A New Route Towered the Insertion of Nano Crystalline Cellulose into Epoxy Resins via Recombinant Proteins

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1. Introduction: Resilin and cellulose as a bio-reinforcing materials for Nano-composites

- **Resilin** is a rubber like protein abundant in arthropods locomotion organs. Resilin has an ultimate **elongation of 300% and resilience of 95%**.
- **Cellulose** fibers consists of highly organized crystalline domains linked together with amorphous regions. The **tensile strength of crystalline cellulose is ~ 500 MPa.**

The **elasticity** of **Resilin** and the **strength of Nano Crystalline Cellulose (NCC)** make them promising elements for enhancing mechanical properties of adhesives.

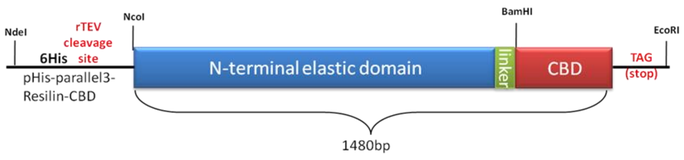
2. Research Objective

Improving mechanical and physical properties of commercial adhesives via incorporation of Resilin variants and NCC.

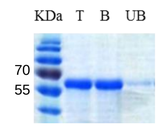
3. Materials preparation

- Recombinant Resilin fused to a Cellulose Binding Domain (**Resilin-CBD**) was cloned and expressed in *E.Coli* bacteria system.

Resilin-CBD construct illustration



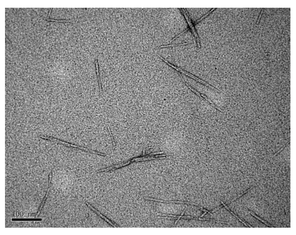
SDS-PAGE analysis shows specific binding of Resilin-CBD to cellulose



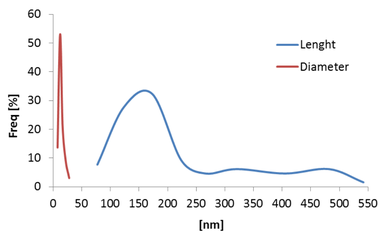
T: Proteins pre cellulose binding, **B**: Resilin-CBD Bound to cellulose, **UB**: Unbound resilin-CBD.

- Acid hydrolysis, heat and sonication treatments to cellulose enabled the extraction of **NCC**.

TEM image of rod-like individual NCC

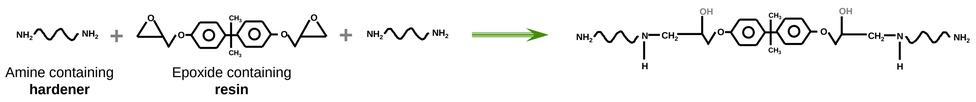


NCC particles size distribution



- Epoxy resin (EPON 828™) and amine-based hardener (EPIKURE™ 3140) were used as two component EPOXY-based adhesive.

General curing reaction of a two component EPOXY system

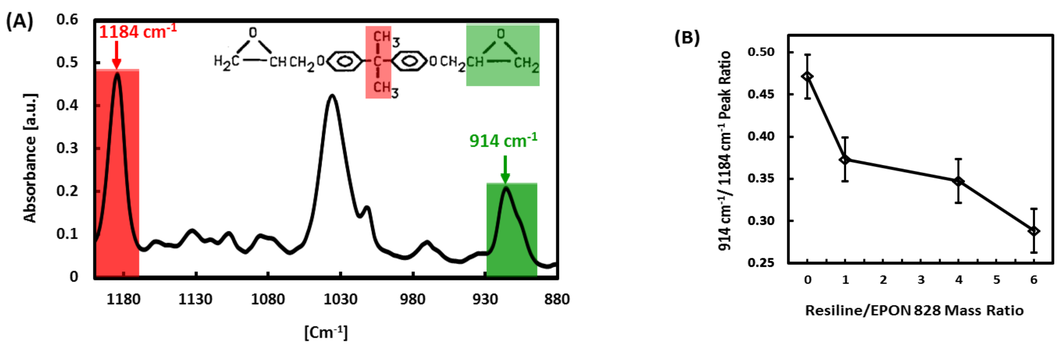


4. Results

Water-based NCC precipitate in EPON resin while Resilin-CBD/NCC is dispersed in it

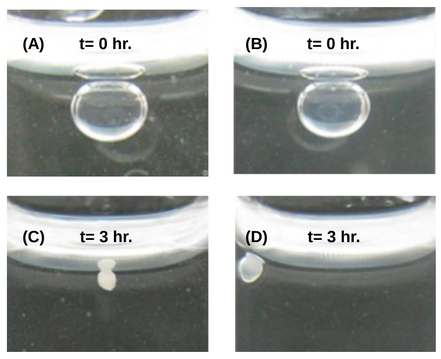
Blending of water based NCC or Resilin-CBD/NCC with EPON 828 results in the formation of a white emulsion **(A)**. Following water evaporation, **pure NCC precipitates** from the EPON phase **(B)**, while **Resilin-CBD/NCC disperse in it** **(C)**.

