NM WRRI Student Water Research Grant Final Report

5 cents is still a lot: new generation of anti-bacterial absorbents based on functionalized cellulose

Student Researcher:
Sahar Qavi
Ph.D. Candidate
Department of Chemical and Materials Engineering
New Mexico State University

Faculty Advisor:
Reza Foudazi
Assistant Professor
Department of Chemical and Materials Engineering
New Mexico State University

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Research problem and research objectives

The national drought mitigation center reports that most regions of New Mexico face abnormally dry or moderate drought conditions from the beginning of current year which is a critical concern for the residents.[1] One of the easiest steps we can take to mitigate the impacts of drought is conserving water. Wise usage of water during drought is always recommended to have more available water for humans, plants, and wildlife. However, all the available water is not clean for human usage.

Water pollution is a growing problem in many locations, especially New Mexico. *E. coli* is one of the top 10 causes of surface water impairment for rivers and streams in NM. The safe limit for fecal coliform bacteria (including *E. coli*) in NM is 0 coliform/100 mL that shows the importance of removing any bacteria from water.[2]

Different methods, such as ultrafiltration and adsorption by granular activated carbon (GAC) have been employed to remove organic constituents and bacteria from water supplies.[3] Membrane filtration needs some post-treatment steps such as chlorination and/or UV treatment to make drinking water antibacterial.[4] Post-treatment steps will result in high cost of pure water production and its availability is limited to large cities. The problem associated with GAC is the variation of pore size depending on the source of carbon which makes its application limited. Additionally, it is not easy to use any type of GAC for getting efficient result since the efficiency is dependent on the source. Besides, only a few carbon filter systems have been certified for the removal of coliform and GAC generally is used in combination with other methods. The main problem that has been addressed is the lack of portable and economic water treatment technologies in rural areas.

Here, we propose that nano-crystalline cellulose (NCC) can be used to make low-cost antibacterial absorbents for water treatment in rural areas. Cellulose is one of the most abundant materials in nature. NCC can be easily obtained by acid hydrolysis of cellulose at moderate temperatures.[5] Functionalization of NCC with quaternary ammonium groups provides antibacterial properties in the final product.[6] Aerogel is a synthetic porous ultralight material derived from a gel, in which the liquid component of the gel has been replaced with a gas.[7] Cellulose aerogels have been used a lot in different fields of science.[8] The objective of present work is to make an anti-bacterial NCC aerogel that can be used as absorbent for water treatment in rural areas, where people have less access to purified water. Proposed adsorbent aerogels are easy to use and promise cleaner water for an inexpensive price.

Methodology

Extracting NCC: Different sources of cellulose can be used for NCC extraction. In this work microcrystalline cellulose (MCC), which is commercially available with a reasonable price ($ 0.15/g), is used as the cellulose source. 1 g of MCC is hydrolyzed with 13.33 ml of sulfuric acid (64% v/v) for 30 minutes at 45°C. Then, the obtained suspension is centrifuged, the supernatant is removed, the precipitate is diluted with deionized (DI). This process is repeated for several times until pH is reached (neutralized) to about 4. Afterwards, NCC solution in water is transferred to dialysis membrane and dialyzed against water for 3 days or until pH becomes constant. Finally, NCC is separated by centrifugation. Freeze-drying is used for drying collected NCC.

Functionalization of NCC with quaternary ammonium group: The functionalization is done under an alkali environment. The concentration of NCC dispersion in DI water is adjusted to 1 wt.%. Sodium hydroxide is added to the suspension while being mixed with a stirrer. After dissolution of sodium hydroxide, a certain amount (20 wt.% of suspension) of 2,3-epoxypropyltrimethylammonium chloride is added to the system, and the temperature is controlled at 85°C for 6 h. Modified NCC as product is collected through centrifugation for several cycles to remove sodium hydroxide. Hydrochloride acid is used for neutralizing the product. Chemical structure of the materials is shown in Figure 1. Figure 2 shows the final structure of modified NCC with quaternary ammonium groups.

