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# Nanoencapsulation of lutein and its effect on mice's declarative memory

Jéssica Thaís do Prado Silva [a], Júlia Maria Tonin Geiss [b], Sara Marchesan Oliveira [c], Evelyne da Silva Brum [c], Sara Cristina Sagae [d], Daniela Becker [e], Fernanda Vitória Leimann [a], Rafael Porto Ineu [a], Gustavo Petri Guerra [b], Odinei Hess Gonçalves [a,*]

[a] Federal University of Technology – Paraná (UTFPR), Post-Graduation Program of Food Technology (PPGTA), PO Box 271, BR 369, km 0.5, 87301-006 Campo Mourão, PR, Brazil
[b] Federal University of Technology – Paraná (UTFPR), Post-Graduation Program of Food Technology (PPGTA), PO Box 271, Avenida Brasil, 4232, 85884-000 Medianeira, PR, Brazil
[c] Graduate Program in Biological Sciences: Toxicological Biochemistry, Federal University of Santa Maria (UFSM), Avenida Roraima n° 1000, 97105-900 Santa Maria, RS, Brazil
[d] State University of Western Paraná (UNIOESTE), Center of Biological and Health Sciences, Rua Universitária n° 2069, 85819-110, Cascavel, PR, Brazil
[e] State University of Santa Catarina (UDESC), Center for Technological Sciences, 89223-100 Joinville, SC, Brazil

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ABSTRACT

Lutein is a xanthophyll carotenoid widely known by its biological properties and low toxicity. When located in the brain, lutein may inhibit damage mechanisms, acting in neural cells maintenance. However, this carotenoid is very sensitive to external agents such as heat, light, pH and oxidation, besides presenting low absorption in gastrointestinal tract due its low solubility in water. Encapsulation procedures have shown promising results to increase lutein stability and bioavailability. In this work, lutein was encapsulated in polyvinylpyrrolidone (PVP) matrix by the dissolution in common solvent method. Nanoparticles were characterized in respect to morphology, water solubility, and interactions between PVP and lutein. *In vivo* tests were carried out in order to investigate the influence of lutein encapsulation on mice's declarative memory. *Ex vivo* tests were also carried out to determine if nanoparticles may cause any inflammatory process *per se*. Results indicated that lutein was successfully encapsulated in PVP while nanoparticles presented spherical shape and uniform size. Encapsulation was able to increase water solubility of lutein by more than 43 times, which may be attributed to the formation of soluble complexes trough hydrogen bonds between lutein hydroxyl group and PVP carbonyl group. *In vivo* studies showed that the administration of free lutein at 100 $mg \cdot kg^{-1}$ and lutein-loaded PVP nanoparticles at 10 and 1.5 $mg \cdot kg^{-1}$ significantly increased mice's object recognition index, meaning that significant lower doses of lutein were needed to achieve the same effect when lutein was encapsulated. *Ex vivo* studies showed that lutein-loaded nanoparticles administration did not alter inflammatory parameters in plasma, liver and brain of mice. In this sense, lutein-loaded PVP nanocapsules showed to be an advantageous alternative to increase water solubility and to improve the memory of mice without causing inflammatory damage *per se*.



## 1. Introduction

Lutein is a dietary xanthophyll carotenoid found in green leafy vegetables and other foods such as corn, persimmon, broccoli and egg yolk [1,2] and it is widely known by its anti-inflammatory [3–5] and antioxidant properties [6–8]. Along with its isomer zeaxanthin, lutein is the major carotenoid present in primate brain [9]. Recently, lutein has been associated with neuroprotective effects [10–12]. According to Erdman Jr. and co-workers [13], lutein located next to neural membranes may influence cognition by maintaining cell viability, acting in inhibiting damage mechanisms, cellular dysfunction and death.

Despite having important biological activities in the brain, this carotenoid is very sensitive to external agents such as heat, light, pH and oxidative conditions, which limit its application in food and pharmaceutical industries [14]. Moreover, low water solubility of lutein lead to low absorption in gastrointestinal tract, compromising its bioavailability [15]. Encapsulation is a procedure that have been applied to bioactive compounds aiming to reduce or prevent degradation processes until the compound of interest reaches the sites where absorption is desired [16]. Also, this technique have shown ability to increase lutein bioavailability [17–19].

