JANELLE TAM - NANOCRYSTALLINE CELLULOSE: A NOVEL, RENEWABLE ANTIOXIDANT

A. BACKGROUND

An excess of free radicals (highly reactive atoms) in the body causes damage to cells and tissues; antioxidants are the body's natural defense. When the body undergoes oxidative stress, free radicals accumulate, resulting in diseases such as cancer and diabetes [1]. Antioxidants are known to prevent disease, support medical treatment, and extend the survival of the terminally ill [2], and thus are extensively used as medical therapies. They are also used as preservatives in food and anti-aging agents in cosmetics. However, there are three problems with current antioxidants: toxicity [3]; low water solubility, leading to low bioavailability [4]; and deterioration over long-term storage. Thus, a stable, non-toxic, water-soluble antioxidant is needed.

Cellulose is the most abundant biocompatible material on earth. Removal of its amorphous regions via acid hydrolysis (polymer degradation via sulphuric acid) yields nanocrystalline cellulose (NCC), a cheap, stable, non-toxic [5], water-soluble biomaterial. With a projected North American market of $250 million, this emerging biomaterial will rejuvenate the struggling Canadian forestry industry. [6] Most importantly, its abundance of surface hydroxyl groups allows for the grafting of strong radical scavengers to enhance its antioxidant properties [7].

The buckminister fullerene (C_{60}) is one such strong antioxidant [8]. A hollow sphere of carbon, it is commercially used as a cosmetic ingredient. Although fullerenes are insoluble in water, they can be modified to increase solubility. Therefore, NCC, or a NCC-C_{60} complex could be an effective antioxidant that is superior to existing radical scavengers.

B. PURPOSE

This project has two goals: (1) to synthesize water-soluble fullerenes and graft them onto NCC; and (2) to compare the antioxidant properties of NCC, NCC-C_{60} complex and other similar polymer-fullerene complexes in order to evaluate the strength of NCC or NCC-C_{60} as antioxidants.

C. HYPOTHESES

Free radicals are quenched by the donation of a proton or electron to balance their lone electrons. The hypothesis is that NCC would be a strong radical scavenger as its crystalline structure, rod-like shape, and nano size allows for an abundance of hydroxyl groups on its surface, which reduce free radicals by donating a proton [9]. It is also conjectured that combining NCC and C_{60} will produce a stronger antioxidant, as fullerenes are excellent radical scavengers due to their abundance of surface π-electrons [10].

D. PROCEDURE

1) Establishing a protocol for testing antioxidant properties

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method is a standard test used to evaluate antioxidant properties. As DPPH radicals are scavenged, the solution turns from purple to yellow; this colour change is monitored by Ultraviolet-visible (UV-Vis) spectroscopy. The components of the experimental setup are summarized in Table 1. Fresh stock solutions of DPPH in alcohol reagent at concentrations of 1mg/mL were prepared daily. Control experiments were performed with ascorbic acid. The results matched literature data [11]. A DPPH calibration curve determined experimentally agreed with published results [12], confirming that the DPPH samples had not deteriorated. Solvent control experiments were run to ensure that the solvent used did not affect the results.

Table 1. Ingredients mixed in a 3mL cuvette for each experiment.

Material	**Volume**
Antioxidant solution	1 mL
Tris-HCl buffer (pH 7)	0.5 mL
Alcohol reagent *(90% ethanol, 10% methanol, 10% isopropyl alcohol)*	1.35 mL
DPPH stock solution	0.15 mL

2) Synthesis of NCC- C_{60} complex

a) Production of water-soluble fullerenes via conjugation of β-cyclodextrin (β-CD) and C_{60}: Saturated solutions of C_{60} in toluene and β-CD in water were vigorously mixed at 25°C for two weeks, so that the C_{60} molecules were encapsulated by β-CD to yield a soluble β-CD-C_{60} complex. The product was dialyzed for two days to remove impurities.

b) Grafting β-CD-C_{60} complex to NCC: A 2.5% (by weight) NCC solution was mixed with sodium persulfate and the β-CD-C_{60} complex, bubbled with argon for one hour, and stirred at 65°C for two days to produce the NCC- C_{60} complex.