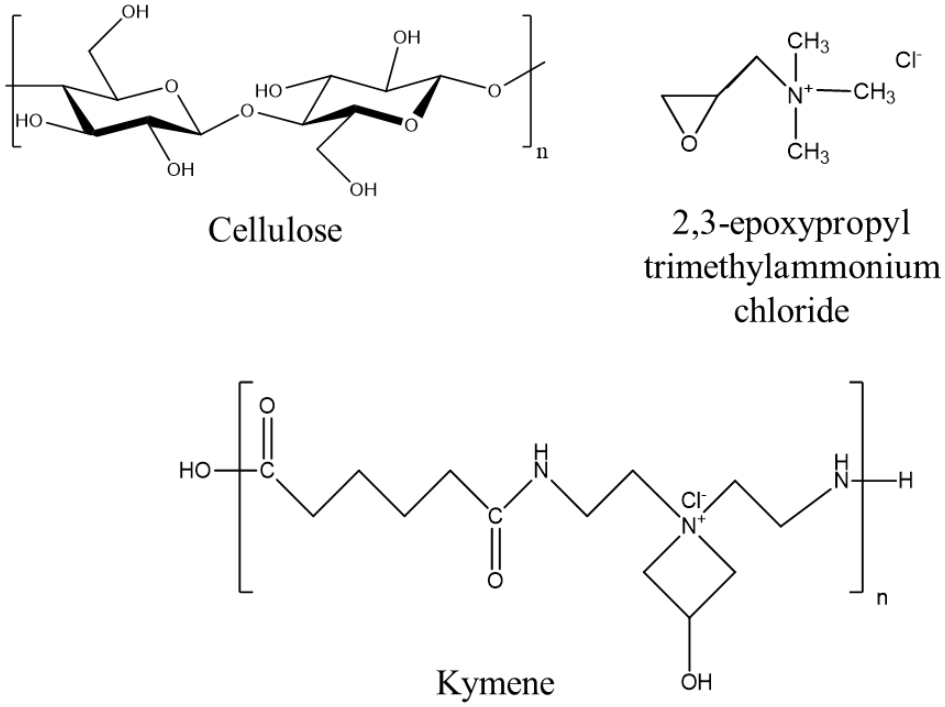

Figure 1. Chemical structure of materials used for functionalizing NCC.

Figure 2. Chemical structure of modified NCC with quaternary ammonium groups.

Making antibacterial aerogels: After preparing the dispersion of functionalized NCC in water in previous step, a cross-linker (Kymene G3-X) is added to the modified NCC suspension under mechanical mixing until a gel-like suspension is obtained. The weight ratio of cross-linker to modified NCC is 0.025. In the final step, ultrasonic bath is used for 10 min for homogenizing the gel. Produced gel is transferred to plastic centrifuge tubes and frozen in liquid nitrogen. Then, samples are transferred to a freeze-dryer at a condenser temperature of -75°C and under a vacuum

of 80 mTorr for 48 hr. After drying, sponge-like samples are obtained, which are kept at 120°C in a vacuum oven for 3h to have the final aerogels. Figure 3 shows a schematic representation of aerogels production process. As shown, a gel is formed after crosslinking of modified NCC with Kymene and solvent extraction results in the formation of interconnected networks with more than 95% air volume, known as aerogel.

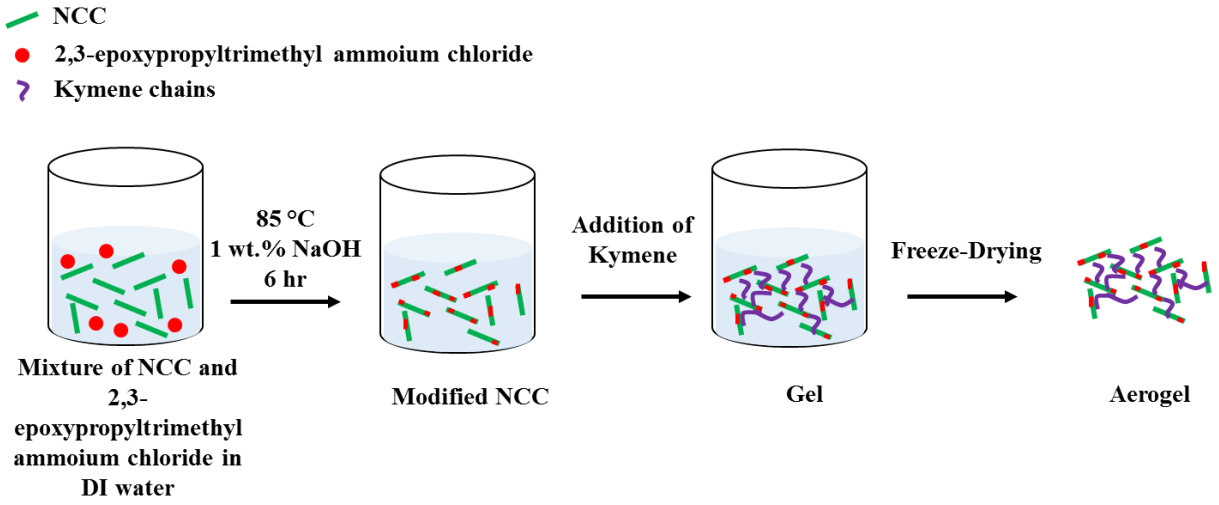

Figure 3. Schematic representation of aerogel formation.

