Dextrin-Microencapsulated Porphyrin

Luminescent Properties

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Photophysical properties of porphyrins in aqueous solutions are strongly affected by aggregation. One possible solution to this problem is to encapsulate the porphyrin into polymeric spheres, to provide an environment where the photosensitizer can be administered in its monomeric form in such treatments as photodynamic therapy. Here we report the microencapsulation of the *meso*-tetrakis(4-sulphonatophenyl) porphyrin ($TPPS_4$) photosensitizer by the ultrasonic spray-drying technique. The encapsulated $TPPS_4$ was morphologically characterized by scanning electron microscopy, and its photophysical properties were studied and compared with those of a physical blend of dextrin and $TPPS_4$. We successfully encapsulated $TPPS_4$ into dextrin microspheres, and the encapsulated photosensitizer displays higher luminescence intensity than that of the prepared physical blends.

Key words: **microencapsulation; spray-drying; porphyrin; luminescence; photodynamic therapy**

Introduction

Photodynamic therapy (PDT) is an efficient therapeutic modality for the treatment of a variety of oncological, cardiovascular, dermatological, and ophthalmic diseases. PDT is based on the use of photosensitizers, which are preferentially taken up and/or retained by diseased tissue.[1–3] Activation of the photosensitizer upon absorption of light at the appropriate wavelength takes the drug from its ground state (^{1}PS) to an excited singlet state ^{1}PS* (FIG. 1). From this state, the drug may decay directly back to the ground state by emitting fluorescence, which is a property that can be used clinically for photodetection. However, to obtain a therapeutic photodynamic effect, the photosensitizer must undergo electron spin conversion to its triplet state (^{3}PS*). In the presence of oxygen, the excited molecule can react directly with a substrate by proton or electron transfer, to form radicals or radical ions that can interact with oxygen to produce oxygenated products (type I reaction). Alternatively, the energy of the excited photosensitizer can be directly transferred to oxygen to form singlet oxygen (type II reaction), which is the most damaging species generated during PDT.[4,5] The generation of cytotoxic species leads to irreversible destruction of the treated tissues. Compared with current treatments, including surgery, radiation therapy, and chemotherapy, PDT is advantageous because it is an effective and selective method of destroying diseased tissues without damaging surrounding healthy tissues.[1]

Porphyrins and other closely related macrocycles, which may contain peripheral substituents or incorporated metal cations, have been used in many investigations as potential photosensitizers for PDT of cancerous diseases.[6,7] $TPPS_4$ (FIG. 2) is considered one of the most promising water-soluble synthetic compounds for application in PDT,[8] and it has been studied and used in clinical experiments.[7,9] From a synthetic and economic point of view, $TPPS_4$ is the most accessible water-soluble porphyrin.[10,11] The following factors are important for the successful application of these compounds in the PDT framework: (1) the photophysical properties of the macrocycles, (2) their affinity for specific biological structures (macromolecules, membranes), and (3) self-aggregation of the macrocycles under physiological conditions, which leads to changes in their photophysical properties as well as in subcellular tissue distribution.[6]

Self-aggregation in aqueousen systems is a common feature of water-soluble porphyrins carrying charged substituents.[6] The polar groups (e.g., sulfonic, amino, or ammonium groups) that convert the porphyrin chromophore into a water-soluble molecule can exhibit strong intermolecular interactions with the center of other porphyrin molecules,

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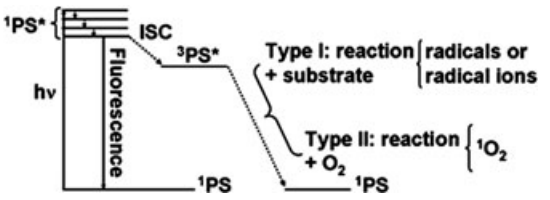

FIGURE 1. Energy pathways in the PDT process.

