J|A|C|S COMMUNICATIONS

Published on Web 09/01/2009

Polyelectrolyte Synthesis and *in Situ* Complex Formation in Ionic Liquids

Martin Gericke, Tim Liebert, and Thomas Heinze*

Centre of Excellence for Polysaccharide Research, Friedrich Schiller University of Jena, Humboldtstraβe 10, D-07743 Jena, Germany

Received June 18, 2009; E-mail: Thomas.Heinze@uni-jena.de

State of the art biotechnological processes use immobilization of biological materials, in particular enzymes, to guarantee durability, to simplify workup steps, and to allow enzyme recovery.[1] Polyelectrolyte complex (PEC) capsules constructed from biocompatible polyelectrolytes are well suited for the entrapment of bioactive species.[2] One potential candidate is the polyanion cellulose sulfate (CS).[3] Although the usefulness of enzyme entrapment in CS-based PEC is well recognized, the multistep process is time-consuming and inefficient preventing broad application. Moreover, it is restricted to water-soluble CS with degrees of substitution (DS) starting from 0.3. Thus, new pathways for synthesis of soluble CS and encapsulation with low DS cellulose derivatives are indispensable. Recently, we reported on the homogeneous sulfation of cellulose in ionic liquids (ILs), which allows adjustment of the DS and recovery of the reaction medium.[4] Here, we demonstrate that the exploitation of ILs may even combine rapid synthesis of CS with PEC formation and enzyme entrapment.

In preliminary experiments, PEC formation with water-insoluble CS of low DS (0.16) was examined using 1-ethyl-3-methyl-imidazolium acetate (EMIMAc) as the solvent for the polyanion. The polycation, poly(dimethyldiallyammonium chloride) (PolyDADMAC), was dissolved in an aqueous 0.9% (w/w) NaCl solution. Adding defined droplets of CS in EMIMAc (concentration 2 to 4%, w/w) to a solution of PolyDADMAC (2 to 4%, w/w) yielded spherically shaped capsules (Figure 1a).

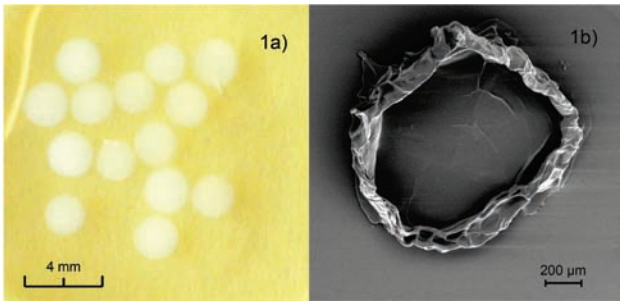

Figure 1. Polyelectrolyte complex capsules prepared from water insoluble cellulose sulfate (a) and scanning electron microscope image of a dried slice from the middle of one capsule (b).

Scanning electron microscopy (SEM) of the PEC capsules after freezing in Tissue-Tek matrix and cutting the middle section into thin slices (20 μm) revealed that the capsules consist of an outer PEC membrane and a hollow inner core (Figure 1b). Hence, the IL-based PEC capsules could act as containers, e.g., for enzyme mediated reactions.

In comparison to PEC capsules prepared in water,[4] the mechanical stability of capsules prepared from water insoluble CS of low DS dissolved in IL appeared to be much greater. This subjective impression obtained during handling of the capsules was confirmed by treatment of PEC spheres in an ultrasonic water bath. Capsules prepared from aqueous CS solution turned turbid after about 30 min and underwent destruction within 2 h. On the contrary, PEC capsules prepared via the novel path were stable even after 24 h of ultrasonic treatment. The increased stability was most likely caused by pronounced formation of hydrogen bonds between the water insoluble CS with high OH content and electrostatic polyanion−polycation interactions.

Although the CS was water insoluble, the capsule formation from IL solution was not caused by a simple precipitation in the aqueous medium but resulted from the formation of a PEC membrane. It was observed that CS solution dropped into water also initially led to the formation of spherical particles, but these pure CS particles disintegrated within several minutes of storage in water. The formation of a polyanion−polycation complex was also confirmed by FT-IR and solid state NMR spectroscopy of freeze-dried PEC capsules (Figures 2 and 3).