Characterization methods: Atomic force microscopy (AFM, Dimension FastScan, Bruker) and transmission electron microscopy (TEM, H-7650, Hitachi High-Technologies Corporations) were used to characterize NCCs. For AFM, some drop of sample was dried on the mica surface and formed film on mica was used for characterization. NCC suspension in DI water was diluted for TEM test and about 5 µL of 2.5% uranyl acetate solution was used for staining. Samples were dried in freeze-dryer for FT-IR measurements that were done using a Perkin Elmer device. Aerogels were cut in small cubes with 2 cm edge and weight of each cube was recorder. Density of aerogel was measured by dividing weight per volume of cubes and average value was reported as density. Acid-base titration was done to measure the surface charge of modified NCC. First, 0.1 M sodium hydroxide (NaOH) and 0.1 M hydrochloric acid (HCl) were prepared. 0.5 gram of modified NCC was dispersed in 40 mL DI water. First, acid was added to the stirred solution dropwise (20 µL each drop) and pH and conductivity of solution was measured each time until pH was very low (pH=3). After that, base was added to the solution (dropwise) until pH=10. Diagrams of pH and conductivity versus acid/base volume were plotted to get the surface charge.

Results, conclusion, and future work

MCC was used as a source for extracting nano-crystalline cellulose. After acid hydrolysis of MCC with 64% sulfuric acid at 45°C, the suspension was centrifuged for several times and NCC solution in water was transferred to dialysis tubes (Figure 4) and was dialyzed against water for a few days until a constant pH was obtained.

Figure 4. Extracted NCC under dialysis.

Figure 5 shows an AFM micrograph obtained from NCCs. Needle like structure of NCCs is clear from AFM result. Besides, we can see that NCCs have a relatively smooth surface and they appear to be one-dimensional. This Figure shows that after the acid hydrolysis, the length of the nano-crystalline cellulose is in the range of 100 nm.

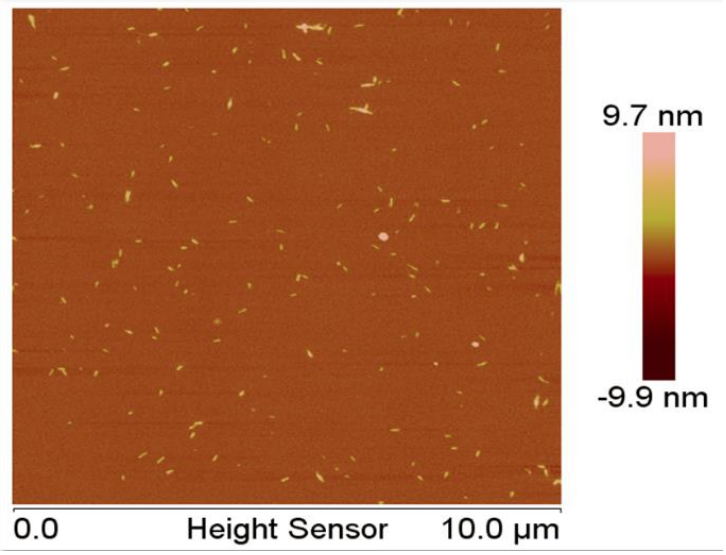

Figure 5. AFM micrograph obtained from extracted NCCs.

TEM images are shown in Figure 6. Average length of NCCs is about 100 nm and their diameter is in the range of 5-15 nm based on TEM micrographs. Needle like structure of NCCs is clear from TEM results. Since, NCC suspension is concentrated, NCCs aggregate on the TEM grid.