3) Quantification and comparison of antioxidant properties

Table 2. Polymer-fullerene complexes tested in Phase 3.

Abbreviation	**Polymer-Fullerene Complex**
PAA-C_{60}	Mono-fullerene end-capped poly(acrylic acid)
C_{60}-PAA-C_{60}	Di-fullerene end-capped poly(acrylic acid)
PEO-b-PAA-C_{60}	Poly(ethylene oxide)-block-poly(acrylic acid)-fullerene
PDMA-C_{60}	Poly(2-(dimethylamino) ethyl methacrylate)-fullerene
PDEA-C_{60}	Poly(2-(diethylamino) ethyl methacrylate)-fullerene

The protocol developed in phase one using DPPH was used to evaluate the antioxidant activity of NCC, NCC-C_{60} and thepolymer-fullerene complexes shown in Table 2. Antioxidant activity was measured via two parameters:

- The rate constant (k), which measures the speed of reaction, was calculated using the formula: $-kt = \ln \frac{A_\infty - A_t}{A_\infty - A_0}$ where t is time and A_∞, A_t, A_0 are the absorbances at infinite time, time t and time zero [13].
- Inhibition activity (IA), which measures the capacity of a molecule to scavenge radicals, is determined via the equation: $IA\ (\%) = \left(\frac{A_0 - A_\infty}{A_0}\right) \times 100$, where A_0 and A_∞ are the absorbances at zero and infinite time three days after experiment was conducted.

All data were normalized to allow for comparisons at the same fullerene concentration.

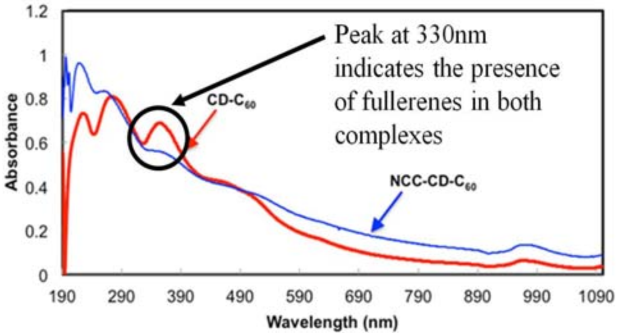
E. RESULTS

1) Establishing a protocol for testing antioxidant properties

The extinction coefficient obtained, 11575 $M^{-1}cm^{-1}$, agreed with previous research [12], as did the UV-Vis spectroscopy result for the ascorbic acid, thus confirming the validity of the protocol. As well, there was a negligible difference (standard deviation of 6.36 x10^{-5}) between the rate constants from different runs performed in different solvents.

