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Artigo

PREPARATION OF PVP HYDROGEL NANOPARTICLES USING LECITHIN VESICLES[#]

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Hydrogels micro, sub-micro and nanoparticles are of great interest for drug encapsulation and delivery or as embolotherapic agents. In this work it is described the preparation of nano and sub-microparticles of pre-formed, high molecular weight and monomer free poly(*N*-vinyl-2-pyrrolidone) encapsulated inside the core of lecithin vesicles. The hydrogel particles are formed with a very narrow diameter distribution, of about 800 nm, and a moderate swelling ratio, of approximately 10.

Keywords: PVP hydrogel nanoparticle; lecithin vesicle; photo-Fenton reaction.

INTRODUCTION

Hydrogel nanoparticles (nanogels) are of interest due to their ability to combine the advantages of biocompatibility, inherent to most hydrogels,[1] and small size.[2] Potential applications of nanogels include controlled drug delivery systems[3] (oral[4] and/or parenteral[5] delivery) and alternative therapies, like embolotherapy.[6]

Nanogels can be obtained from monomer polymerization in presence of difunctional monomers, either in w/o emulsions[7,8] or using the core of reverse micelles as formatting system.[9] Alternatively, nanogels can be prepared by inclusion in reverse micelles of pre-formed polymers, followed by crosslinking.[10] Vesicles also can be used to obtain hydrogels nanoparticles, by monomer encapsulation followed by polymerization[11] or gelation of encapsulated polymers, generally induced by sol-gel temperature transitions[12] or ionic crosslinking,[13] typically without removal of the lipid bilayer.[14] These gel-like vesicles work as cell models, since they have elastic modulus comparable to that of cell cytoplasm[14] and are considered artificial cytoskeletons.[14,15] Vesicles containing poly(*N*-isopropylacrylamide) are good examples. This polymer responds to temperature changes forming physical gels on a reversible process, even within vesicles.[16] This temperature induced sol-gel transition within the vesicle mimics cell stiffening.[12] Hydrogel-liposome assemblies (lipobeads) can also be used as drug delivery systems.[17] The lipid bilayer is often left intact and the encapsulated polymer is not crosslinking.

Vesicles have advantages compared to other systems for producing nanogels, since these assemblies permit more diameter control alternatives.[18] The particle diameter can be controlled varying the vesicles diameter from a few nanometers to millimeters and can be obtained with a narrow size distribution. Vesicles can be prepared using low amounts of non-toxic solvents and this compares favorably with other methods such as emulsion polymerization. The major component of a vesicle preparation is water and a non-toxic surfactant can be chosen, thereby resulting in a biocompatible preparation.

Poly(*N*-vinyl-2-pyrrolidone) hydrogels can be prepared by several straightforward methodologies.[19-22] One of these methods involves the use of photo-Fenton reaction[21], using ferric ions, hydrogen peroxide and UVA radiation.[22]

Here we describe the use of lecithin vesicles as formatting system to obtain poly(*N*-vinyl-2-pyrrolidone) hydrogel particles, using photo-Fenton or Fenton reactions for crosslinking.

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[#]This paper is dedicated to Prof. Hans Viertler

EXPERIMENTAL

Materials

Egg phosphatidylcholine was purified from egg yolks as described by Maximiano *et al*..[23] Soy lecithin was obtained from crude soy lecithin capsules by purification with the same method used for egg phosphatidilcholine.[23] Dioctadecyldimethylammonium chloride (DODAC) was obtained from the bromide salt (DODAB; Aldrich) after ion exchange with a Dowex-21K resin (Fluka) in the chloride form in methanol. 1,2-Dipalmitoyl-3-trimethylammonium-propane chloride (DPTMA) was synthesized as described previously[24] as bromide salt, which was exchanged for chloride as indicated for DODAC. Poly(*N*-vinyl-2-pyrrolidone) (PVP) (Plasdone K90, $\overline{M}_w$ = 1,300,000) was kindly donated by BASF. $FeCl_2$, $FeCl_3$ and H_2O_2 30% (Aldrich), NaCl (Merck) and CH_2Cl_2 (Synth Brazil) were analytical grade and used as received. Water was deionized.