so that their positive (metal cation or protonated nitrogens) or negative (deprotonated nitrogens) charges can be neutralized.[10] Also, when a porphyrin is dissolved in water, this macrocycle can form noncovalent dimers or larger aggregates in bulk solution or on the surface of oppositely charged molecules (other macrocycles, cyanines, polypeptides, proteins, nucleic acids, or the components of mitochondrial membranes).[6]

Aggregation strongly affects the spectral and energetic characteristics of porphyrins, thus reducing quantum yields and excited-state lifetimes (singlet and/or triplet). One possible solution to this problem is to encapsulate the porphyrin in biodegradable polymeric microspheres to provide an environment where the photosensitizer can be administered in a monomeric form.[1]

Naturally occurring porphyrin systems are protected and preserved in their monomeric form by biological polymers (proteins), such as the prosthetic group heme in hemoglobin and mioglobin.[14] Encapsulation is a technique in which a membrane encloses small particles of solid, liquid, or volatile compounds with the objective of offering protection to the core material from adverse environmental conditions such as undesirable effects due to light, moisture, and oxygen, thus contributing to an increase in the product shelf life.[15] Also, the wall material protects the sensitive active (or core) against the effects of temperature, pH, and chemical incompatibilities; modifies physical and chemical characteristics; prevents the loss of volatile actives; and controls active release.[16,17]

Although several methods are available for encapsulation of actives in polymeric matrices, spray-drying has been widely used in the pharmaceutical industry.[18] Spray-drying is by definition the transformation of a pumpable feed from a fluid state (solution, dispersion, or emulsion) into a dried particulate form by spraying the feed into a hot drying medium.[16,17] Indeed, this technique is a one-step process for turning a liquid feed into a powder product, thus minimizing handling while reducing the powder bulk weight and size.[19] It is a continuous and one-cycle operation

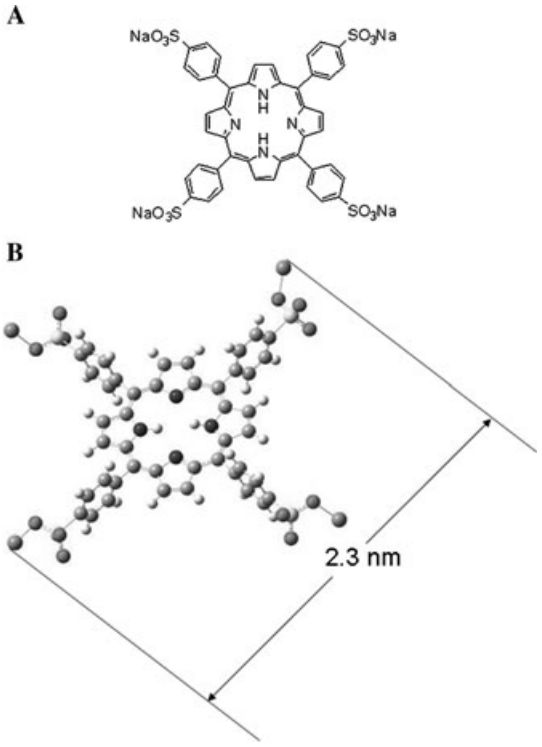

FIGURE 2. $TPPS_4$ **(A)** molecular structure and **(B)** optimized structure obtained by semiempirical calculations (PM3).[12,13]

consisting basically of a sequence of four steps: atomization, mixing of spray and air, evaporation, and product separation.[16,20,21] Spray-drying can be adapted to the creation of a matricial system of the spherical type, starting from complex liquid mixtures comprising an active material dissolved, dispersed, or emulsified with a polymer in an aqueous or organic solvent.[22] Common carriers for these encapsulation processes include carbohydrates, gums, and cellulose esters and ethers. In particular, polysaccharide-based microparticles have gained much more attention in the development of microparticulated systems because they offer flexibility with respect to the desirable drug release profile, they are cost-effective, and they have broad regulatory acceptance. Also, polysaccharidic biodegradable matrices are of interest because the degradation of natural products occurs naturally in the human body.[23]