2) Synthesis of NCC- C_{60} complex

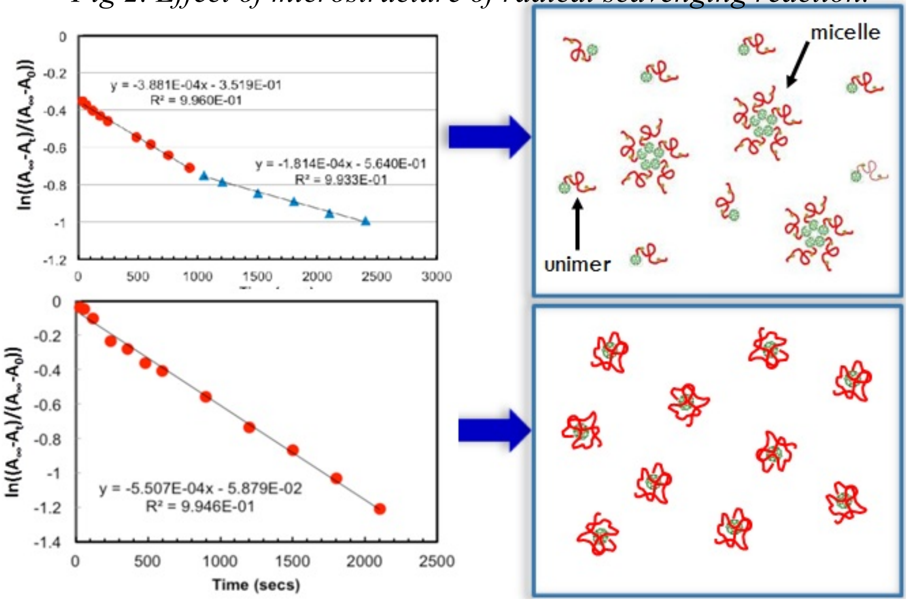
Fig 1. UV-Vis spectra of both complexes



Both synthetic experiments (2a and 2b) produced homogenous, brown colored solutions even after dialysis, when unreacted fullerenes were removed, confirming that fullerenes (responsible for the brown color) were present in both complexes. A peak at 330 nm, the characteristic absorption line of fullerenes, on the UV-Vis spectra of both complexes provided evidence for the success of the grafting reaction (Fig. 1).

3) Quantification and comparison of antioxidant properties

Fig 2. Effect of microstructure of radical scavenging reaction.



Analyses of kinetic curves of polymer-fullerene complexes (to obtain the rate constants) indicated a double exponential trend, showing that there were two reactions: an initial fast one followed by a slower reaction. This is likely due to the presence of both unimers and micelles (Fig. 2) in the solution. Polymer-fullerene complexes were amphiphilic, and the concentrations used during experimentation were higher than the critical micelle concentrations (CMCs). Thus, a mixture of unimers and polymers was present. Because unimers are more accessible to free radicals, they react first and at a faster rate. Radicals then diffuse to the hydrophobic micellar cores and react with the fullerenes, yielding the second rate constant. A subsequent experiment on a pure unimeric solution revealed a single exponential trend, which supports this explanation.

The NCC-C_{60} complex had the fastest radical scavenging reaction rate, and thus was the most efficient at scavenging radicals (Fig. 3). NCC showed a slightly slower reaction rate, confirming the hypothesis. The NCC-C_{60} complex displayed moderate inhibition activity, followed closely by NCC (Fig. 4). However, its capacity for scavenging radicals can be increased by grafting more fullerenes onto its surface.

Fig 3. Rate constants of all materials tested.

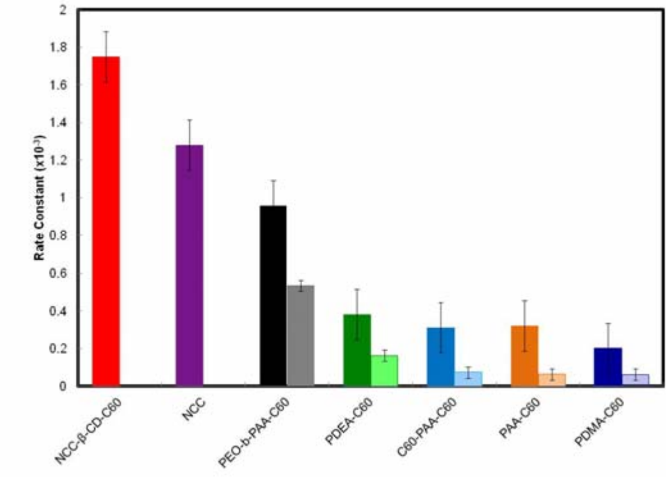
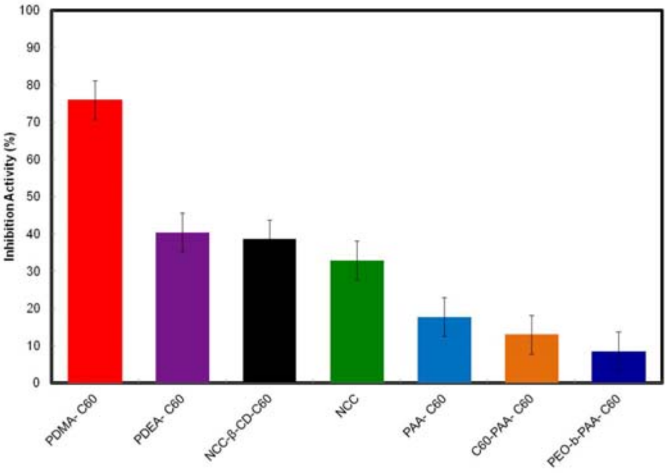


Fig 4. Inhibition activities of all materials tested.