Methods

PVP encapsulation in liposome

PVP containing vesicles were obtained as follows (Figure 1): 0.5 mL of a CH_2Cl_2 solution containing 50 mg of egg or soy phosphatidylcholine (100 mg mL^{-1}) were injected into 5 mL of PVP aqueous solution (80 g L^{-1}), containing 17 mmol L^{-1} $FeCl_2$ maintained at 50 ºC. CH_2Cl_2 solution was injected with a syringe adapted in a KD Scientific Inc. Model KDS120 Push-Pull Pump, equipped with a fine-gauge needle (No 3D). During injection, a N_2 stream was bubbled into PVP solution, which continued after liposome formation until removal of residual solvent. Liposome suspension was centrifuged at 22,800 × g for 30 min (Hitachi Himac CR20B2 centrifuge; Hitachi Ltd.) at 25 ºC to remove non-entrapped polymer from the external phase. The liposome-containing pellet was washed twice by centrifugation under same conditions in saline solution (51 mmol L^{-1}). The washed pellet was suspended on 20 mL of $NaCl_{(aq)}$.

PVP concentration inside vesicles was estimated using Lugol method.[25] Resulting PVP complex with Lugol Reagent (PVP-I_3^-) absorbs at 400 nm, and the PVP concentration was obtained using appropriate calibration curve. The encapsulation yield (EY) was calculated using Equation 1.

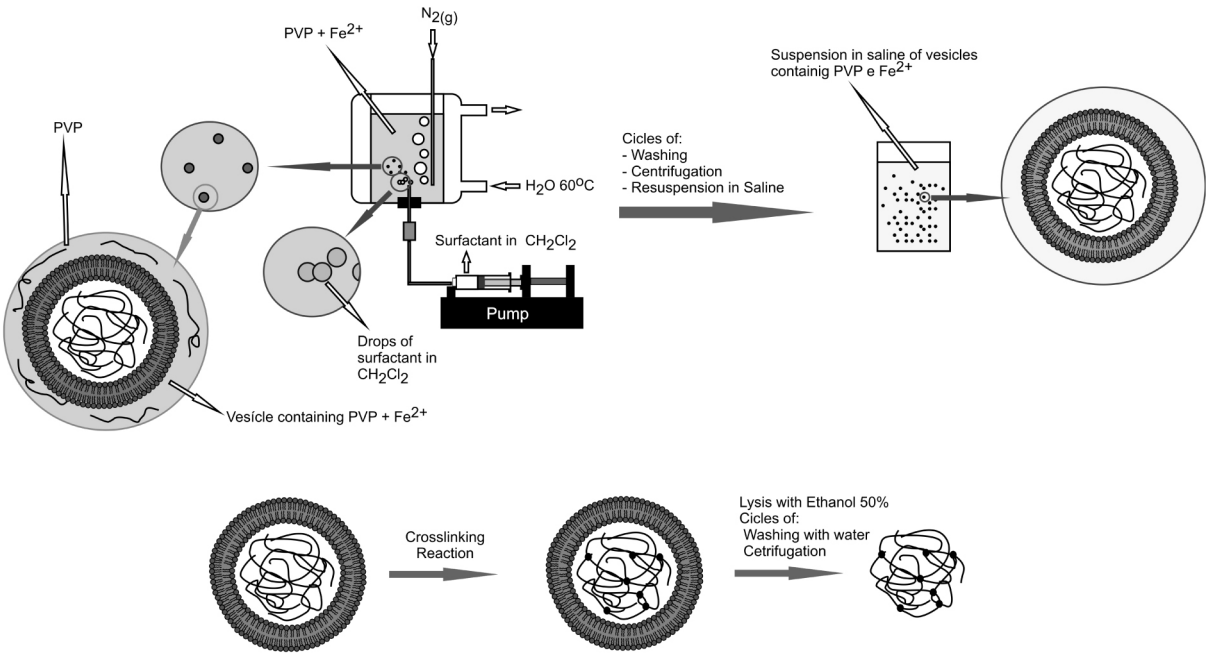

Figure 1. *Production of PVP hydrogel particles*

$$EY\ (\%) = \frac{m_{PVP}^{vesicles}}{0.4} \times 100 \quad (1)$$

where $m_{PVP}^{vesicles}$ is the mass of encapsulated PVP in the vesicles pellet and 0.4 is the mass of PVP in the initial solution (in g).