In this work we report the microencapsulation of the $TPPS_4$ photosensitizer in dextrin by an ultrasonic spray-dryer developed by our group.[24] The encapsulated $TPPS_4$ was morphologically characterized by scanning electron microscopy (SEM), and its photophysical properties were studied and

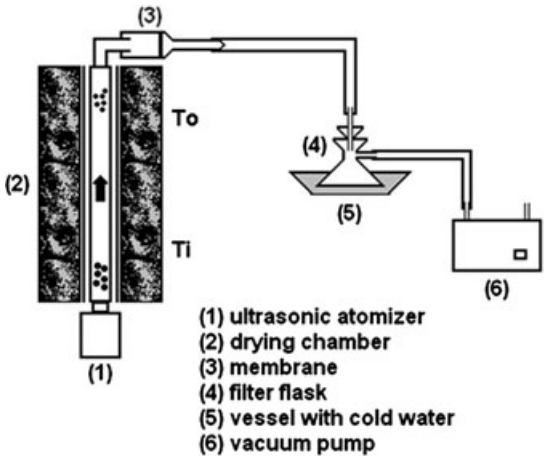

FIGURE 3. Scheme of the cocurrent ultrasound spray-drying system.[24]

compared with those of a physical blend of dextrin and $TPPS_4$.

Experimental Method

Feed Solution

To prepare the feed solution, we added 1.0 g of dextrin (ACROS®, Morris Plains, NJ) to 40.0 mL of water. The mixture was then stirred and heated at 70°C for 30 min to promote dextrin solubilization. This solution was stirred for 24 h at room temperature. Later, 10.0 mg of $TPPS_4$ (Mid Century®, Chicago, IL) was dissolved in the solution at room temperature, and the final volume was completed to 50.0 mL. The final solution was stirred for 24 h at room temperature.

Microencapsulation of $TPPS_4$ in Dextrin

The microencapsulation process was accomplished using a previously described ultrasonic spray-dryer system (FIG. 3).[24] In this process, micro- and nanodroplets from the feed solution were produced and dried inside a vertical tubular furnace. The inlet and outlet temperatures, 280°C and 275°C, respectively, for the drying chamber were chosen according to the polymer thermal features.

Physical Blend Preparation

To evaluate the effect of microencapsulation on the porphyrin photophysical properties, we prepared physical blends of dextrin and $TPPS_4$. We used the following $TPPS_4$/dextrin ratios: 0.2, 0.3, 0.8, and 1.0% (all wt/wt). Each preparation was obtained by adding dextrin and porphyrin into a porcelain crucible, followed by homogenization with methanol. The crucible was later heated to promote methanol evaporation.