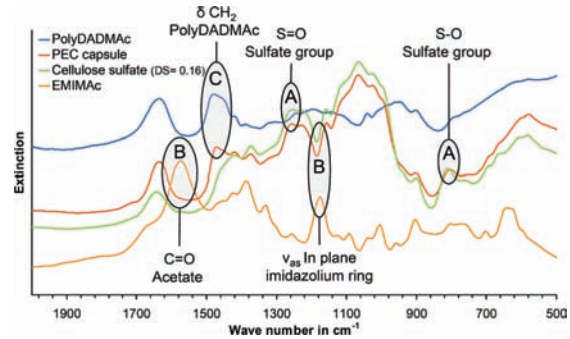

Figure 2. FT-IR spectra of cellulose sulfate, polyelectrolyte complex capsules, poly(dimethyldiallyammonium chloride) (PolyDADMAC), and 1-ethyl-3-methyl-imidazolium acetate (EMIMAc). Characteristic regions for (A) sulfate groups, (B) EMIMAc, and (C) PolyDADMAC are marked with circles.

The FT-IR spectrum of the PEC strongly resembled the CS spectrum. Characteristic peaks originated from sulfate groups could be found in both spectra (region A) at 1256 cm^{-1} (ν SO_2) and 805 cm^{-1} (δ SO). Additionally, a signal for the pyrolidinium ring of PolyDADMAC (1480 cm^{-1}; δ CH_2) occurred as a shoulder in the PEC capsule spectrum (region C).[5] This band was consistent with the PEC formation. The absence of characteristic FT-IR bands for EMIMAc (region B) at 1175 cm^{-1} (ring ν_{as} from: (N)CH_2, CH_3(N)CN, and CC) and 1574 cm^{-1} (ν C═O from acetate) in the spectrum of PEC capsules indicated that no residual IL existed.[6] It has to be noted that the carbonyl stretching band of the acetate ion, usually located at ∼1750 cm^{-1}, was shifted to a lower value for EMIMAc due to electronic interactions with the organic cation and was therefore difficult to assign.[6] In the CP/MAS $^{13}C\{^1H\}$ NMR spectrum of freeze-dried PEC capsules (Figure 3), no signals for the three aromatic carbon atoms of the imidazolium ring were found in the expected

10.1021/ja905003r CCC: $40.75

range 120−140 ppm. Thus, within the experimental error it can be concluded that no imidazolium cations were attached to the PEC capsules. Surprisingly, peaks corresponding to a carbonyl carbon atom were detected at ~180 ppm in the CP/MAS $^{13}C\{^1H\}$ NMR spectrum, in contrast to the FTIR measurements where no clear assignment was possible. Obviously the polypyrolidinium cations in the PECs were neutralized not only by CS and chloride counterions but also by a considerable amount of acetate ions that originated from the IL.

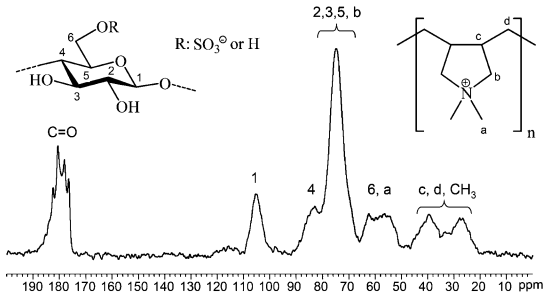

Figure 3. CP/MAS $^{13}C\{^1H\}$ NMR spectrum of freeze-dried polyelectrolyte complex capsules.

Enzyme entrapment was attempted with glucose oxidase (GOD), a catalyst for the oxidation of D-glucose to δ-gluconolactone, a reaction widely exploited in food industries and as a diagnostic tool for glucose monitoring.[7,8] GOD was successfully immobilized in the IL-based capsules by suspension of the enzyme in CS/EMIMAc solution prior to PEC formation. The activity of the encapsulated GOD and permeation of the PEC membrane by low-molecular weight substrates were studied in the presence of the horseradish peroxidase (HRP)/2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) system.[9] Despite the fairly harsh conditions, the entrapped GOD remained intact (Figure 4). Within 1 min after GOD containing PEC capsules were added to an O_2-saturated ABTS/glucose/HRP solution the color changed to deep green. The GOD capsules showed an enzyme activity of 45.4 ± 2.8 mU/capsule and contained 3.8 ± 0.5 μg of GOD per capsule. These values corresponded to a specific activity of 12.1 ± 2.4 mU/μg for the encapsulated GOD, which was almost identical to the value 12.3 ± 3.1 mU/μg reported for PEC capsules prepared from water-soluble CS.[4] Compared to free GOD this corresponded to relative activities of 14%, which is equivalent to the GOD activity measured for other encapsulating techniques, e.g., for CS/alginate/poly(methylene-co-guanidine) capsules (relative activity: 13%).[2] Consequently, the membrane properties that determined matter transfer between the inner core of the PEC capsule and the outer medium were comparable to those of common capsules from water-soluble CS.