Resilin-CBD covalently react with the EPOXY resin



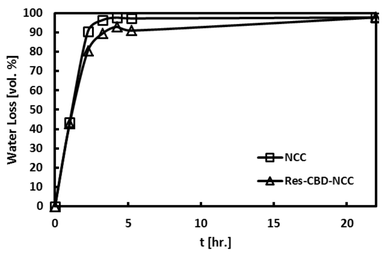
Zoom-in FTIR spectrum of pristine EPON 828 showing the epoxide ring band at a wave number of 912 cm^{-1} and the C-C stretching internal reference band at 1184 cm^{-1} **(A)**. Epoxide ring opening reaction progress as a function of Resilin to EPON 828 mass ratio **(B)**.

Water evaporation kinetics of aqueous Resilin-CBD/NCC drop placed in EPOXY resin implies on surface reaction between Resilin-CBD and the EPON resin

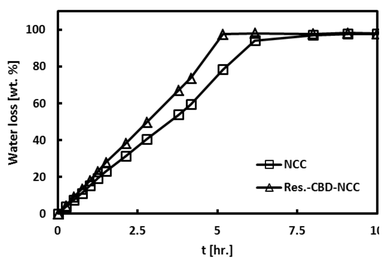


Drops of water-based NCC and Resilin-CBD/NCC placed in EPON 828 phase before evaporation **(A and B)** and following 3 hours of evaporation at 70˚C **(C and D)**.

Reaction between the Resilin-CBD amine groups and the epoxide groups retards the water evaporation rate in the emulsion state.



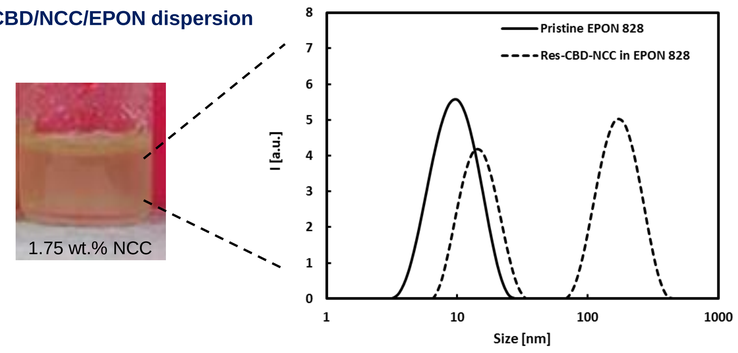
Evaporation kinetic of water from NCC and Resilin-CBD/NCC suspensions through the EPON 828 phase.



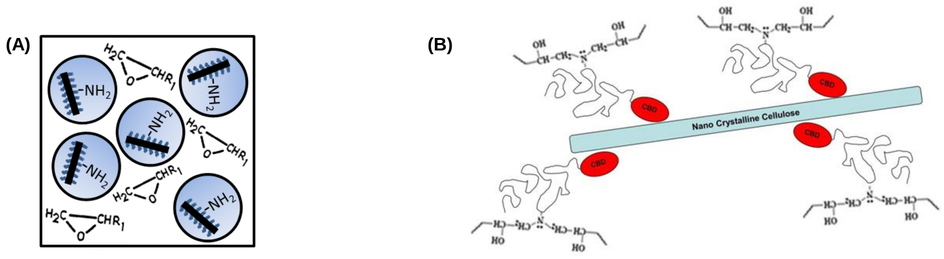
Evaporation kinetics of water from pure NCC and Resilin-CBD/NCC suspension.

Dynamic light scattering of Resilin-CBD/NCC/EPON shows expected particles size distribution of ~200 nm

Resilin-CBD/NCC/EPON dispersion

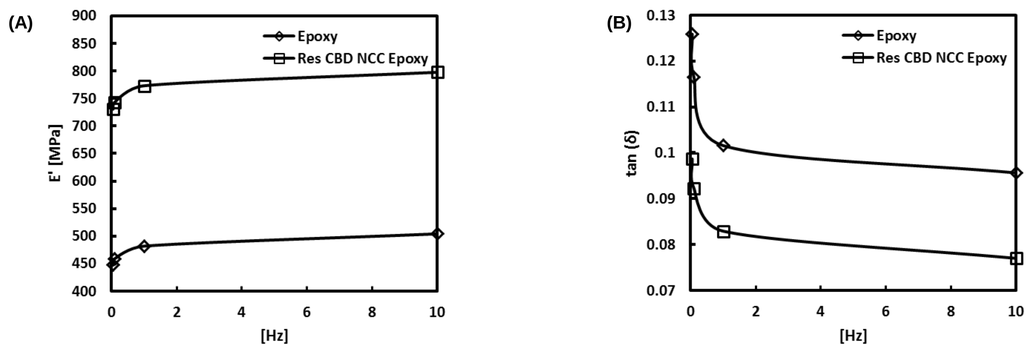


Proposed mechanism of the insertion of NCC into EPOXY via Resilin-CBD



Interface interaction between the emulsion's aqueous droplet phase and the epoxy resin organic continuous phase **(A)**. As water evaporates, Resilin-CBD/NCC that covalently interact with the epoxide monomers are inserted into the epoxy resin organic phase **(B)**.

DMA of the bio-nanocomposites shows enhancement in storage modules and elasticity compared to pristine epoxy



Storage modulus **(A)** and loss tangent **(B)** of the bio-nano-composite cured EPOXY and pristine cured EPOXY during a frequency sweep.

5. Summary

- Resilin-CBD/NCC were incorporated and cured in EPOXY adhesive system
- Mechanism of Resilin-CBD/NCC binding to EPON resin was proposed
- The effect of Resilin-CBD/NCC on epoxy's mechanical properties were demonstrated.