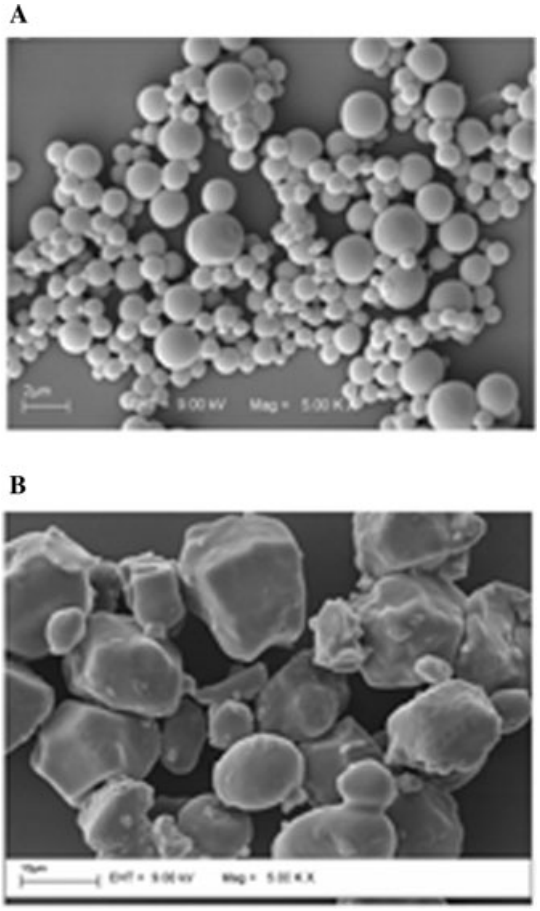

FIGURE 4. SEM images of $TPPS_4$ **(A)** loaded in dextrin microspheres (0.7% wt/wt) and **(B)** physically blended (0.8% wt/wt).

Characterization

Morphological

$TPPS_4$–dextrin microspheres prepared by spray-drying and one physical blend (0.8%) were morphologically characterized by SEM (Zeiss *Evo® 50*; Cambridge; Cambridgeshire England; operated at 9 kV). For SEM image acquisition, the powder was dispersed in chloroform under ultrasound stirring, supported on an aluminum stub, and coated with a gold thin layer by using a sputtering system after drying. Sphere diameter size distribution was estimated from SEM images.

Encapsulation Efficiency and Photosensitizer Ratio Associated with the Microspheres

Experiments were carried out in triplicate. Samples (~10.0 mg) of microencapsulated $TPPS_4$ were washed with 1.0 mL of methanol. Then, the samples were

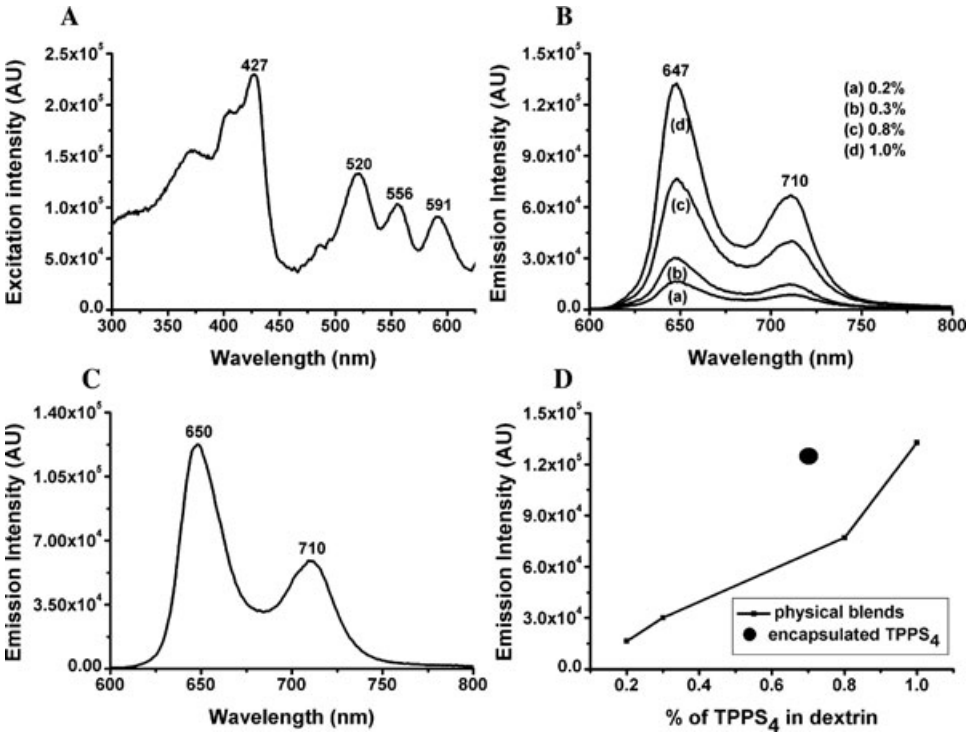

FIGURE 5. (A) Excitation spectrum of microencapsulated $TPPS_4$ (0.7% wt/wt) ($\lambda_{em} = 650$ nm). Emission spectra of **(B)** physically blended $TPPS_4$ and **(C)** microencapsulated $TPPS_4$ (0.7% wt/wt). **(D)** Comparison between the emission intensities of microencapsulated and physically blended $TPPS_4$ at 650 nm ($\lambda_{exc} = 520$ nm).