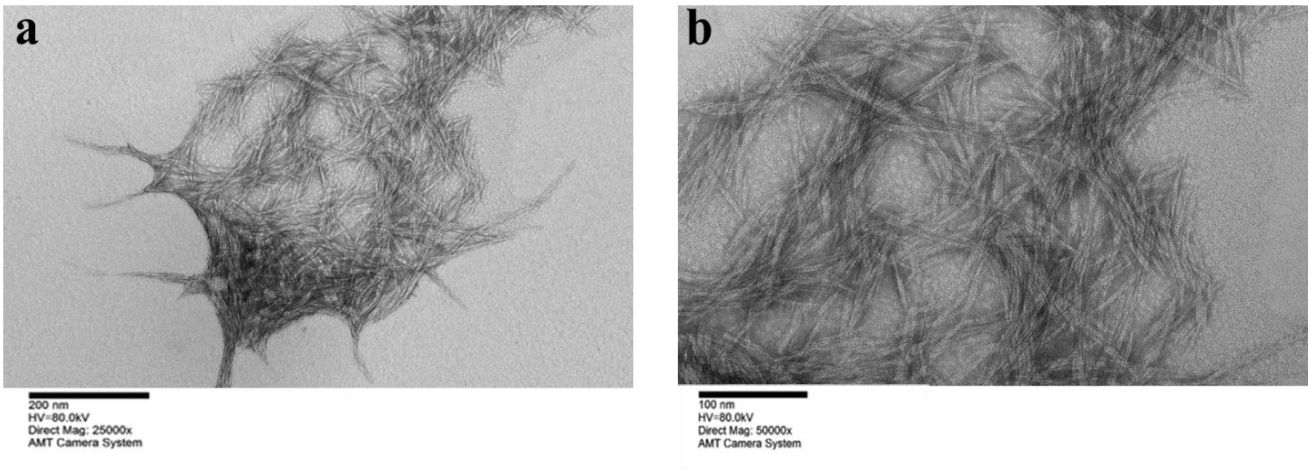

Figure 6. TEM images of suspension of extracted NCCs, (a): 25000X magnification, scale bar 200nm, (b): 50000X magnification, scale bar 100nm.

Since the nano-crystalline structure of obtained product is confirmed by AFM and TEM, we modified the resultant NCCs with quaternary ammonium groups. As mentioned in previous section, 2,3-epoxypropyltrimethylammonium chloride is used to functionalize NCCs with quaternary ammonium groups.

FT-IR was used as a characterization method to compare pure NCC with the modified one (Figure 7-9). The peak at 2916 cm^{-1} is stretching vibration band from C-H, the peak at 1386 cm^{-1} is from C-H of -CH_3 stretching vibration band, and anti-symmetric absorption peak of C-O-C is located in 1050cm^{-1}. The characteristic absorption peak at 1510 cm^{-1} and 2850 cm^{-1} shows the bending stretching of quaternary ammonium group that indicates NCC has been successfully modified.[9]

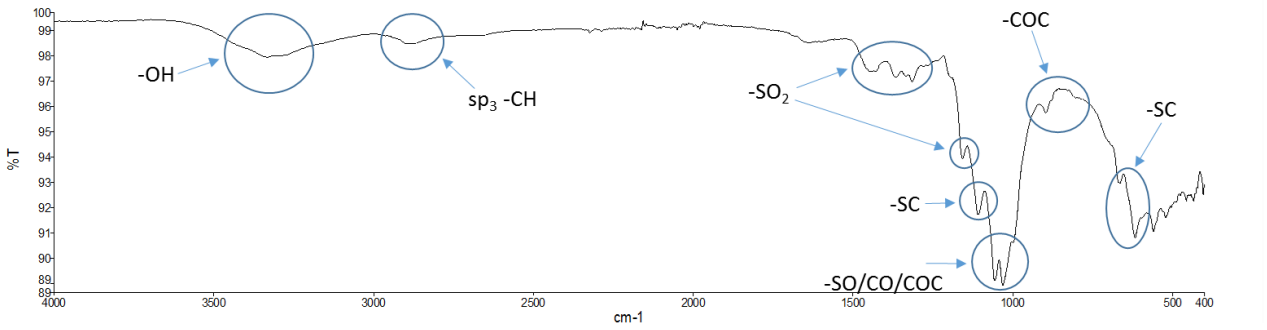

Figure 7. FTIR spectra of NCC.

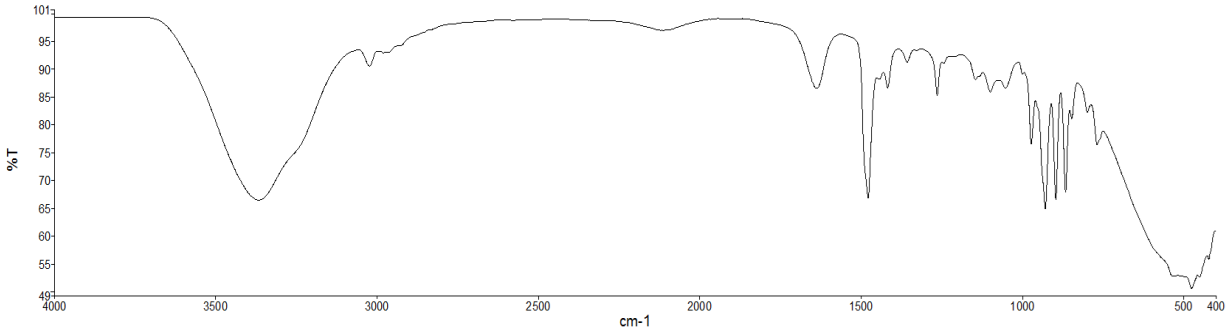

Figure 8. FTIR spectra of 2,3-epoxypropyl trimehylammonium chloride.

