Activation of Psyllium and Cellulose as Iron Chelators

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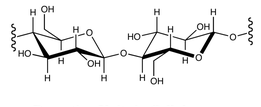
Introduction:

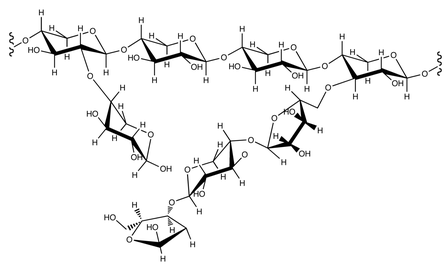
Hereditary Hemochromatosis (HH) is a genetic disorder in which the body absorbs excess amounts of iron from the diet[2]. This excess iron cannot be excreted, and once storage proteins are saturated iron in organs and joints. Free iron *in vivo* has the propensity to participate in redox reactions resulting in free radical damage[3]. HH can contribute to a variety of health ailments including joint pain, arthritis, impotency, and liver cirrhosis[2]. The most common form of treatment for HH is therapeutic phlebotomy, an effective yet unpleasant process. This work proposes that by manipulating the polysaccharide molecules of common dietary supplements with iron chelating agents, this new supplement could reduce the amount of bioavailable iron and reduce the frequency of phlebotomy.

Cellulose is one the most common organic compound found on earth[1]. It makes up approximately 33% of all plant matter. Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units[4]. Cellulose is not a compound that can be easily digested in the human body. It can be broken down to a certain extent, but it is normally referred to as indigestible dietary fiber. It is seen as a bulking agent that can help compile waste together and remove it from the body[5].

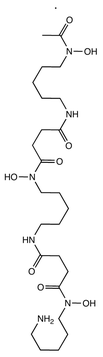
Psyllium is derived from the plant *Plantago ovata* and is used in many over-the-counter dietary fiber supplements. The structure of psyllium is a complex, branched, water-soluble polysaccharide. The backbone of the molecule is composed of monomers of xylose, which are b-1→4 and 1→3 linked. The backbone substituents include the simple sugars L-arabinose, D-xylose, D-glucuronic acid, and D-galactouronic acid. Psyllium attaches and excretes wastes in the human body,



Repeating Units in Cellulose



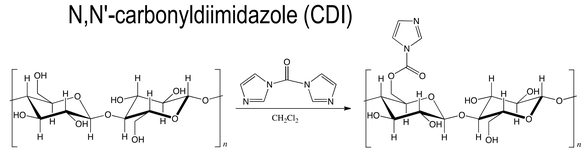
Approximate Repeating Units in Psyllium



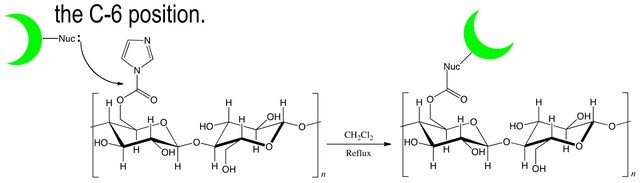
Deferoxamine

Synthesis of Chelating Agents:

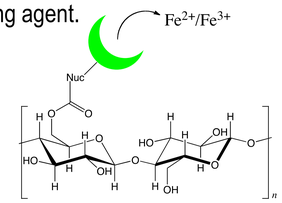
Step 1: Activate cellulose backbone with N,N'-carbonyldiimidazole (CDI)



Step 2: Nucleophile from chelating agent attaches through carbonyl. Also possible to have an SN2 attack at the C-6 position.



Step 3: Sequestering of iron species by attached chelating agent.



Experiments

Step 1: Approximately 5 grams psyllium was ground and mixed with 0.3379 grams (2.0E-3 moles) of CDI.
Step 2: The mixture was paced in a round bottom flask with at least ten milliliters of methylene chloride. The round bottom flask (with mixture) was attached to a reflux condenser apparatus. A stir bar was used to keep the mixture approximately 40 degrees Celsius.
Step 3: One monitored over a 48-hour period and one was monitored over a 96-hour period.
Step 4: CDI was added immediately and approximately 0.325 grams (3.0E-3 moles) of GABA (gamma amino butyric acid) was added after 24 or 48 hours following activation.
Step 5: Products were collected and filtered for future titrations.

