“Polyelectrolyte Synthesis and In Situ Complex Formation in Ionic Liquids”

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Supporting Information

Experimental Part

Materials

1-Butyl-3-methylimidazolium chloride (BMIMCl, for synthesis, purity ≥ 98%, charge: EQ4003579 624) was purchased from Merck. 1-Ethyl-3-methyl-imidazolium acetate (EMIMAc, BASF-quality, purity ≥ 90%, lot&filling code: S40470 10707B17) was received from Fluka. Poly(dimethyldiallyammonium chloride) (PolyDADMAC, MW= 200 000-350 000 g/mol, 20 wt.-% in water), *N*,*N*-Dimethyl formamide (DMF, water free), SO_3-pyridine, glucose oxidase (GOD, prepared from Aspergillus Niger, activity: 86 U/mg) and horseradish peroxidase (HRP, peroxidase II from horseradish, activity: 224 U/mg) were purchased from Sigma Aldrich. 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was obtained from SERVA, Heidelberg. All chemicals were used as received.

Spruce sulfite pulp (SSP, $[\eta]_{Cuen}$= 435 cm^3/g, MW_{Cuen}= 254 700 g/mol) was purchased from Fluka and dried for 3 hours at 100°C in vacuum prior to use. Intrinsic viscosity, [η] and molecular weight (M) were determined in cupriethylendiamine hydroxide (Cuen).[4]

Measurements

A CHNS 932 Analyzer (Leco) was used for elemental analyses. The average degree of substitution (DS) was calculated from the sulfur content according to the equation:

$$DS = \frac{S\% \cdot 162.1}{3207 - 102.1 \cdot S\%}$$

The FT-IR spectra were recorded on a Nicolet AVATAR 370 DTGS spectrometer with KBr-technique. The enzyme activity measurements were performed on a Lambda 10 UV/Vis spectrometer from Perkin Elmer. A LS 50B fluorescence spectrophotometer from Perkin Elmer was used for the quantitative GOD determination.

Tests of the mechanical stability of PEC capsules were performed with an ultrasonic bath (Transonic T460H, frequency: 35 kHz, energy input: 72 W) from Elma GmbH & Co KG.

CP/MAS $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer at 100.58 MHz using a 4 mm MAS double resonance probe and ZrO_2 rotors. The measurements were carried out at 6.5 kHz MAS. The cross polarization (CP) contact time was 1 ms, 40 k scans were accumulated, and the recycle delay was set to 2 s. Adamantane was used as an external reference.

For scanning electron microscopy (SEM) imaging of the polyelectrolyte (PES) capsules were frozen in Tissue-Tek and frozen slices (thickness 20 µm) were prepared at -20°C with a Microm HM 500. The slices were defrosted in bidistilled water, and after drying sputtered with gold using a Bal-Tec SCD 005. The samples prepared in that way were used for SEM on a Zeiss Leo 1350 Gemini FEG microscope.

Preparation of cellulose sulfate

Cellulose sulfate (CS) was synthesized in BMIMCl/DMF as described in detail in a previous work.[4] In brief, SSP was dissolved in BMIMCl. Subsequently, the solution was diluted with DMF, cooled to room temperature and reacted with SO_3-pyridine. Finally, aqueous workup with NaOH and precipitation in isopropyl alcohol/water (9:1) yielded CS with varying average degrees of substitution (DS) from 0.16 to 0.58.

Preparation of polyelectrolyte complex capsules from cellulose sulfate

For capsule preparation, CS (DS = 0.16, water insoluble) was dissolved in EMIMAc (1-4 wt.-%) and added in drops through a syringe (diameter 0.4 mm) to a stirred PolyDADMAC solution (1-4 wt.-% in 0.9% NaCl solution). After 30 min stirring in the precipitation bath the capsules were removed, washed, and stored in saline solution. PEC capsules from water soluble CS (DS = 0.36 and 0.58) were prepared in the same manner but by dissolving CS in 0.9% NaCl (2-4%). Additionally, CS particles were prepared by dropping CS/EMIMAc solution (DS = 0.16) into NaCl solution.

Preparation of glucose oxidase containing polyelectrolyte complex capsules

GOD-PEC capsules were prepared according to the general procedure described above. CS/EMIMAc solution (2 wt.-%; DS = 0.16; water insoluble) containing GOD (3.75 mg/g CS solution) was added in drops into a 2 wt.-% solution of PolyDADMAC in phosphate buffer (pH 6). After 30 min stirring in the precipitation bath, the capsules were removed, washed, and stored in buffer solution for 5 h. In order to determine the enzyme activity, a GOD containing PEC capsule was placed in a cuvette, equipped with a magnetic stirrer, together with 1.5 ml of ABTS testing solution (0.16 M glucose, 0.9 mg/ml ABTS and 3 U/ml HRP in O_2-saturated phosphate buffer with pH 6). The enzyme activity (EA), specified in international units (U, 1 U = conversion of one µmol substrate per minute) was determined according to the formula:

$$EA = \frac{\Delta A \cdot V}{\varepsilon \cdot d}$$

with ΔA being the increase of absorbance at a wave length of 405 nm, V the volume of the added testing solution, ε the absorption coefficient of ABTS (ε= 36.8 cm^2/µmol) and d the

thickness of the cuvette (d= 1 cm). The absorbance was determined over a period of 2 min. 8 different capsules were measured and the mean value was calculated.

GOD content was determined by treating the freeze dried capsule with 1.5 ml KH_2PO_4/H_2SO_4 buffer (pH 1.5) for 15 min. The fluorescent flavin adenine dinukleotide (FAD) coenzyme, liberated from the capsules by acid treatment, was quantified via fluorescence spectroscopy (light emission: 520 nm, fluorescence excitation: 460 nm). The total amount of GOD encapsulated was determined with the aid of a GOD-calibration series (7 concentrations in the range of 0.5 to 20 µg/ml GOD). The measurement was performed with 8 capsules and the mean value was calculated.

Preparation of polyelectrolyte complex capsules directly from sulfation mixture

1 g (6.2 mmol) SSP was dissolved in 9 g BMIMCl at 80°C. 10 ml DMF were added to the cellulose solution and the mixture was cooled to 25°C under vigorous stirring. 589 mg (3.7 mmol) SO_3-pyridine complex dissolved in 2 ml DMF were added to the cellulose solution and the reaction mixture was stirred for 2 h at 25°C. In order to determine the DS, 5 ml of the reaction mixture were removed and CS was isolated by precipitation and purified according to the general procedure described above yielding a DS of 0.19 The remaining reaction mixture was dropped through a syringe (diameter 0.4 mm) into a precipitating bath containing PolyDADMAC (4 wt.-%) in physiological NaCl solution and PEC capsules were obtained. After 30 min the capsules were removed, washed, and stored in saline solution. PEC-GOD capsules were prepared in the same manner by mixing 3.95 mg GOD with 1 g of the reaction mixture and dropping the solution into a PolyDADMAC precipitating bath (4 wt.-%).

References

(4) Gericke, M.; Liebert, T.; Heinze, T. Macromol. Biosci. 2009, 9, 343-353.