PVP entrapped polymerization

To crosslink PVP inside the vesicles, 4 mL of H_2O_2 diluted on $NaCl_{(aq)}$ (51 mmol L^{-1}) was added to the PVP-containing vesicles ($[H_2O_2]_{final}$ = 200 mmol L^{-1}).[22] As Fe^{2+} can be oxidized to Fe^{3+} inside vesicles before H_2O_2 addition, to promote crosslinking by photo-Fenton reaction[21] H_2O_2 containing vesicles suspension was submitted to 12 h of UV radiation (360 nm). The hydrogel particles were purified by lysing vesicles washing 3× with ethanol 50%. The resulting pellet was resuspended in water and freeze-dried.

Dynamic light scattering measurements

The determination of particles size distribution was carried out by dynamic light scattering (DLS), using a spectrometer from Brookhaven Instruments. The average hydrodynamic diameters were obtained from the unimodal distribution of particles analyzed by Zeta PALS Particle Sizing Software Version 2.29.

Scanning electron microscopy (SEM)

Particles were analyzed by SEM (FEG-SEM, model FEG 7401F, from Jeol). Samples were prepared by fixing the particles (freeze-dried powder) on a double-face copper tape and the specimens were analyzed without coating.

Swelling ratio determination (Q)

Swelling ratio (Q) determination was done by weighing the swollen particles pellet in an analytical balance (m_{swo}), followed by freeze drying and weighing the dry pellet (m_{dry}). Q was calculated directly by Equation 2.

$$Q = \frac{(m_{swo} - m_{dry})}{m_{dry}} \quad (2)$$

RESULTS AND DISCUSSION

Polymer encapsulation

The efficiency of vesicle encapsulation depends on polymer size and decreases with the increase of polymer molecular weight.[26,27] Szoka and Papahadjopoulos[28] found that encapsulation efficiency decreases with the increase of molecular weight of the encapsulated protein. Dominak *et al.*[26] observed the same inverse relationship between encapsulation efficiency and molecular weight studying polyethylene glycol and dextran encapsulation in giant vesicles. To encapsulate PVP, various surfactants were tested, including lecithin from egg yolk and soybeans and cationic surfactants. Table 1 shows the surfactants and their respective PVP encapsulation yield. As each vesicle in solution can have different encapsulation values,[26] it is important to emphasize that PVP inclusion was measured by estimating the total amount that was incorporated and therefore the incorporation represents an average over all vesicles.

Table 1. PVP encapsulation in obtained vesicles

Surfactant	EY (%)
Egg lecithin	0.8 ± 0.1
Soy lecithin	2.0 ± 0.3
DPPC	0.6 ± 0.1
DODAC 1% + soy lecithin	0.4 ± 0.1
DPTMA 1% + soy lecithin	2.0 ± 0.4

The best encapsulation efficiency was obtained with soy lecithin vesicles. Lecithins of different sources exhibit distinct properties.[23,29] In particular the length of the apolar portion of the molecule and the insaturation of the alkyl chain will result in vesicles with different properties.[30] The method used to obtain vesicle encapsulated PVP also contributes to high encapsulation efficiency.[18]

Since the method used to crosslink the encapsulated polymer requires long irradiation times (photo-Fenton reaction), the addition

of cationic surfactants decreases vesicle aggregation during irradiation by increasing vesicle repulsion. Two saturated surfactants where tested: DODAC and DPTMA. Encapsulation efficiency was higher with DPTMA than with DOCAC. DODAC contains two octadecyl chains linked to the tetraalkylammonium group; in DPTMA the acyl chains are attached to a propanediol moiety linked to tetraalkylammonium group (Figure 2). Bilayer packing defects are expected[31,32] upon incorporation of DODAC to lecithin causing a decrease in polymer entrapment. Measurements of polymer leak showed that entrapped PVP was retained after several washes (supernatant PVP concentration was measured as $<10^{-4}$ g L^{-1} for DPPC vesicles and as 0.02 g L^{-1} for DODAC/lecithin vesicles, after 5 wash cycles).