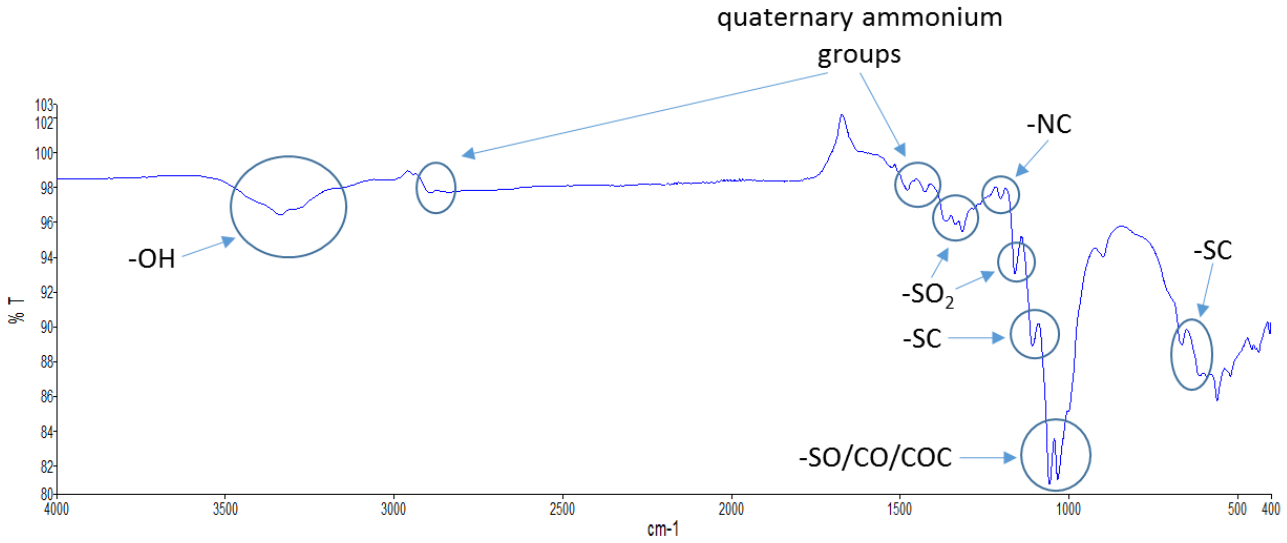

Figure 9. FTIR spectra of quaternarized NCC.

Based on Figure 7 and 8, most of the peaks associated with functional groups are very close to the ones of NCC and in order to confirm functionalization more clearly, titration method was used.

Acid-base titration was performed to measure the charge density of functionalized NCC. Figure 10 and Figure 11 show the corresponding titration data versus base and acid volume for NCC before modification. Titration data of modified NCC is shown in Figure 12 and 13.

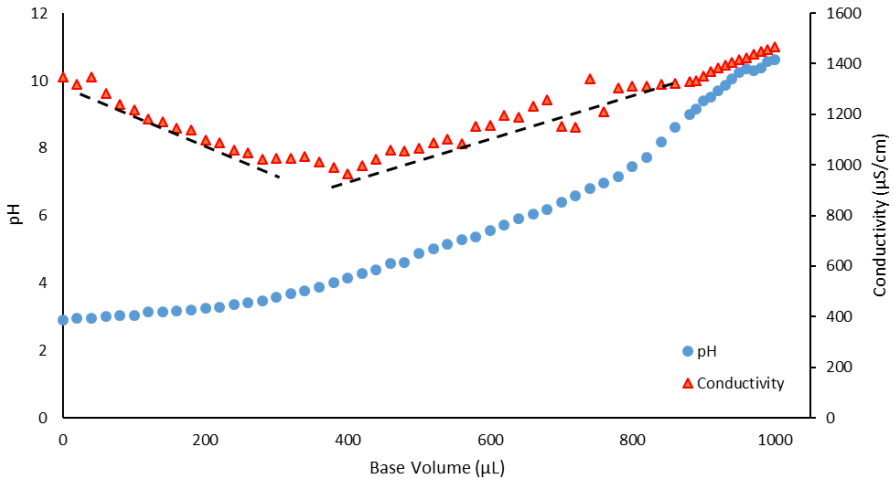

Figure 10. pH and conductivity of unmodified NCC plotted versus base volume.