centrifuged and the methanol containing nonencapsulated $TPPS_4$ was removed; this washing process was repeated four times. The amount of nonencapsulated $TPPS_4$ was evaluated by measuring the absorbance at 417 nm. Later, 1.0 mL of distilled water was added to each sample and left to stand for 24 h. After sonication for 5 min, the samples were centrifuged and the encapsulation efficiency and photosensitizer ratio were determined by measuring the absorbance at 417 nm.

Luminescence Spectroscopy

Emission spectra of microencapsulated and physically blended $TPPS_4$ were recorded at room temperature on a Spex Triax 550 Fluorolog III spectrofluorometer (Edison, NJ). All the solid samples were supported in glass capillary tubes, and their emissions were acquired perpendicularly to the excitation.

Absorption Spectroscopy

Absorption spectra of microencapsulated and physically blended $TPPS_4$ were recorded on an Ocean Optics® USB4000 spectrometer (Dunedin, FL).

Results and Discussion

$TPPS_4$ Microencapsulation and Morphological Characterization

From the SEM photomicrographs (FIG. 4) one can see that the produced particles are spherical and exhibit a smooth surface. Diameter size distribution was measured from the SEM image. A total of 1000 particles were analyzed, and the average diameter was $0.93 \pm 0.40\ \mu m$.[25] The encapsulation efficiency (total amount of encapsulated porphyrin/total amount of porphyrin) is 97% (wt/wt), and the photosensitizer ratio (total amount of porphyrin/total amount of dextrin–porphyrin) associated with the microspheres is 0.7% (wt/wt).

One of the physical blends of $TPPS_4$ and dextrin (0.8% wt/wt) was also analyzed by SEM. These particles are larger than the microencapsulated ones and do not a have defined form.

Luminescence Spectroscopy

Initially, the microencapsulated $TPPS_4$ excitation spectrum was acquired (FIG. 5A). Emission spectra of microencapsulated and physically blended $TPPS_4$,

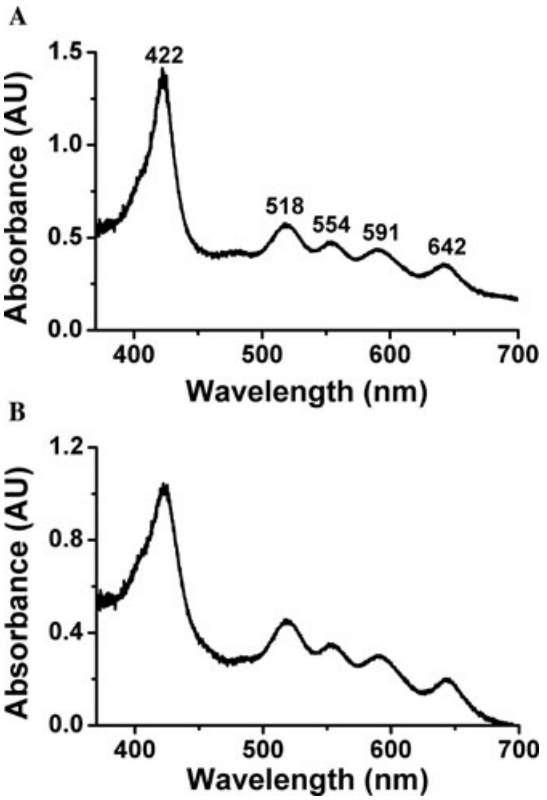

FIGURE 6. Absorption spectra of $TPPS_4$ **(A)** loaded in dextrin microspheres (0.7% wt/wt) and **(B)** physically blended (0.8% wt/wt).

recorded at room temperature, are presented in FIGURE 5. FIGURE 5B shows the luminescence emission of $TPPS_4$ in different concentrations (0.2, 0.3, 0.8 and 1.0% [all wt/wt]) in the physical blends with dextrin. These spectra display the same characteristic emission bands of the free-base porphyrins in aqueous solution at 650 and 710 nm, indicating that no structural change has occurred. However, the emissions from the physical blend samples are not as intense as the encapsulated ones because of the porphyrin concentration quenching in the blends.

