). Silva et al. (2005) showed that *P. umbellata* liquid extracts were not degraded by UVB radiation. On the other hand, Costa et al. (2011) showed that different kinds of liquid *P. umbellata* preparations (percolate and ultrasound extracts) were strongly degraded by UVA and visible light. Solid state photostability, in general, is higher than that in liquid state (Tonnesen, 2001) and several variables may affect light penetration in a solid material, such as particle size, type of excipients, color and crystalline structure (Tonnesen, 2001).

In the present work, *P. umbellata* extracts were incorporated into microcrystalline cellulose (MCC) pellets and the photostability of the active compound, 4-NC, was evaluated. *P. umbellata* hydroetanolic extract showed a total solid content of 2.36±0.16% (w/v); the ethanol amount in the preparation was 32.72±0.14% (w/w). The concentration of 4-NC in the percolate was 1.053 mg/mL. In order to incorporate higher amounts of 4-NC in the pellets without compromising their preparation and physical characteristics, the percolate was concentrated in a rotary evaporator and used as the agglomeration liquid in the pellet preparation. The concentrated extract had 6.03 mg/mL of 4-NC. Due to the high hydrophobicity of 4-NC and it's tendency to precipitate in the concentrated extract upon removal of ethanol, colloidal silicon dioxide and polysorbate 80 were added to the percolate before the evaporation step in order to maintain the homogeneity of the concentrated

extract. The ratio between plant solids and adjuvants (colloidal silicon dioxide and polysorbate 80) in the concentrated extract was 2.3:1 (w/w). The total solid content in the concentrated extract was 13.44 % (w/v).

Dried pellets showed a narrow size distribution with almost 97% of pellets retained on three sieve fractions (850 µm, 7.03%; 1000 µm, 76%; and 1400 µm, 13.79%). The calculated average diameter was 1245 µm. Pellets showed low friability (<0.5%) which allowed the fluidized bed coating procedure without the formation of an excessive amount of fine powder.

Data from the photostability assay (Figure 1) showed that 4-NC content in the percolate quickly decreased after 12 h of light exposure. The incorporation of the plant extract into microcrystalline cellulose pellets resulted in a discrete improvement on 4-NC photostability after 12 h of exposure ($p$<0.05), but no significant differences were observed after 24, 36 or 48 h of irradiation. This observation can be attributed to the movement of the liquid during the extrusion and drying processes. During extrusion, the liquid content retained in the internal structure of the microcrystalline cellulose pellets is squeezed to the surface of the particle (Dukic´-Ott et al., 2009). This behavior may cause an accumulation of 4-NC on the surface of the pellets, thus more accessible to light irradiation and photodegradation. Drug concentration versus time of light exposure were plotted and the best fit curve resulted in a second order equation. Correlation coefficients and second order K values (rate constant) are presented in Table 2. No degradation of 4-NC was observed in liquid or solid samples kept in the absence of light (covered with aluminum foil), which also indicated that the temperature in which the assay was carried out did not influence on 4-NC degradation.

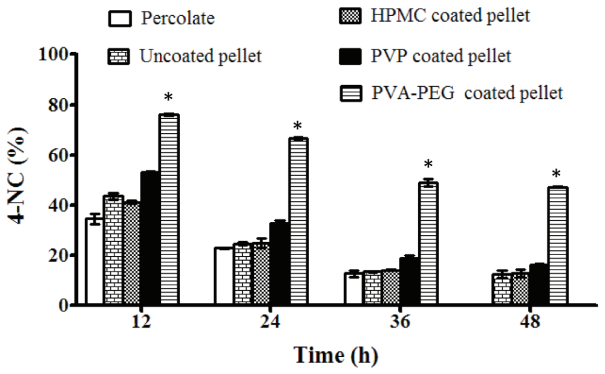


**Figure 1.** Photostability of 4-NC from *Pothomorphe umbellata* liquid extract (percolate), uncoated and coated (3% weight gain) microcrystalline cellulose pellets. Mean values indicated by bars bearing asterisks differ significantly ($p$<0.05) from that of the other samples at the same period of time.

Second order k value for *P. umbellata* pellets coated with HPMC (Opadry YS-1-7007) was very similar to the values obtained from irradiated uncoated pellets (Table 2) with no significant differences between HPMC coated and non-coated pellets ($p$>0.05) (Figure 1).

Calculated $k$ value for PVP-K30 coated pellets was lower than $k$ values for uncoated and HPMC coated pellets (Table 2), however, the differences in 4-NC content in the pellets were small (Figure 1). On the other hand, pellets coated with the copolymer PVA-PEG (Kollicoat®) resulted in a significant improvement ($p$<0.05) on 4-NC photostability. Table 2 shows a strong decrease in k values for all PVA-PEG formulations which is reflected in the higher concentration of 4-NC in all time points tested (Figure 1). The superior performance of PVA-PEG coating can be attributed to the nature of the polymeric material. Porter et al. (2009) discussed that copolymers showed better adhesive properties and are able to form higher quality films on the surface of tablets and granules, which is in agreement with our results.

**Table 2.** Photodegradation $k$ values and $r^2$ of second order kinetic model applied to the different formulations of *Pothomorphe umbelata* extracts.

| Formulation | $k$ values | $r^2$ |
|---|---|---|
| Percolate | 0.0018 | 0.9640 |
| Uncoated pellet | 0.0016 | 0.9631 |
| HPMC 3% coated pellet | 0.0015 | 0.9707 |
| PVP 3% coated pellet | 0.0011 | 0.9715 |
| PVA-PEG 3% coated pellet | 0.0002 | 0.9587 |
| PVA-PEG 6% coated pellet | 0.0001 | 0.9670 |
| PVA-PEG 9% coated pellet | 0.0001 | 0.9766 |

Figure 2 shows the scanning electron micrographs of PVP and PVA-PEG coated pellets. It can be noticed that pellets coated with PVA-PEG had a ticker and more regular coating layer, which probably contributed to a more efficient barrier against light penetration, and consequent photodegradation.