There are many techniques available to encapsulate lutein. Among them, the dissolution in common solvent method is a viable and economic method to enhance dissolution and bioavailability of poorly water soluble compounds. Besides, the mechanism of solid dispersion formation may influence the solid-state characteristics, changing the dissolution profile [20,21].

Water soluble carriers such as high molecular weight polyethylene glycols and polyvinylpyrrolidone (PVP) are the most common carriers

* Corresponding author.
*E-mail address:* odinei@utfpr.edu.br (O.H. Gonçalves).

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used to solid dispersion preparation [22]. PVP is an amorphous polymer able to form complexes with other substances and it is soluble in common organic solvents [23]. The biocompatible and nontoxic nature of PVP turns it into a promising material for biotechnological applications [24]. However, it is worth investigate the actual improvement in the biological activity of lutein due to encapsulation because encapsulation procedures are often expensive and complex. It is also important to determine if nanoparticles are safe to be used. The objective of this work was to encapsulate lutein in PVP nanoparticles and investigate its effect on *in vivo* mice's declarative memory. Nanoparticles were produced by the dissolution in common solvent method and characterized in respect of the interactions between lutein and PVP. In addition, mice tissues were collected to determine if nanoparticles may cause inflammatory processes.

## 2. Material and methods

### 2.1. Material

Lutein (90% purity, kindly gifted by Pincredit Bio-tech Co.), polyvinylpyrrolidone (PVP, 40,000 $g \cdot mol^{-1}$, Sigma-Aldrich), Tween 80 (Dinâmica) and ethanol (99.5%, Neon) were used in the nanoparticles preparation. Ethyl acetate (99.5%, Neon) and methanol (99.8%, Neon) were used as solvent in the nanoparticles solubility test. Olive oil was used for free lutein administration in mice by oral gavage. Sodium acetate, glycine, acetic acid, sodium citrate, (Vetec - Brazil), Hexadecyltrimethylammonium bromide (HTAB), 3,3′,5,5′-tetramethylbenzidine solution (TMB) and p-nitrofenil-2-acetamide-β-D-glucopyranoside (Sigma-Aldrich) were used to determine myeloperoxidase (MPO) and NAGase activities.

### 2.2. Preparation of the lutein-loaded PVP nanoparticles

Nanoparticles were prepared by the dissolution in common solvent method according to Karavas and co-workers [21] with minor modifications as follows. Initially, PVP was dissolved in ethanol under magnetic stirring until the obtainment of translucent solutions. After that, lutein and Tween 80 were added to this solution, remaining in mild agitation for five minutes. The obtained mixture was sonicated for 15 min under pulse condition of 30 s on and 10 s off (Fisher Scientific, 120 W and 1/8′ tip). Finally, the solvent was evaporated in a circulation oven at 40 °C for 24 h. Formulations were prepared with the following mass proportions of PVP:lutein: 0:1, 2:1, 4:1, 6:1, 8:1, 10:1, 12:1 and 14:1 (m:m).

### 2.3. Nanoparticle characterization

Morphological characterization of the nanoparticles was performed using Transmission Electron Microscopy (TEM; JEOL model JEM 2100, 200 kV). Diluted samples were dripped onto 300 mesh parlodium covered copper grids. Grids were dried at room temperature and stained with osmium tetroxide for 4 h. Thermal properties of the nanoparticles were investigated by Differential Scanning Calorimetry (DSC, Perkin Elmer 4000). Samples were accommodated in sealed aluminium pans under nitrogen flow (50 $mL \cdot min^{-1}$) and heated from 20 to 360 °C at 20 $°C \cdot min^{-1}$. Fourier Transform Infrared (FTIR) spectra were acquired using a Frontier Perkin Elmer equipment in potassium bromide pellets, with resolution of 1 $cm^{-1}$ from 4000 to 400 $cm^{-1}$. X-ray diffraction analyses (XRD, Bruker, D8 Advance) were carried out from 3° to 60° (2θ) at 5.9° $\cdot min^{-1}$, using Cu Kα radiation generated at 40 kV and 35 mA. For contact angle measures, nanoparticles were dispersed in ethanol and dripped onto a glass slide in duplicate. Ethanol was allowed to evaporate under dry atmosphere protected from light for 48 h. Finally, plates were evaluated in a goniometer (Hamé-Hart) using drop of diiodomethane on the sample surface. A total of 10 measured for each glass slide carried out.

