Functionalizing Cellulose Nanocrystals for Targeted and Sustainable Applications of Agriculture Agents

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The United Nations released a report that agricultural production needs to double by 2050 in order to keep up with population growth (United Nations, 2009). The most common method of increasing agricultural yield is the use of agricultural agents such as pesticides, herbicides, and fertilizers. Unfortunately, as little as 10 % of pesticides actually make it into plants; instead, these chemicals enter the ecosystem through run-off or air, where they can harm wildlife, plant life, and people (Zhang et al., 2020). New methods of delivering these agricultural agents are needed to meet agricultural demands while reducing the risks to environmental and public health. Several studies have focused on the use of lignocellulosic nanomaterials as carriers for agrochemicals (Lima et al., 2021). The purpose of this research is to successfully attach and detect agricultural agents on the surface of cellulose nanocrystals (CNCs) for utilization as a targeted-delivery system into plant cells.

CNCs are derived from cellulose, which is an organic, non toxic, and abundant biopolymer that can be extracted from sources like trees, cotton, and biomass wastes. Cellulose is mechanically extracted from the hemicellulose and lignin portions of the polymer and hydrolyzed using acid or enzymes to create CNCs. CNCs have a readily tunable surface chemistry as well as nanoscale dimensions that allow them to be taken up easily by plants through roots or stomata (Puppala et al., 2023). Sequence alignment comparing CLRDV-AL to the previously isolated strains from South American countries indeed showed some mutations within the domain known for VSR function (Agrofoglio et al., 2019).

The herbicide chosen for this research was 2,4- dichlorophenoxyacetic acid (2,4D), which is widely used across the world to kill broadleaf weeds in lawncare or agricultural settings. Microbes in the soil break down 2,4D, but the high concentrations released into the environment by broadcast spraying can still cause issues because of its toxicity to plant and animal life ("2,4D," 2023). More efficient delivery of 2,4D to weeds promises to reduce the amount needed and the risk to the environment.

A novel reaction scheme, depicted in Figure 1, was developed using some common organic chemistry pathways and biochemical reagentsto produce 2,4D-CNC. Both the 2,4D and CNC used in this reaction were obtained from a commercial supplier. The sulfuric acid hydrolysis used to create the CNCs leaves some sulfate half-ester groups on the surface along with the expected hydroxyl groups seen in Figure 1, but there has been no evidence that they substantially impact the reaction process. The CNC was diluted to an initial dispersion concentration that would prevent gelling and allow even mixing during the reaction. The reagents were added in excess to maximize attachment, so purification of the conjugated CNC in the form of centrifugation or dialysis had to occur after each step. First, an epoxy ring is added to the surface using epichlorohydrin. Then, ammonia opens the ring and adds a primary amine that can react with 2,4D in the presence of EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) and sulfo NHS (sulfo-(N-hydroxysulfosuccinimide)).

Because of CNCs' structure and tendency to aggregate, it has been difficult to achieve a high reaction conversion. The synthesis scheme was carried out with varying initial dispersion concentrations of 1 wt.% and 2 wt.% CNC to determine if reagents' side reactions with water were negatively impacting substitution.

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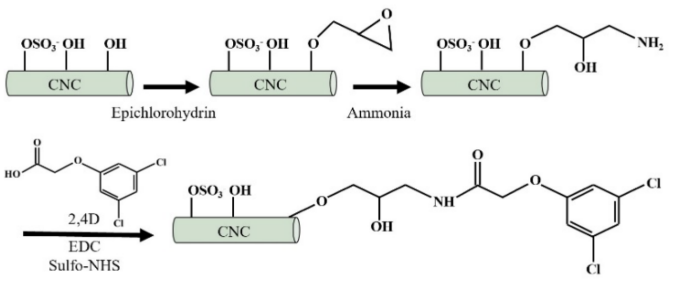

Fig. 1. 2,4D-CNC reaction scheme.

Various characterization techniques—including thermogravimetric analysis (TGA), Ultraviolet-visible spectroscopy (UV-vis), attenuated total reflectance infrared spectroscopy (ATR-IR), and a couple elemental analysis methods—were used to identify 2,4D after forming the conjugated CNC product in order to determine a method that could be used to quantify the degree of attachment. Many methods could identify the presence of 2,4D because nitrogen and chlorine are present in the added functional groups but not in the original CNC material. Unfortunately, the complexity of the conjugated material prevented most methods from being able to determine the quantity of 2,4D added. As chlorine was the only element solely present in 2,4D, an elemental analysis technique, energy dispersive X-ray spectroscopy (EDS), was ultimately used to quantify the degree of substitution of 2,4D through chlorine content. EDS gives the weight percentage concentration of the elements present in a sample. The degree of substitution could be determined for each reaction through chlorine weight percent using Equation 1 (Akhlaghi et al., 2015).

$$DS = \frac{162 \times \%Cl}{3545 - 221.04 \times \%Cl} \quad (1)$$

Where DS is degree of substitution and %Cl is weight percent of chlorine. The results of this method are listed in Table 1.

Table 1 Chlorine weight percent and degree of substitution for the different 2,4D-CNC products.

Initial Dispersion Concentration (wt. %)	Cl (wt. %)	DS (%)
1	0.027 ± 0.007	0.12
2	0.050 ± 0.01	0.23

The increase in degree of substitution with increasing initial dispersion concentration indicates that the hypothesis of water side reactions may be correct. In the future, initial dispersion concentration will be increased further to find the reaction or substitution limits.

Samples of 2,4D-CNC have been sent to collaborators for testing on plants. After receiving results from them, there will be opportunities to improve the materials and draw conclusions that could influence future agrochemical nanocarrier work. These findings could be applied to other pesticides as well as fertilizers and other growth agents. By utilizing organic nanomaterials, plants can be used to enhance agricultural production and minimize the amounts of toxic chemicals in the environment.

Statement of Research Advisor

Elise Collins is an excellent researcher who has been an integral part of this project. She has an outstanding ability to synthesize knowledge from the literature and develop an efficient research plan. Her synthesis and analytical chemistry skills enabled us to progress the research more quickly. In particular, her research into the effects of CNC concentration on the degree of 2,4D substitution was a key contribution to the work.
-Virginia A. Davis, Samuel Ginn College of Engineering

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Authors Biography

Elise G. Collins is a senior-year student pursuing a B.S. degree in Chemical Engineering at Auburn University. She has played key research roles in optimizing reaction conditions and exploring characterization methods.

Delaney E. Clouse is a graduate student in the Department of Chemical Engineering at Auburn University. She received a B.S degree in chemistry at the University of Southern Mississippi. Her work focuses on the use of cellulose nanocrystals in agricultural applications.

Dr. Virginia A. Davis is the Daniel F. and Josephine Breeden Professor in Chemical Engineering. Her research interests include sustainable nanomaterials, polymers, additive manufacturing, and STEM education.