

**Design and Test Improved Assays for Endoglucanases and Exoglucanases
Subcontract No. XDH-0-30009-03
under Prime Contract No. DE-AC36-99GO10337**

**Technical Status Report
Covers the period October 5, 2000 – December 5, 2000**

Submitted by University of California, Davis

During this period, work was performed on task 4 and the deliverable for task 4 is provided in draft form.

Task 4.

Develop the HPSEC-MALLS method for analysis of insoluble high-molecular weight cellulose using DMAc/LiCl solvent system. Substrates used for this task will include cotton cellulose or bacterial cellulose (4 months, 09/01/00 – 01/01/01).

Deliverable for Task 4.

Submit a standard procedure for preparing solutions of high-molecular weight cellulose in LiCl/DMAc. Report on the usefulness of the HPSEC-MALLS for characterizing cellulose (determination of molecular weight averages, polydispersity indices, and radii of gyration) (5/31/01).

Pertaining to **Task 4** of the UC Davis Statement of Work, the HPSEC-MALLS method is being developed for analysis of cellulosic materials in DMAc/LiCl solvent system.

The first step in conducting the SEC analysis of cellulose is the development of a reliable dissolution procedure that would allow preparing true cellulose solutions stable over a long time. The solvent system should exhibit no degradation of cellulose during the dissolution process, and must be reasonably inert with respect to SEC packing material.

Earlier, we determined that the DMAc/LiCl solvent system is compatible with all these requirements, and identified the dissolution method described by Timpa (1991) as the most promising of the literature procedures. After conducting a number of optimization experiments, we were able to formulate a standard procedure for preparing solutions of cellulose from different sources in DMAc/LiCl (**part of deliverable for Task 4**).

According to the standard procedure, dissolution is carried out within a single vial without sample transfer except for final dilution and filtration. The protocol of the standard procedure is attached to this report. The description of cellulosic materials used in dissolution experiments is given below.

High-molecular weight **cotton cellulose** was obtained from Prof. Roy Cantrell, Department of Agronomy and Horticulture, New Mexico State University. Cotton is the purest form of naturally occurring cellulose, but it contains several impurities such as cotton wax (0.3-1.0%), pectin, mainly in the outer layers (0.5-1.2%), and protein residues from the protoplasm (Wood, 1988).

Before purification, the cotton sample (about 75 g) was grinded in the Wiley mill to 20-mesh size. Waxes were extracted in a Soxhlet apparatus with 1,1,1-trichloroethane (TCE) according to AATCC Test Method 97 (ATCC Technical Manual, 1999). Protein and pectin impurities were extracted by hot alkali according to Wood (1988). The alkali treatment was carried out in the absence of oxygen to minimize depolymerization.

Bacterial cellulose (BC) from *Acetobacter xylinum* was obtained in the form of Cellulon fiber from Weyerhaeuser. One of the attractive features of BC is the absence of polysaccharide impurities, lignin and waxes *within* its network. BC may contain small amounts of such impurities as bacterial cells, organic acids, salts, residual sugars and metabolites.

Cellulon fiber is usually isolated and purified from the fermentation broth using a lysis with hot alkali followed by several washing and dewatering steps, and contains about 95% cellulose with small amounts of cell wall fragments and mineral salts. To remove these impurities, the cellulose suspension in distilled water was homogenized using a laboratory homogenizer (Eastern Industries Division, Model 3A) with subsequent washing and centrifugation steps repeated four times.

In addition to cotton and bacterial cellulose, three other samples were included in dissolution experiments for subsequent analysis by the HPSEC-MALLS: **purified cotton** cellulose (absorbent cotton balls, Fisher, milled to 20 mesh), **Avicel PH101** microcrystalline cellulose (FMC Corp., 50 μm), and **Solka Floc 300 FCC** amorphous cellulose (Fiber Sales & Development Corp., 22 μm). The inclusion of these samples in our experiments provided a broad range of physico-chemical properties of analyzed cellulosic materials.

It was shown that DMAc/LiCl had the capability of dissolving cellulose samples differing in molecular weight (MW) and degree of crystallinity. Longer activation and dissolution times were necessary for high-molecular weight cellulose samples with higher degree of crystallinity. Cellulose chain length and crystallinity also determined the maximum amount of cellulose that could be dissolved. Avicel (low MW, high crystallinity) and Solka Floc (high MW, low crystallinity) were dissolved at 6.0 mg/ml after ~16-h incubation with DMAc/8% LiCl at 50°C. On the other side, cotton cellulose (high MW, high crystallinity) could be dissolved only at 3 mg/ml following 10-days incubation at 50°C and 2-days incubation at room temperature. Bacterial cellulose, which had intermediate between Solka Floc and cotton cellulose chain length and crystallinity, was dissolved at 6.0 mg/ml after ~24-h incubation.

References

- AATCC Test Method 97-1995. Extractable content of greige and/or prepared textiles. In: *AATCC Technical Manual*. American Association of Textile Chemists and Colorists, Research Triangle Park, N. C., 1999, 139-140
- Timpa, J. D. Application of universal calibration in gel permeation chromatography for molecular weight determinations of plant cell wall polymers: Cotton fiber. *J. Agric. Food Chem.* 1991, **39**, 270-275
- Wood, T. M. Preparation of crystalline, amorphous, and dyed cellulose substrates. *Methods in Enzymology*. 1988, **160**, 19-25

Standard procedure for preparing solutions of cellulose from different sources in DMAc/LiCl solvent system

1. Place 30 mg of cellulose (on a dry weight basis) into 10-ml ReactiVial. **Dry** the cellulose overnight at room temperature (25°C) under vacuum (25-30 Hg) in the oven with a desiccant. The ReactiVial should be covered with paper filter instead of Teflon disk. In case of cotton cellulose, start with 15 mg of dry material.
2. Add 5 ml of DMAc dried with molecular sieves and a conical magnetic stirrer into the ReactiVial, and place the ReactiVial in a heating block. Increase the temperature to 150°C and maintain with stirring for 2 h to **activate** cellulose with hot vapors of DMAc. Initial concentration of cellulose suspension is 6 mg/ml (3 mg/ml for cotton).

To obtain more effective dissolution, the vial should be kept *open* until the temperature of 150°C is reached. This allows any remaining moisture to be driven off while the system is still maintained below the boiling point of DMAc (166°C). After reaching 150°C, the vial should be capped to allow the exposure to hot DMAc vapors.

3. Allow the mixture to cool to 100°C, and add 0.400 g of dry LiCl (oven-dried LiCl should be stored in a desiccator). This will give an initial LiCl concentration of 8% w/v. Shake vial by hand and return to the heating block. Maintain the mixture with stirring at 100°C for 1 h. Lower the temperature of the block to 50°C, and stir the samples at this temperature for 12-240 h. If necessary, stir the mixture for another 6-24 h at room temperature (23-25°C) until all solids are completely **dissolved**.
4. Quantitatively transfer the solutions to 50-ml volumetric flasks and **dilute** to 50 ml with dry DMAc. Final cellulose concentration is 0.6 mg/ml in DMAc/0.8% LiCl (0.3 mg/ml for cotton cellulose).
5. **Filter** the solutions through a solvent-resistant Teflon disposable filter into 15-ml test tubes using vacuum sampling manifold (Millipore).
6. Transfer the filtered samples into 20-ml scintillation vials and **store** at 4°C.