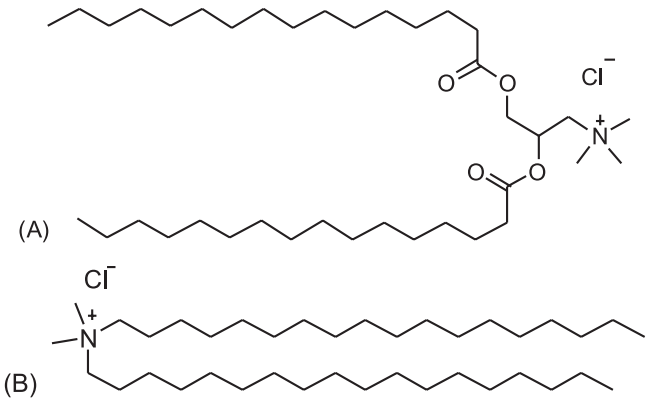

Figure 2. *Structures of (A) DPTMA and (B) DODAC*

Crosslinking reaction

Two methods were used to crosslink the polymer. The Fenton reaction, an efficient method to obtain PVP hydrogels with good mechanical properties,[22] has been used to obtain PVP nanogels with superabsorbent properties.[10] Photo-Fenton reaction, a photochemical analog of Fenton reaction, also produces PVP hydrogels[21] (Scheme 1).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \qquad \text{Fenton reaction}$$

$$Fe^{3+} + HO^{-} \rightarrow FeOH^{2+}$$
$$FeOH^{2+} + H_2O_2 \xrightarrow{h\nu} Fe^{2+} + OH^{\bullet} \qquad \text{photo-Fenton reaction}$$

Scheme 1. *Hydroxyl radical generation from Fenton and photo-Fenton reactions*

The Fenton reaction is interesting because it allows a fast route to obtain PVP hydrogels with any desired format.[33] However, this reaction requires Fe^{2+} which autoxidizes. On the other hand, photo-Fenton reaction uses Fe^{3+} ions, H_2O_2 and UV radiation (λ = 365 nm). Since the vesicles can scatter and react with H_2O_2 (lipoperoxidation), the UV dose and H_2O_2 concentrations were higher than those described.[21]

Fenton and photo-Fenton reactions did not change significantly the vesicles diameter distribution (Figures 3 and 4). This finding does not imply that the lipid bilayer remains unchanged during crosslinking reaction, since hydroxyl radical is known to react with unsaturated acyl chains.[34]

Particle characterization

When conventional Fenton reaction was used no pellet was obtained after vesicle´s lysis, evidencing absence of crosslinking, probably due Fe^{2+} depletion via its autoxidation. A phosphorus-free yellow pellet was obtained after crosslinking by photo-Fenton reaction and vesicles lyses.[39] This pellet was resuspended in water and analyzed by SEM and DLS. Results demonstrated the presence of a formless particulate material with dimensions comparable to vesicles (Figures 5 and 6).

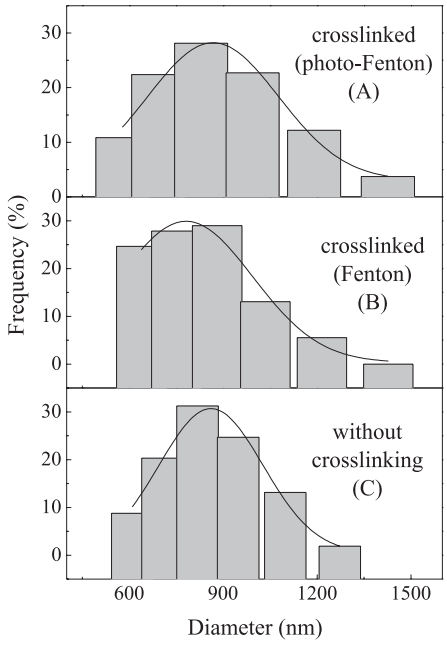

Figure 3. *Diameter distribution of egg lecithin vesicles containing PVP. (A) vesicles submitted to Fenton reaction; (B) vesicles submitted to photo-Fenton reaction and (C) vesicles prior to crosslinking reaction*