### 2.4. Interaction between PVP and lutein

The spectrophotometric method suggested by Karavas and co-workers [21] was used to verify the magnitude of the interaction between the wall material (PVP) and the encapsulated compound (lutein) [21]. A lutein solution in ethanol was prepared (0.05 $mg \cdot mL^{-1}$) followed by the gradually addition of PVP in order to obtain different mass proportions of PVP:lutein, ranging of 0:1 to 16:1 (m:m). Each obtained system was analysed by UV–Vis spectrophotometry (Ocean Optics, USB-650-UV-VIS Red Tide) at the maximum absorption peak of lutein (446 nm).

Eq. (1) was used to correlate PVP proportion in the system with the interaction intensity (F) between PVP and lutein [21], where $A_S$ and $A_0$ represent the absorbance value at 446 nm of the PVP:LUT system and pristine lutein, respectively.

$$F = \frac{A_S - A_0}{A_0} \quad (1)$$

### 2.5. Phase-solubility studies of lutein-loaded PVP nanoparticles

In order to verify the influence of PVP proportion in the solubility of the nanoparticles in water, formulations were evaluated according to the procedure described by Mura and co-workers [25]. Aliquots of each formulation were transferred to test tubes so that 5 mg of lutein was present in each tube. After that, 7 mL of water was added and the test tubes were stirred during 1 h at 25 °C. Solutions were filtered through Millipore membrane filter (0.45 μm) and 500 μL of the filtrate was lyophilized (Liotop, L101). Lastly, 2 mL solution of methanol:ethyl acetate 1:1 (v:v) was added to the lyophilized samples and were evaluated by UV–Vis spectrophotometry at 446 nm. The amount of lutein that passed through the filtration membrane (0.45 μm) was considered water soluble since particles below that average size tend to remain stable without noticeable precipitation.

### 2.6. In vivo and ex vivo studies

#### 2.6.1. Animals

Adult male Swiss mice (90–60 days of age; $n = 61$) were used, weighting between 25 and 30 g each, from the Central Animal Laboratory at the State University of West Paraná. Animals were housed in polypropylene cages of 41 × 34 × 16 cm, under light controlled conditions (light/dark cycles of 12 h) at (22 ± 2) °C, with food and water *ad libitum*. The animal use protocol followed the Official Ethical Guidelines of the Brazilian Government and the Ethics Committee on Animal Use (CEUA) of UNIOESTE and UTFPR. All experimental protocols were designed to minimize the number of animals used and their suffering, according to the project approved by the Ethics Committee, ordinance no. 2729/2014 – GRE.

Animals were divided into 6 groups and each group was fed by oral gavage during 14 days as following: saline solution (10 $mL \cdot kg^{-1}$), olive oil (10 $mL \cdot kg^{-1}$), pristine lutein (100 $mg \cdot kg^{-1}$) and lutein-loaded PVP nanoparticles with 8:1 (m:m) PVP:LUT mass proportion (1.5, 5.0 and 10.0 $mg \cdot kg^{-1}$).

#### 2.6.2. Object recognition task

The object recognition task was carried out according to the protocol described by Bevins and Besheer [26], which is composed of three distinct phases: habituation, training and testing, each one with duration of 10 min.

Crossing and rearing were calculated in the habituation session. In training session, mice were introduced to two identical objects and in testing session, one of the objects was replaced for a new one. The time spent to explore the familiar object (A) and the new object (B)