FIGURE 5C shows the emission spectrum of microencapsulated $TPPS_4$, which also displays the same characteristic emission bands of free-base porphyrins in aqueous solution at 650 and 710 nm. As before, the ratio of $TPPS_4$ associated with the dextrin microspheres is 0.7% (wt/wt). However, this sample presents higher luminescence intensity than that of a more concentrated physical blend (0.8% wt/wt of $TPPS_4$). This increase is shown in FIGURE 5D and can be explained on the basis of particle form.

SEM images (FIG. 4) reveal a more uniform shape and a smaller microsphere size than those of the physical blend. Microencapsulation is responsible for a more efficient distribution of $TPPS_4$ molecules in the spheres, leading to higher emission. Because aggregation strongly affects the spectral and energetic characteristics of porphyrins, one possible solution to this problem is to encapsulate the porphyrin into biodegradable polymeric microspheres, thus providing an environment where the photosensitizer is in its monomeric form.

Absorption Spectroscopy

This analysis was performed to investigate the possible changes on $TPPS_4$ absorption spectra. The spectra of microencapsulated and physically blended $TPPS_4$ are shown in FIGURE 6.

The spectra presented in FIGURE 6 agree with the typical electronic absorption spectrum of free-base porphyrins. This spectrum consists of a strong transition to the second excited state at about 400 nm (the Soret band) and four weak transitions to the first excited state from 500 to 700 nm (the Q bands).[26]

Conclusion

We successfully encapsulated $TPPS_4$ into dextrin microspheres, which led to a higher luminescence intensity than that of the physically prepared blends. When the photosensitizer is microencapsulated in biodegradable spheres, it is protected from the environment and can be administered in PDT in its monomeric form. Also, the developed spray-drying method is suitable for drug encapsulation and its desired controlled-release action.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. KONAN, Y.N., R. GURNY & E. ALLÉMANN. 2002. State of the art in the delivery of photosensitizers for photodynamic therapy. J. Photochem. Photobiol. B **66:** 89–106.
2. ENGELMANN, F.B., S.V.O. ROCHA, H.E. TOMA, *et al.* 2006. Determination of n-octanol/water partition and membrane binding of cationic porphyrins. Pharm. Nanotechnol. **329:** 12–18.
3. KO, Y.-J., K.-J. YUN, M.-S. KANG, *et al.* 2007. Synthesis and in vitro photodynamic activities of water-soluble fluorinated tetrapyridylporphyrins as tumor photosensitizers. Bioorg. Med. Chem. Lett. **17:** 2789–2794.