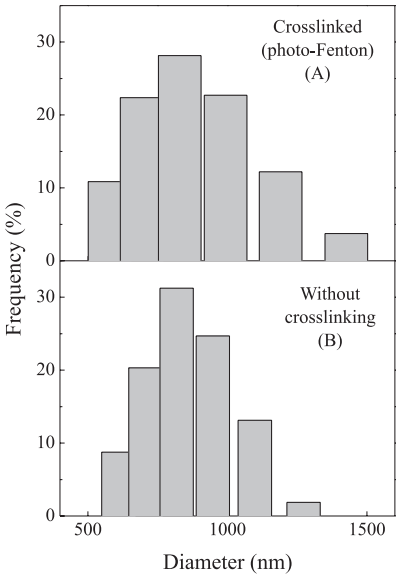

Figure 4. *Diameter distribution of soy lecithin vesicles containing PVP. (A) vesicles submitted to photo-Fenton reaction and (B) vesicles prior to crosslinking reaction*

The diameter of nano and sub-microgel and nanogel particles can be controlled by choosing the vesicle preparation method. Polyacrylamide nanogels (from monomers) covered by phospholipids bilayers obtained by detergent depletion[35] or freeze-thaw cycles followed by extrusion[36]/sonication[11,17] presented diameters of 100 and 200 nm, respectively. Larger nanogels (about 450 nm) are obtained from encapsulation of 20% dextran hydroyethylmethacrylate solution ($\bar{M}n$= 19000) inside vesicles obtained by phospholipids film hydration, followed by extru-

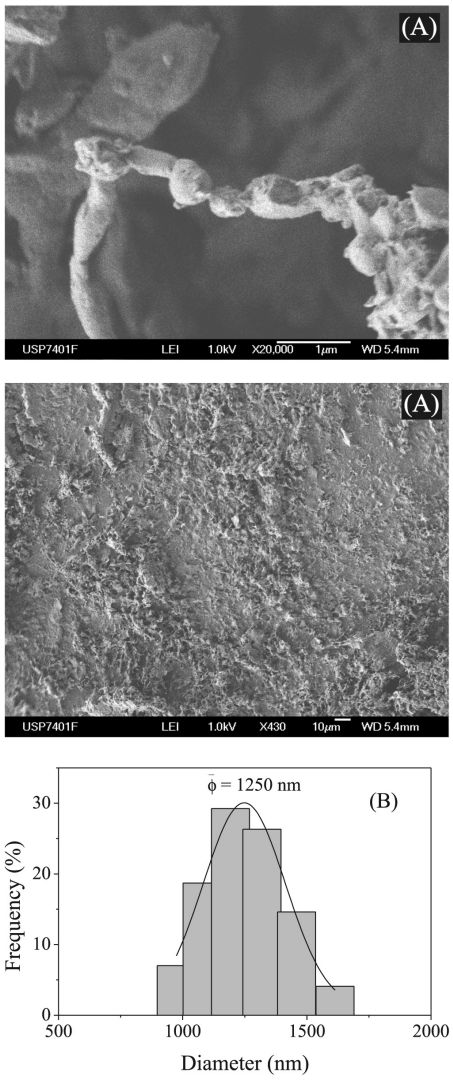

Figure 5. *SEM image of freeze-dried particles obtained from egg lecithin vesicles (A) and DLS particles diameter distribution (B)*