Reactions with cellulose:
Step 1: Repeated with use of cellulose.
Step 2: Repeated with use of toluene and methylene chloride.
Step 3: Reaction monitored over 24 hours.
Step 4: CDI was added immediately, and GABA was added after 24 hours.
Step 5: Repeated.

Reactions with psyllium and perchloroformate:
Step 1: Repeated with use of 125 microliters of perchloroformate instead of CDI.
Step 2: Repeated with use of toluene.
Step 3: Reaction was monitored over a 5 day period. Each day, for 3 days, 125 microliters of perchloroformate was added to the mixture. A sample was taken each day prior to the new addition.
Step 4: Deferoxamine (DFO) was added to the mixture that was leftover after the removal of the daily sample.
Step 5: Repeated.

Hydroxyl groups on psyllium/cellulose were modified to produce a carbamate with a good leaving group. A nucleophilic amine (GABA) or iron chelator (DFO) was then attached to the activated psyllium/cellulose fibers. GABA was not used as an iron chelator, but was used to quantify the number of activation sites on the psyllium/cellulose fibers through titration with NaOH. The equivalent weight, or grams of psyllium for every mole of activated sites, was determined by titrating the acid group of GABA with 0.1004M NaOH.

Activated with GABA (Reaction done in Methylene Chloride)

Grams of Activated Psyllium	Volume NaOH (mL)	Moles of H+	Grams/mole
0.2024	0.58	6.049x10-5	3345.79
0.2030	0.65	6.779x10-5	2994.32
0.1029	0.40	4.18x10-5	2466.44
0.1025	0.40	4.18x10-5	2456.86

Grams of Cellulose Activated	Volume of NaOH (mL)	Moles of H+	Grams/mole
0.2000	0.80	8.344x10-5	2426.89
0.2022	0.85	8.865x10-5	2280.75
0.1089	0.95	1.035x10-4	1052.63
0.1093	1.00	1.043x10-4	1047.94

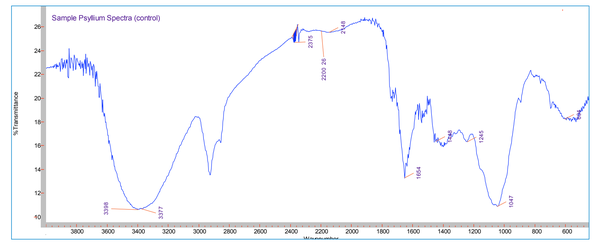
Activated with GABA (Reaction done in Toluene)

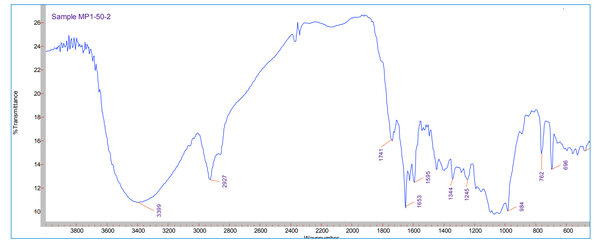
Grams of Activated Cellulose	Volume of NaOH (mL)	Moles of H+	Grams/mole
0.2020	0.95	9.908x10-5	2038.65
0.2020	1.00	1.043x10-5	1936.72
0.1048	0.75	9.800x10-5	1339.73
0.1048	0.85	8.866x10-5	1182.11

Controls

Psyllium	Volume of NaOH (mL)	Moles of H+	Grams/mole
0.1994	0.25	2.608x10-5	7647.17
0.2023	0.25	2.585x10-5	7825.92
0.1047	0.22	2.295x10-5	4562.89
0.1044	0.20	2.086x10-5	5004.79

Cellulose	Volume of NaOH (mL)	Moles of H+	Grams/mole
0.2009	0.30	3.129x10-5	6420.58
0.2020	0.32	3.338x10-5	6052.25
0.1048	0.24	2.503x10-5	4186.64
0.1054	0.25	2.608x10-5	4042.19





IR Spectra: Comparison of the unactivated psyllium (top) and the CDI activate psyllium (bottom) shows the appearance of an ester-like carbonyl at 1741 cm^{-1} and peaks in the fingerprint region that could be attributed to the aromatic imidazole.

Ongoing research: Iron (II & III) binding studies are being performed on DFO modified cellulose. The activated cellulose is placed in dialysis tubing and then placed in a vessel containing a known concentration of iron solution. At several time intervals samples are taken, worked up to form an bipyrridine complex, and concentration is determined against a calibration curve.

References and Acknowledgements:

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