In contrast to other sulfation routes for cellulose, homogeneous sulfation in ILs required no special workup procedure. No side products or solubilizing groups, such as nitrite or acetate moieties, which are usually introduced first to guarantee even accessibility to the cellulose chains, had to be removed in an isolation and purification step.[10] These facts in conjunction with the promising results discussed above indicated it was reasonable to attempt a one-pot approach for the *in situ* sulfation, PEC formation, and encapsulation. Thus, cellulose was converted to CS with a DS of 0.19 by homogeneous sulfation in a mixture of 1-butyl-3-methyl-imidazolium chloride and DMF without isolation or neutralization of CS. The reaction mixture was directly dropped into an aqueous solution of PolyDADMAc.

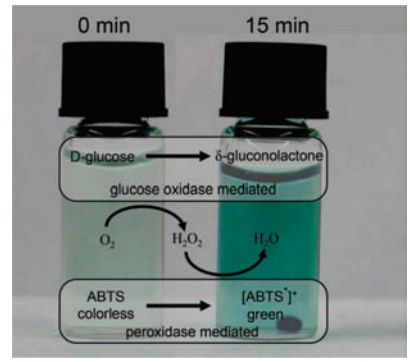

Figure 4. Testing solutions for glucose oxidase activity before and 15 min after the addition of glucose oxidase capsules.

Very stable spherical PEC capsules were obtained, whereas only precipitation occurred when water was used. Finally, all steps were combined. Homogeneous sulfation in an IL/cosolvent mixture was carried out. After the reaction was completed, GOD was carefully suspended in the mixture at room temperature, and the system was dropped into the solution of the polycation giving stable and spherical capsules. After several washing steps, low molecular weight compounds were removed. The presence of the enzyme in the PEC capsules was confirmed by the HRP/ABTS assay and showed the entrapped enzyme was active.

The results illustrated that encapsulation of an enzyme, namely GOD, could be carried out in a one-pot procedure that started from a solution of cellulose in ILs. This highly efficient process yielded enzyme loaded capsules with improved mechanical stability. The application of the IL-based technique for other enzymes is currently under investigation to illustrate the generality of this approach. Lipases can be applied because it has been reported that these enzymes retain their activity after dispersion in ILs.[11] Furthermore, it is possible to prevent enzyme denaturation by treatment with polyethylene glycol or by precoating the enzyme with task specific ILs.[11,12]

Acknowledgment. The financial support of "Fachagentur Nachwachsende Rohstoffe e.V." (Project 22021905) is gratefully acknowledged. We thank F. Steiniger (FSU Jena) for SEM and S. Hesse-Ertelt (FSU Jena/TITK Rudolstadt) for NMR studies.

Supporting Information Available: Full experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hanefeld, U.; Gardossi, L.; Magner, E. *Chem. Soc. Rev.* **2009**, *38*, 453–468.
(2) Vikartovská, A.; Bučko, M.; Mislovičcová, D.; Potoprstý, V.; Lacík, I.; Gemeiner, P. *Enzyme Microb. Technol.* **2007**, *41*, 748–755.
(3) Dautzenberg, H.; Schuldt, U.; Grasnick, G.; Karle, P.; Müller, P.; Löhr, M.; Pelegrin, M.; Piechaczyk, M.; Rombs, K. V.; Gunzburg, W. H.; Salmons, B.; Saller, R. M. *Ann. N.Y. Acad. Sci.* **1999**, *875*, 46–63.
(4) Gericke, M.; Liebert, T.; Heinze, T. *Macromol. Biosci.* **2009**, *9*, 343–353.
(5) Müller, M.; Kessler, B.; Richter, S. *Langmuir* **2005**, *21*, 7044–4051.
(6) Kiefer, J.; Obert, K.; Bösmann, A.; Seeger, T.; Wasserscheid, P.; Leipertz, A. *ChemPhysChem* **2008**, *9*, 1317–1322.
(7) Rauf, S.; Ihsan, A.; Akhtar, K.; Ghauri, M. A.; Rahman, M.; Anwar, M. A.; Khalid, A. M. *J. Biotechnol.* **2006**, *121*, 351–360.
(8) Wong, C. M.; Wong, K. H.; Chen, X. D. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 927–938.
(9) Ukeda, H.; Fujita, Y.; Ohira, M.; Sawamura, M. *J. Agric. Food Chem.* **1996**, *44*, 3858–3863.
(10) Heinze, T.; Liebert, T. Koschella, A. *Esterification of Polysaccharides*; Springer Laboratory: 2006.
(11) Armand, M.; Endres, F.; MacFarlane, D. R.; Ohno, H.; Scrosati, B. *Nat. Mater.* **2009**, *8*, 621–629.
(12) Turner, M. B.; Spear, S. K.; Kolbrey, J. D.; Rogers, R. D. *Biomacromolecules* **2004**, *5*, 1379–1384.

JA905003R