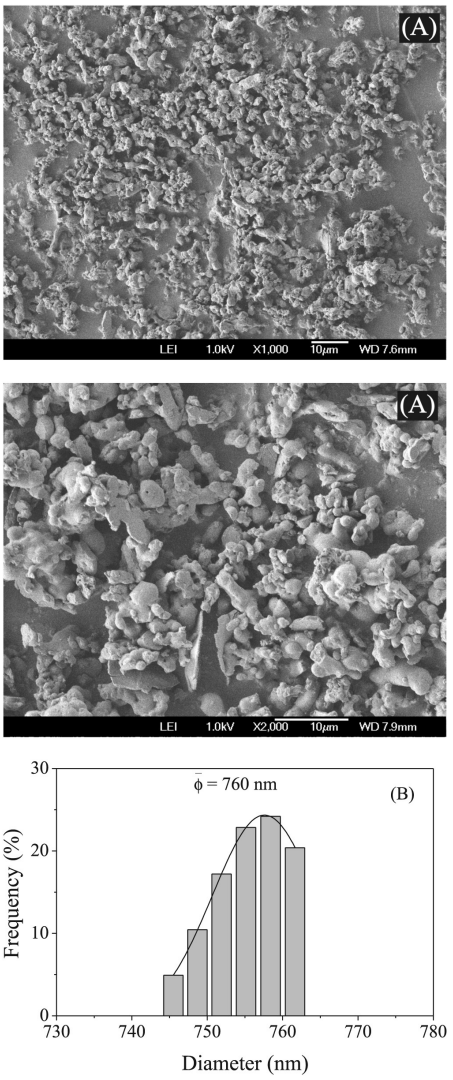

Figure 6. *SEM image of freeze-dried particles obtained from soy lecithin vesicles (A) and DLS particles diameter distribution (B)*

sion.[37] Poly(ethylenedioxythiophene)/poly(styrenesulfonate)[13] and poly(*N*-isopropylacrylamide)[12,16] microgels, in conjunction with lipid bilayers, are obtained by injecting a polymer solution inside GUVs, followed by freeze-thaw cycles and electroporation. The vesicle diameters, in these latter cases, vary from 5 to 100 μm.

The hydrogel particles obtained here showed no defined morphology, in spite of being obtained from spherical vesicles. Vesicles may be destabilized during crosslinking by H_2O_2 oxidation and ensuing lipoperoxidated[38] (Figure 7). Fluctuation of crosslinking density, known to influence hydrogel particle morphology, may also account for the lack of defined shape.[13] Non spherical particles have also been reported with other nanogels prepared using vesicles.[17,37]

Particle swelling was estimated directly and the swelling ratio (*Q*) was 10, i.e., particles absorb only 10 times their mass in water. This is a strong indication that the crosslinking density is high since *Q* is inversely proportional to the ability of absorbing water.

Swelling of nanogels formatted from vesicles has seldom been discussed in literature. In the case of poly(*N*-isopropyl acrylamide) hydrogels and copolymers, coagulation of particles was observed instead of the variation in swelling. Kasakov *et al.* have extensively studied this systems[11,17,36] mainly in regard to the interaction between particle and liposome. Since the hydrogel used is thermo- and pH-responsive, coagulation was observed upon heating, including coagulation of the lipid bilayer, which, after

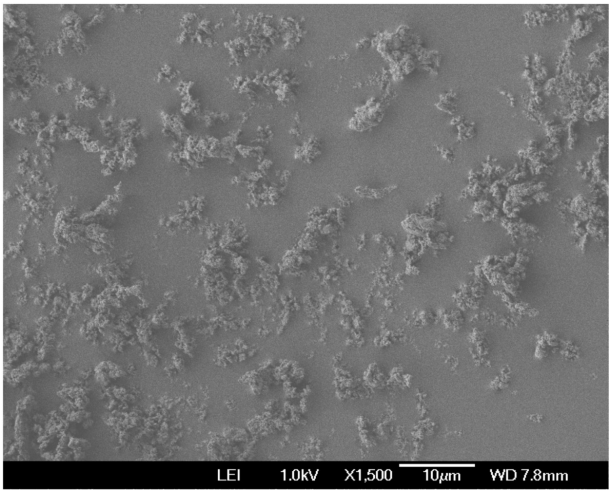

Figure 7. *SEM image of particles obtained from soy lecithin vesicles with 10% DPTMA*

cooling, surround the entire set of nanoparticles in a reversible process.[36] The authors also observed an increase in diameter with pH change: 100 nm at pH 6.5 to 300 nm at pH 3.0, with dimerization of particles.[17]

CONCLUSION

Injected lecithin vesicles can encapsulate high molecular weight PVP. The encapsulated polymer can be crosslinked by photo-Fenton reaction and hydrogel nano and sub-microparticles can be isolated. Spherical morphology was not achieved, but the produced hydrogel particles have a narrow diameter distribution compatible with the vesicles diameter distribution.

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