F. CONCLUSIONS

NCC is an efficient antioxidant! A new and more efficient free radical scavenger (NCC-C_{60}) was developed that performs better than many other polymer-fullerene complexes. As well, the role of microstructure (formation of unimers and micelles) in free radical scavenging reactions was elucidated. NCC and NCC-C_{60} fulfill the requirement of a stable, biocompatible antioxidant. No research on the antioxidant properties of NCC has been reported. Thus, these exciting findings open up vast possibilities for this renewable resource. Since cellulosic materials are excellent carbon sinks for capturing CO_2, NCC will reduce our dependence on conventional carbon sources (e.g. crude oil) and help to address the environmental and energy problems facing our world.

G. ACKNOWLEDGEMENTS

I thank Dr. Yao Zhaoling, for her mentorship, Dr. Liu Juewen, for the use of his UV-Vis Spectrophotometer, Dr. Brad Bass, for his invaluable help, and Sanofi for financial support.

H. REFERENCES

[1] Ames, B. N. "Oxidants, Antioxidants, and the Degenerative Diseases of Aging."*Proceedings of the National Academy of Sciences* 90.17 (1993): 7915-922. Print.

[2] Blot, William J., J.Y. Li, P. R. Taylor, W.D. Guo, S. Dawsey, G.Q. Wang, C.S. Yang, S.F Zheng, M. Gail, G.Y. Li, Y. Yu, B.Q. Liu, J. Tangrea, Y.H. Sun, F.S. Liu, J.F. Fraumeni, Y.H. Zhang, and B. Li. "Nutrition Intervention Trials in Linxian, China: Supplementation With Specific Vitamin/Mineral Combinations, Cancer Incidence, and Disease-Specific Mortality in the General Population." *Journal of the National Cancer Institute.* 85.18 (1993): 1483-491. Print.

[3] Valentao, P., E. Fernandes, F. Carvalho, P. B. Andrade, R. M. Scabra, and M. L. Bastos. "Antioxidative Properties of Cardoon (Cynara Cardunculus L.) Infusion against Superoxide Radical, Hydroxyl Radical, and Hypochlorous Acid." *Journal Agriculture Food Chemistry* 50 (2002): 4989-993. Print.

[4] Mitri, Khalil, Ranjita Shegokar, Sven Gohla, Cecilia Anselmi, and Rainer H. Müller. "Lutein Nanocrystals as Antioxidant Formulation for Oral and Dermal Delivery."*International Journal of Pharmaceutics* 420 (2011): 141-46. Print.

[5] Kovacs, Tibor, V. Naish, B. O'Connor, C. Blaise, F. Gagné, L. Hall, V. Trudeau, and P. Martel. "An Ecotoxicological Characterization of Nanocrystalline Cellulose." *Nanotoxicology* 4.3 (2010): 255-70. Print.

[6] Savoie, David. "Nanocrystalline Cellulose Is Breathing New Life into Canada's Forestry Industry." *Nanocrystalline Cellulose Is Breathing New Life into Canada's Forest Industry*. University Affairs, 31 Aug. 2011. Web. Feb.-Mar. 2012. <http://www.universityaffairs.ca/-nanocrystalline-cellulose-is-breathing-new-life-into-Canadas-forest-industry.aspx>.

[7] Klemm, Dieter, F. Kramer, S. Moritz, T. Lindström, M. Ankerfors, D. Gray, and A. Dorris. "Nanocelluloses: A New Family of Nature-Based Materials." *Angewandte Chemie International Edition* 50.24 (2011): 5438-466. Print.

[7] Krusic, P. J., E. Wasserman, P. N. Keizer, J. R. Morton, and K. F. Preston. "Radical Reactions of C60." *Science* 254.5035 (1991): 1183-185. Print.