Pellets coated with higher amounts of PVA-PEG were also obtained (6 and 9% of weight gain). The increase in the amount of polymer on the surface of the pellets contributed significantly ($p$<0.05) to the photoprotective effect of this coating (Figure 3), leading to a 50% reduction of the degradation kinetics ($k$) as shown in Table 2. After 48 h of light exposure, 4-NC concentration in the pellets coated with 6% and 9% PVA-PEG was 59.83% and 61.32%, respectively (Figure 3). Degradation rate constants for both formulations were identical (Table 2). In light of this, the formulation of pellets coated with 6% PVA-PEG can be considered the most adequate preparation

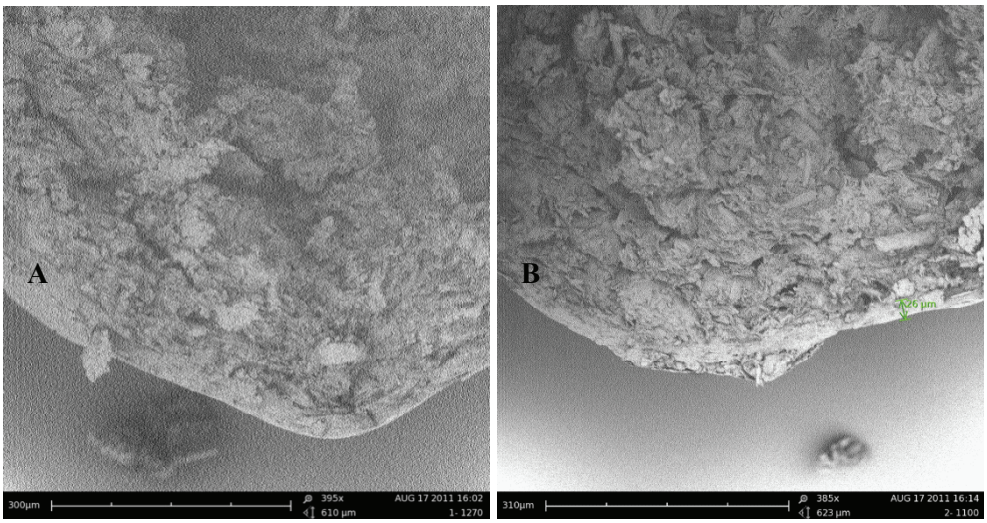


**Figure 2.** SEM micrographs of PVA-PEG coated pellets (a) and PVP K-30 coated pellets (b). Both samples were coated until 3% weight gain.

in this study, since it was able to promote a higher photoprotection of 4-NC with an intermediate amount of polymer film coating. All the formulations tested were far superior to the *P. umbellata* liquid extract in promoting 4-NC stability, since no measurable concentration of 4-NC was found in the percolate samples following 48 h of light exposure. In addition, the final concentration of 4-NC in the PVA-PEG coated pellets was approximately five times higher than that from the uncoated particles (Figure 3). PVA-PEG graft copolymer is a water-soluble film-forming agent used for the manufacture of immediate-release solid dosage forms, thus differences in dissolution rates from coated and non-coated pellets cannot be expected.

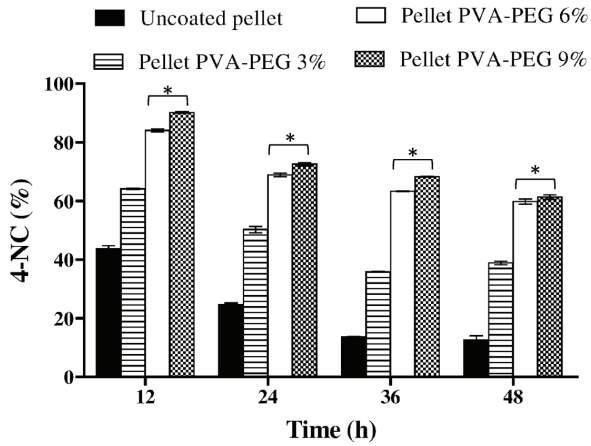


**Figure 3.** Photostability of 4-NC from *Pothomorphe umbellata* uncoated and PVA-PEG coated pellets (3, 6 and 9% weight gain). Mean values indicated by bars bearing asterisks differ significantly ($p$<0.05) from that of the other samples at the same period of time.

## Conclusion

The inclusion of *Pothomorphe umbellat*a (L.) Miq. (Piperaceae) extract into microcrystalline cellulose pellets obtained by extrusion-spheronization technique was not able to improve 4-NC photostability after 48 h of light irradiation, however, polymeric film coating significantly improved the photostability of 4-NC in all formulations. The stabilization effect was dependent on the type and amount of polymer coating used. Pellet coating with PVA-PEG copolymer resulted in a more uniform coating layer and in a higher photoprotective effect on 4-NC stability. Pellets coated with PVA-PEG (6% weight gain) can be considered as a viable multiparticulate solid drug delivery formulation for the photolabile constituents of *P. umbellata* root extracts.

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***Correspondence**

Eliana Martins Lima
Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade Federal de Goiás
Praça Universitária, Praça Universitária c/ 1ª. Avenida, Qd. 62, 74.605-220 Goiânia-GO Brazil
emlima@farmacia.ufg.br
Tel: +55 62 3209 6039