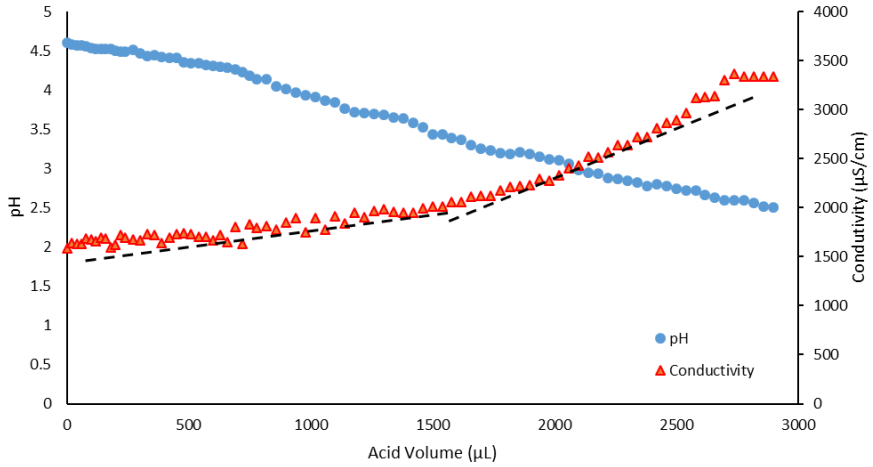

Figure 11. pH and conductivity of unmodified NCC plotted versus acid volume.

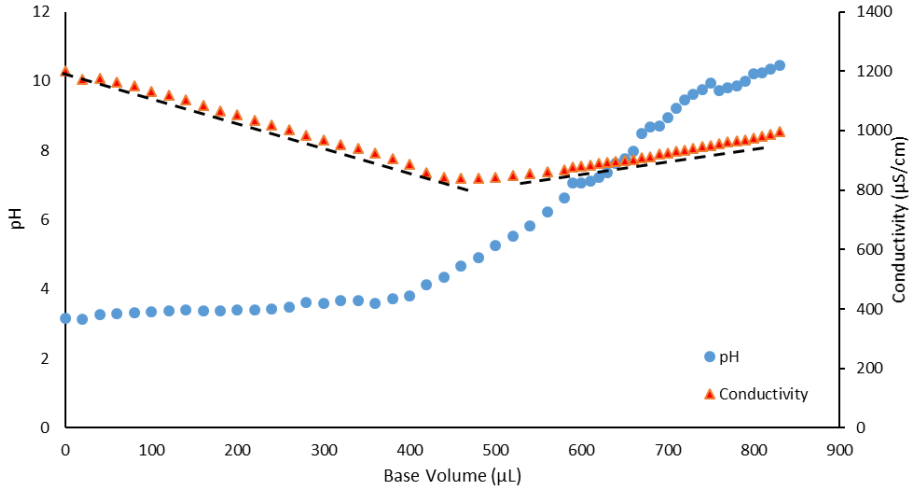

Figure 12. pH and conductivity of modified NCC plotted versus base volume.

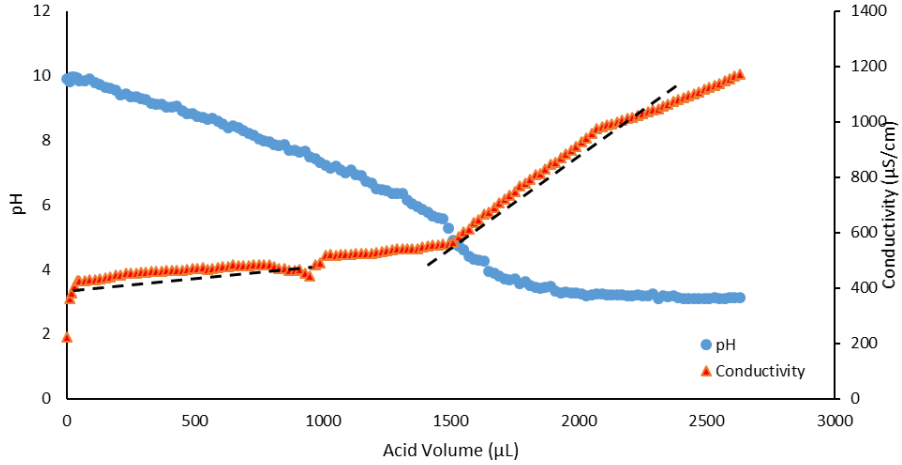

Figure 13. pH and conductivity of modified NCC plotted versus acid volume.