4. Triesscheijn, M., P. Baas, J.H.M. Schellens, *et al.* 2006. Photodynamic therapy in oncology. Oncologist **11:** 1034–1044.
5. Simioni, A.R., C. Vaccari, M.I. Re, *et al.* 2008. PHBHV/PCL microspheres as biodegradable drug delivery systems (DDS) for photodynamic therapy (PDT). J. Mater. Sci. **43:** 580–584.
6. Synytsya, A., A. Synytsya, P. Blafkova, *et al.* 2007. Interaction of meso-tetrakis(4-sulphonatophenyl)porphine with chitosan in aqueous solution. Spectrochim. Acta A Mol. Biomol. Spectrosc. **66:** 225–235.
7. Aggarwal, L.P.F. & I.E. Borissevitch. 2006. On the dynamics of the TPPS4 aggregation in aqueous solutions. Successive formation of H and J aggregates. Spectrochim. Acta A Mol. Biomol. Spectrosc. **63:** 227–233.
8. Aggarwal, L.P.F., M.S. Baptista & I.E. Borissevitch. 2007. Effects of NaCl upon TPPS4 triplet state characteristics and singlet oxygen formation. J. Photochem. Photobiol. A **186:** 187–193.
9. Gonçalves, P.J., L. De Boni, N.M. Barbosa Neto, *et al.* 2005. Effect of protonation on the photophysical properties of meso-tetra(sulfonatophenyl) porphyrin. Chem. Phys. Lett. **407:** 236–241.
10. Rubires, R., J. Crusats, Z. El-Hachemi, *et al.* 1999. Self-assembly in water of the sodium salts of meso-sulfonatophenyl substituted porphyrins. New J. Chem. **23:** 189–198.
11. Ribó, J.M., J.-A. Farrera, M.L. Valero, *et al.* 1995. Self-assembly of cyclodextrins with meso-tetrakis(4-sulfonatophenyl)porphyrin in aqueous solution. Tetrahedron **51:** 3705–3712.
12. Frisch, M.J., G.W. Trucks, H.B. Schlegel, *et al.* 2004. Gaussian 03, Revision C.02, Gaussian, Inc., Wallingford, CT.
13. Gaussview 3.0, Gaussian, Inc., Carnegie Office Park-Building 6, Pittsburgh, PA 15106, USA.
14. Stryer, L. 1996. Bioquímica. Guanabara Koogan, Inc. Rio de Janeiro, RJ.
15. Xie, Y.-L., H.-M. Zhou & Z.-R. Zhang. 2007. Effect of relative humidity on retention and stability of vitamin A microencapsulated by spray drying. J. Food Biochem. **31:** 68–80.
16. Braga, G.K. & W.P. Oliveira. 2007. Manufacturing drug loaded chitosan microspheres by spray drying: development, characterization, and potential use in dentistry. Dry. Technol. **25:** 303–310.
17. Loksuwan, J. 2007. Characteristics of microencapsulated b-carotene formed by spray drying with modified tapioca starch, native tapioca starch and maltodextrin. Food Hydrocolloid. **21:** 928–935.
18. Desai, K.G.H. & H.J. Park. 2005. Preparation and characterization of drug loaded chitosan-tripolyphosphate microspheres by spray drying. Drug Dev. Res. **64:** 114–128.
19. Chiou, D. & T.A.G. Langrish. 2007. Development and characterisation of novel nutraceuticals with spray drying technology. J. Food Eng. **82:** 84–91.
20. Ré, M.I. 1998. Microencapsulation by spray drying. Dry. Technol. **16:** 1195–1236.
21. Freitas, S., H.P. Merkle & B. Gander. 2004. Ultrasonic atomisation into reduced pressure atmosphere—envisaging aseptic spray-drying for microencapsulation. J. Control. Release **95:** 185–195.
22. Ré, M.I. 2006. Formulating drug delivery systems by spray drying. Dry. Technol. **24:** 433–446.
23. Desai, K.G. 2007. Properties of tableted high-amylose corn starch-pectin blend microparticles intended for controlled delivery of diclofenac sodium. J. Biomater. Appl. **21:** 217–233.
24. Luz, P.P., A.M. Pires & O.A. Serra. 2007. A low-cost ultrasonic spray dryer to produce spherical microparticles from polymeric matrices. Quim. Nova. **30:** 1744–1746.
25. ImageJ 1.38 bundled with Java 1.6.0_02.
26. Spellane, P.J., M. Gouterman, A. Antipas, *et al.* 1980. Electronic spectra and four-orbital energies of free-base, zinc, copper, and palladium tetrakis(perfluoro phenyl)porphyrins. Inorg. Chem. **19:** 386–391.