[8] Wepfer, Scott. Topical Anesthetic Formulation. Patent 0269393. 22 Nov. 2007. Print.

[9] Wiegand, Cornelia, P. Elsner, U.C. Hipler, and D. Klemm. "Protease and OS Activities Influenced by a Composite of Bacterial Cellulose and Collagen Type I in Vitro." *Cellulose* 13 (2006): 689-96. Print.

[10] Bondet, V., W. Brand-Williams, and C. Berset. "Kinetics and Mechanisms of Antioxidant Activity Using the DPPH Free Radical Method." *Lebensm.-Wiss. U.-Technol.* 30 (1997): 609-15. Print.

[11] Nenadis, N., and M. Tsimidou. “Observations on the Estimation of Scavenging Activity of Phenolic Compounds Using Rapid 1,1-Diphenyl-2-picryhydrazyl (DPPH^{-}) Tests.” *Journal of American Oil Chemists' Society* 79.12 (2002): 1191-1195. Print.

[12] Foti, M. C., C. Daquino, and C. Geraci. “Electron-Transfer Reaction of Cinnamic Acids and Their Methyl Esters with the DPPH^{-} Radical in Alcoholic Solutions." *Journal of Organic Chemistry* 69 (2004): 2309-2314. Print.

[13] Sin, S. L., L. H. Gan, K. C. Tam, and Y. Y. Gan. "Photochemical and Thermal Isomerizations of Azobenzene-Containing Amphiphilic Diblock Copolymers in Aqueous Micellar Aggregates and in Film." *Macromolecules* 38 (2005): 3943-948. American Chemical Society, 4 Feb. 2005. Web. 6 Sept. 2011.

I. BIBLIOGRAPHY

- Beckman, Kenneth B., and Bruce N. Ames. "The Free Radical Theory of Aging Matures." *Physiological Review* 78.2 (1998): 547-81. *Physiological Reviews*. Web. 12 Dec. 2011. <http://physrev.physiology.org/content/78/2/547.abstract>.
- Blois, Marsden S. "Antioxidant Determinations by the Use of a Stable Free Radical." *Nature* 181.4017 (1958): 1199-2000. *Nature Publishing Group: Science Journals, Jobs, and Information*. Nature. Web. 12 Dec. 2011. <http://www.nature.com/nature/journal/v181/n4617/abs/1811199a0.html>.
- Braca, Alessandra, Nunziatina De Tommasi, Lorenzo Di Bari, Cosimo Pizza, Matteo Politi, and Ivano Morelli. "Antioxidant Principles from Bauhinia Tarapotensis." *J. Nat. Prod.* 64 (2001): 892-95. Print.
- Habibi, Youssef, Lucian A. Lucia, and Orlando J. Rojas. "Cellulose Nanocrystals: Chemistry, Self-Assembly, and Applications." *Chemical Reviews* 110.6 (2010): 3479-500. Print.
- Liang, Bin, Minglin Jin, and Hongbo Liu. "Water-soluble Polysaccharide from Dried Lycrium Barbarum Fruits: Isolation, Structural Features and Antioxidant Activity." *Carbohydrate Polymers* 83 (2011): 1947-951. Print.
- Murthy, C. N., and K. E. Geckeler. "The Water-Soluble β-Cyclodextrin-[60]Fullerene Complex." *ChemCommunication* 32.41 (2001): 1194-1195. Print.
- Prakash, A., F. Rigelhof, and E. Miller. "Antioxidant Activity." *Medallion Laboratories Analytical Progress*. Web. 6 Sept. 2011.
- Zeynalov, Eldar B., Norman S. Allen, and Nazilya I. Salmanova. "Radical Scavenging Efficiency of Different Fullerenes C60-C70 and Fullerene Soot." *Polymer Degradation and Stability* 94 (2009): 1183-189. Print.
- Zhu, Jianfei, and Moucheng Wu. "Characterization and Free Radical Scavenging Activity of Rapeseed Meal Polysaccharides WPS-1 and APS-2." *Journal of Agricultural and Food Chemistry* 57 (2009): 812-19. Print.