Encapsulation of laccase in chitosan carriers by spray drying technique

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INTRODUCTION AND OBJECTIVE

Chitosan is one of the most frequently used biomaterial. Chitosan microparticles can not only protect encapsulated drug molecules from degradation, but also improve their uptake and bioavailability (Agnihotri 2004). Spray dried microspheres made of pure chitosan cannot be kept suspended in aqueous media for a long time because of their swelling and dissolution. Therefore, non cross-linked chitosan microspheres prepared by spray-drying technique are unsuitable for the purpose of controlled drug delivery systems. Commonly used cross-linking agents (e.g. formaldehyde) are toxic and thus not suitable in pharmaceutical applications (Aral 1998). However, the cross-linking of chitosan by the more benign tripolyphosphate (TPP) anions has been recently reported (Arora 2010). The effect of composition (in particular, the TPP/chitosan ratio) and cross-linking method (ex-situ vs. in-situ) on the properties of chitosan microspheres formed by spray drying by 2-fluid/3-fluid nozzle was previously described (Kašpar 2012). The particle size distribution, particle morphology, zeta potential and swelling behavior have been systematically investigated. A novel cross-linking method, based on in-situ contact of TPP and chitosan solutions directly at the nozzle orifice (3-fluid nozzle), has been developed and compared with a more conventional approach of spray-drying a pre-mixed TPP/chitosan colloid solution using a 2-fluid nozzle.

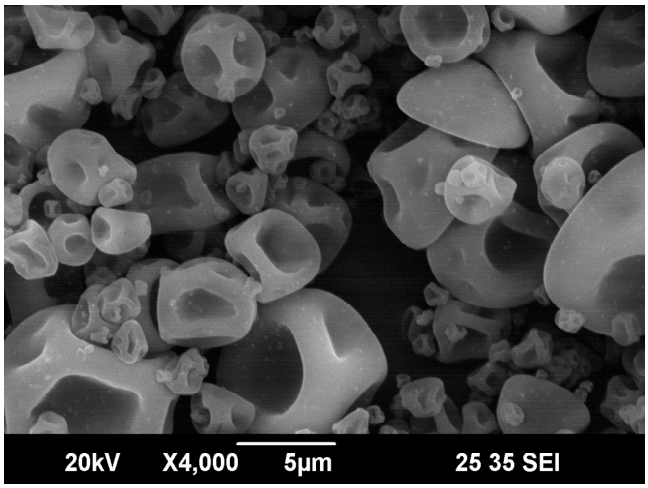

Fig. 1 SEM image of spray-dried chitosan microparticles

The main objective of the present work is encapsulation of enzyme laccase in chitosan particles and evaluation of its activity. Laccase (EC 1.10.3.2) is an enzyme belonging to the family of copper-containing oxidases, which are widely distributed in fungi or higher plants (Zhang 2009). It can oxidize ortho- and para-diphenols, polyphenols, anilines and a number of inorganic ions with the concomitant reduction of molecular oxygen to water.

MATERIALS AND METHODS

Low molecular weight chitosan (75-85 % deacetylated), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) and STPP - sodium tripolyphosphate (>98 % purity) were purchased from Sigma-Aldrich (Germany), acetic acid from Penta (>99 % purity), sodium acetate anhydrous (>99%) was purchased from Fluka, sodium phosphate dibasic dodecahydrate (>99 %) and potassium phosphate monobasic (>99.5 %) from Fluka. Laccase obtained from wood rotten fungi *Trametes hirsute* was a kind gift from TU Graz.

Preparation of chitosan microparticles by spray drying

Chitosan solutions (0.5% w/v) were prepared by dissolving the required amount of chitosan in 25 ml of aqueous solutions of acetic acid (2 %) at room temperature. After complete dissolution of the polymer, the required amount of laccase was added. Cross-linking was achieved by adding different volumes (0-2 ml) of a 1% (w/v) TPP solution drop-wise with a syringe (type 25Gx1") to the chitosan solution under constant stirring and then further stirred at 650 rpm for 15 min to ensure complete cross-linking of chitosan. After addition of TPP the solution changes from transparent to opalescent. Opalescence occurs due to the formation of chitosan-TPP nanoparticles. The colloidal suspension of cross-linked chitosan nanoparticles was then spray-dried by a 2-fluid nozzle to form microspheres with uniformly dispersed laccase. The volume of added laccase was 25 up to 200 µL. The solutions were spray dried using the Buchi B-290 laboratory spray drier with a high performance cyclone in open mode configuration. The values of operation parameters that ensured stable and robust process for the entire range of compositions were: aspirator rate 90%, total liquid flowrate 6.4 ml/min, and atomizing gas flow rate 414 normlitres/hr. Inlet temperature was varied in a range 100 - 190°C. The outlet temperature was typically 40°C (for inlet temperature 100°C) to 85°C (inlet temperature 190°C). An example of the spray dried particles is shown in Fig. 1.