From the intersection points of the linear segments of the ionic conductivity plot before and after the equivalent point (or breakpoint), it is possible to graphically determine the volume (µL) of titrant required to fully protonate all functional groups on modified NCC.[10] By multiplying this value by its concentration (molarity), and referring to the initial NCC mass, the charge density of the polymer (µmol /g) can be calculated. Based on data in Figure 9, the volume in which the conductivity remains constant is approximately 20 µL. Therefore, surface is calculated as $\frac{(20\ \mu L)(0.1\frac{mol}{L})}{0.5\ g} = 4\frac{\mu mol}{g}$. Also, the acid less than 50 µL in Figure 10. Therefore, surface charge is calculated as $\frac{(50\ \mu L)(0.1\frac{mol}{L})}{0.5\ g} = 12\frac{\mu mol}{g}$. This small amount of surface charge in unmodified NCC samples is attributed to SO_3^- groups resulted from acid hydrolysis process.

From Figure 11, the volume in which conductivity is constant is 100 µL. Therefore, surface charge is calculated as $\frac{(100\ \mu L)(0.1\frac{mol}{L})}{0.5\ g} = 24\frac{\mu mol}{g}$. From Figure 12, the volume in which conductivity is constant is 500 µL. Therefore, surface charge is calculated as $\frac{(500\ \mu L)(0.1\frac{mol}{L})}{0.5\ g} = 120\frac{\mu mol}{g}$. Comparing titration data of unmodified and modified samples is an evidence that functionalization has been successfully done on NCC samples.

Kymene is used as cross-linker to maintain the stability of final aerogels. Aerogels are made by freeze-drying the suspension of functionalized NCC and Kymene at 80 mTorr for 48 hr. For further cross-linking, the product is kept at oven at 120 °C for 3hr.

Figure 14 shows the photos of aerogel after freeze-drying. Samples are super-light and spongy. Figure 15 shows the SEM images of synthesized aerogels with highly porous structure.

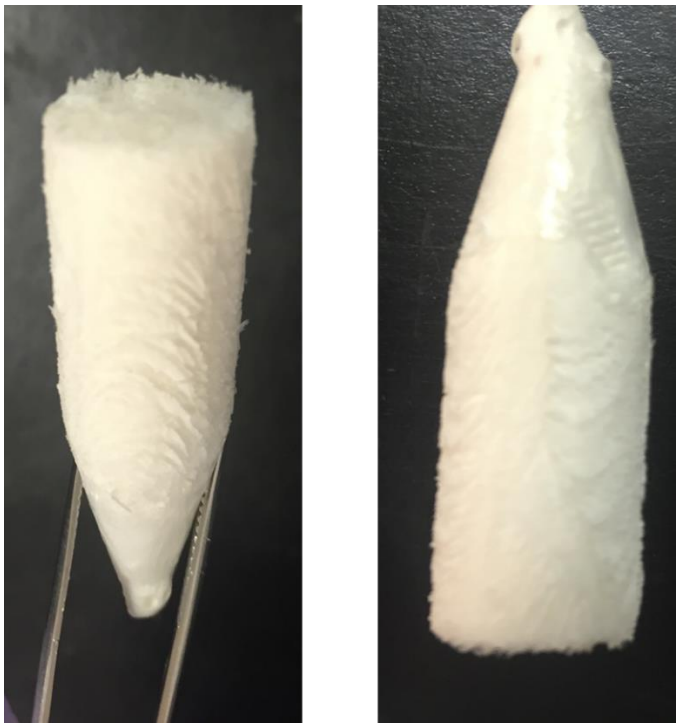

Figure 14. Aerogels samples after freeze-drying step.