Enzymatic assay

Specific laccase activity for ABTS is defined as the quantity of ABTS radicals formed per unit time and per amount of laccase. The specific laccase activity was found to be 0.0051 ± 0.0002 $\mu mol.min^{-1}.\mu L^{-1}$. The activity of chitosan powder with immobilized laccase was determined by uv/vis spectrophotometric measurements. A given amount of particles was placed inside a cuvette filled with 2.8 ml of acetate buffer (pH 5) using an envelope made of filtrate paper that keeps chitosan microparticles but is fully permeable for ABTS.

RESULTS AND DISCUSSION

Influence of drying temperature

Influence of drying temperature on laccase activity and sample properties has been investigated. At higher temperatures (160 – 190°C) laccase has been significantly deactivated (air outlet temperature > 70°C). Suitable inlet temperature had been chosen 130°C (dry, nonstick product). The results of the experiment are showed on Fig. 2.

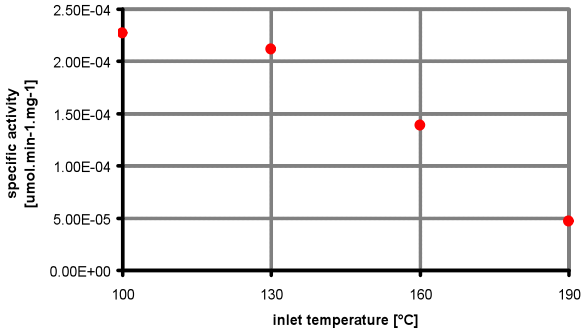

Fig. 2 Dependence of specific laccase activity on inlet drying temperature

Influence of laccase amount and temperature on enzymatic reaction

Finally, the activity of chitosan powder with different laccase content and influence of temperature on enzymatic activity have been investigated [Fig. 3]. It can be seen that the reaction temperature has a significant influence on enzymatic activity. In fact, it has been reported in the literature (Zhang 2009) that the optimum temperature for laccase *T. hirsute* is around 50°C, which is consistent with our findings.

CONCLUSIONS

Laccase was successfully encapsulated in a cross-linked chitosan matrix. The most suitable temperature for spray drying process was determined 130°C. During these conditions was powder product dry without tendency to stick on the wall of drying chamber and specific activity was very close to values of activity for sample dried at 100°C. TPP/chitosan ratio 0.08 does not affect significantly enzymatic compared to non-crosslinked chitosan particles, which were dissolved and the enzyme released to the surroundings. An increase in TPP/chitosan leads to a reduction of swelling behavior and to decrease of enzymatic activity of immobilized enzyme.

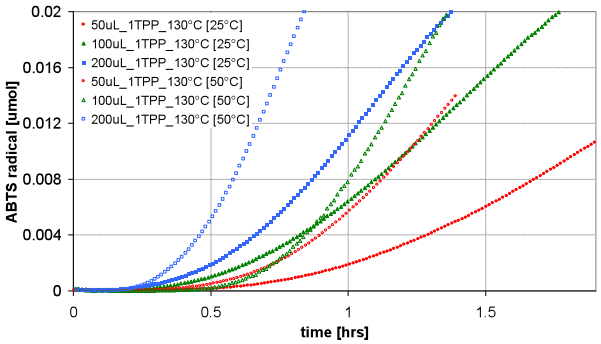

Fig. 3 The enzymatic activity of immobilized laccase at 25°C and 50°C

The next step will be the preparation of core-shell structured particles by three-fluid nozzle, where laccase will be immobilized in shell and substrate in the core of particles. This kind of structured particles will subsequently be used for applications such as the detection of antioxidants.

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