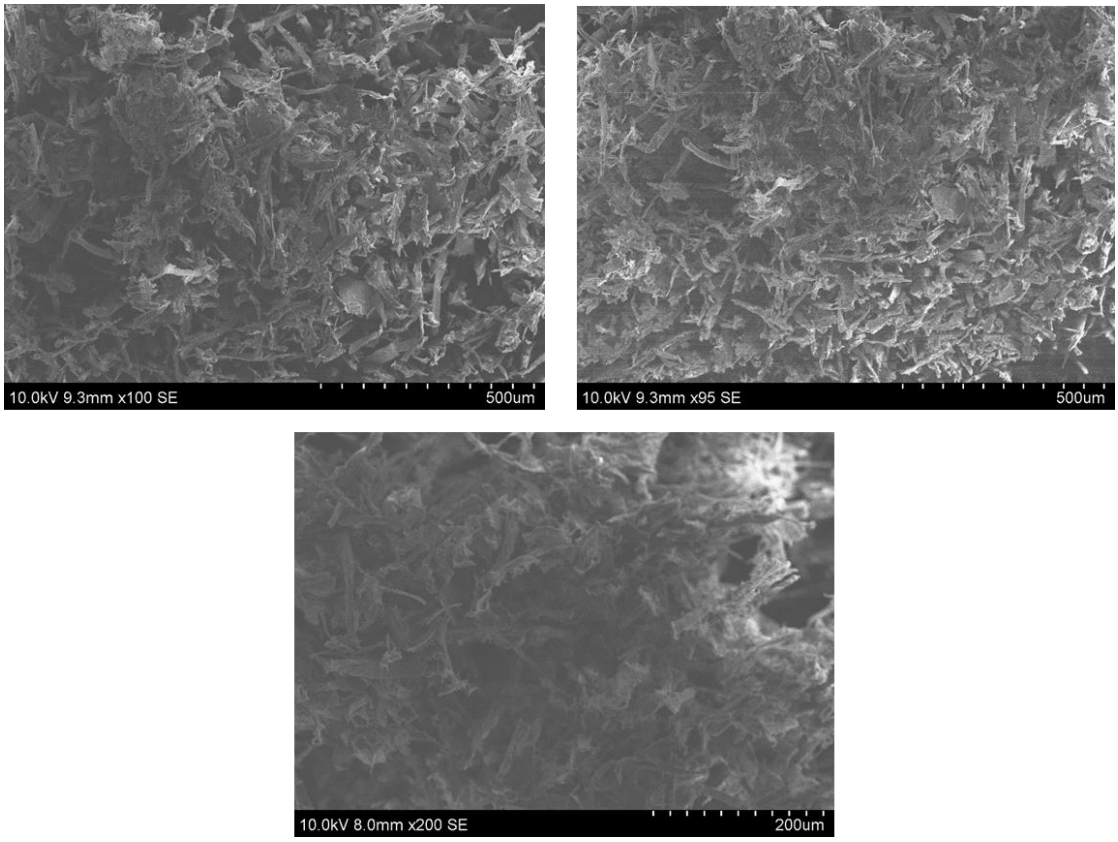

Figure 15. SEM images of synthesized aerogels. Porous structure is evident.

For the future work, we are going to activate the surface of aerogels and measure their surface area again. It is expected to have a much higher surface area in the samples. Density of aerogels and (NCC+crosslinker) mixture was measured and calculated as 0.017 ± 0.002 g/cm^3 and 1.31 g/cm^3, respectively. Considering air voids as the porosity and mixture law, pore fraction of synthesized aerogels can be calculated based on the following equation:

$$\rho_a \varphi + \rho_m (1 - \varphi) = \rho_{ag}$$

where, ρ_a, ρ_m, and ρ_{ag} are air density, (NCC+crosslinker) mixture density, and aerogel density, respectively. φ represents the volume fraction of air. Based on the literature,[11] $\rho_a = 0.0012$; therefore,

$$0.0012\varphi + 1.31\,(1 - \varphi) = 0.017 \;\; \rightarrow \varphi = \; 98.79$$

Therefore, porosity of aerogels is 98.79 %. In other words, 98.79% of aerogel's weights is air. This results confirm the successful production of aerogels.

In conclusion, highly porous aerogels were synthesized using nano-cellulose crystals as precursor. Aerogels show a considerable charge density that is attributed to quaternary ammonium groups with antibacterial properties. For the future work, BET adsorption test can be done to measure the surface area of the aerogels. Besides, culturing E.coli on the surface of aerogel will be performed to determine the extent of the anti-bacterial activity of synthesized aerogels.

Beneficiaries of research

Present research has a great potential for commercialization. The City of Las Cruces, NM and other municipalities may benefit from the results of this research, as well as water agencies such as the New Mexico Water Resources Research Institute (NM WRRI) and the Bureau of Reclamation (BoR). The published results will be made available to those in research and will provide a better understanding of antibacterial materials synthesis with low cost. This research could also be of interest to people in rural areas in the US southwest and New Mexico as well as all over the world.

List of presentations

- 60th Annual New Mexico Water Conference, NMWRRI, Taos, NM, October 8-9, 2015.

List of publications and reports

N/A

List of other students or faculty members involved

- Sicilee Macklin, undergraduate student, Department of Chemical and Materials Engineering, NMSU.

Special recognition awards or notable achievements

N/A

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