Using chitosan to create a biodegradable, antimicrobial plastic alternative to aid in the reduction of plastic waste in hospitals

Summary:

- Chitosan is a product of the organic compound chitin. It was used in this project to make biodegradable and antimicrobial plastic films to reduce harmful plastic waste from hospitals.
- The films were strengthened using citric acid (CA) and tripolyphosphate (TPP) as cross-linkers. Sodium hydroxide (NaOH) was used as an alkali treatment to strengthen the films.
- Chitosan was dissolved in acetic acid to make the films.
- Water absorption, saline absorption, tensile strength and antimicrobial testing was carried out on each film.
- The best performing films were the NaOH, CA + NaOH & CA + TPP films
- They had greatly enhanced tensile strength; CA + NaOH films had almost twice the tensile strength of the control film.

Introduction:

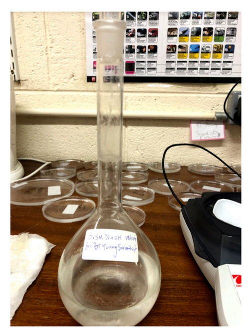
- Our project aims to use the naturally occurring polymer chitosan to create an antimicrobial, biodegradable plastic alternative to reduce harmful plastic waste from hospitals.
- Plastics are extremely harmful to wildlife in oceans and on land, killing millions of animals yearly.
- As hospitals require single-use plastics for sterility, we used chitosan to create a plastic film that will biodegrade.
- Chitin is one of the most common organic compounds and polysaccharides on Earth, found in shells of various crustaceans, as well as the shells of various insects, and in cell walls of fungi.
- Its derivative, chitosan, is formed by the deacetylation of chitin and is more soluble in acids.
- As chitosan is produced from renewable sources, reuses waste from fishing, is biodegradable, antimicrobial and non-toxic, it is a promising substance in the development of green materials.
- Chitosan is mechanically weak, so our project uses citric acid and tripolyphosphate as cross-linkers to strengthen the films. Sodium hydroxide was used as an alkali treatment
- Crosslinking links polymer chains by covalent bonds, and strengthens the material, along with increasing water resistance..

Method:

Making the Films

A control chitosan film, a tripolyphosphate (TPP) chitosan film, a sodium hydroxide (NaOH) chitosan film, a citric acid (CA) chitosan film, a citric acid and tripolyphosphate (CA + TPP) chitosan film, and a citric acid and sodium hydroxide (CA + NaOH) chitosan film were made.

- Chitosan was dissolved in 2% acetic acid solution and stirred with heat, until dissolved.
- The solution was poured into petri dishes and left for four days until the solutions had evaporated and films had been formed.
- Citric acid was added before the solutions were poured into the petri dishes and was dissolved. These films were then heated in an oven at 50°C for the crosslinking reaction to occur.
- To create the TPP and NaOH films, non-citric acid containing films were dipped in a 1% tripolyphosphate solution or a 0.5 M sodium hydroxide solution.
- To create the CA + TPP and CA + NaOH films, crosslinked citric acid containing films were dipped in a 1% tripolyphosphate solution or a 0.5M sodium hydroxide solution.



Film Tests:

Water/Saline Absorption Tests

- For the water and saline tests, the films were left to soak in water and saline for 5 minutes, 20 minutes, 60 minutes, 24 hours, and 1 week. The initial weight and the weight after being left in solution were compared.

Tensile Strength Tests

- Easysense™ software was used to determine the tensile strength of the different plastic films by adding 1N weights to each film of 1 x 5 cm^2 dimensions on a retort stand until they broke.

Antimicrobial Tests

- E.coli was plated on agar plates and discs of 5mm of the different plastics were added on the plates. The plates were then checked for any zones of inhibition for the next three days.

Results:

A one way ANOVA is used when more than two samples need to be compared regarding a response variable; i.e. to compare the water absorption of more than two types of biodegradable plastic to see if there was a significant difference or not. If a significant difference was shown, we conducted the Fisher's Least Significant Differences (LSD) test to check which means were different.

Water Absorption Tests

24-hour tests

One way ANOVA

Source	SS	df	MS	F	Significance
Between samples	3.484	4	0.871	72.58	$p < 0.05$
Within samples	0.362	30	0.012		
Total	3.846	34			

Mean increase in mass of films with Fisher's Least Significant Difference test

Films	Mean increase in mass (g)	n
Control (A)	N/A	N/A
TPP (B)	0.849	7
NaOH (C)	0.0543*	7
Citric Acid (D)	0.08	7
Citric Acid + TPP (E)	0.0443*	7
Citric Acid + NaOH (F)	0.0629*	7

The NaOH chitosan films, the citric acid + TPP chitosan films, and the citric acid + NaOH chitosan films had the best performance

Saline Tests

24-hour tests

One way ANOVA

Source	SS	df	MS	F	Significance
Between samples	1.186	4	0.297	39.55	$p < 0.05$
Within samples	0.225	30	0.00751		
Total	1.411	34			

Mean increase in mass of films with Fisher's Least Significant Difference test

Films	Mean increase in mass (g)	n
Control (A)	N/A	N/A
TPP (B)	0.513	7
NaOH (C)	0.0314*	7
Citric acid (D)	0.253	7
Citric acid + TPP (E)	0.0571*	7
Citric acid + NaOH (F)	0.0529*	7

The NaOH chitosan films, the citric acid + TPP chitosan films, and the citric acid + NaOH chitosan films had the best performance.

1-week tests

One way ANOVA

Source	SS	df	MS	F	Significance
Between samples	4.53	4	1.133	44.09	$p < 0.05$
Within samples	0.77	30	0.0257		
Total	5.3	34			

Mean increase in mass of films with Fisher's Least Significant Difference test

Films	Mean increase in mass (g)	n
Control (A)	N/A	N/A
TPP (B)	1.059	7
NaOH (C)	0.209*	7
Citric acid (D)	0.67	7
Citric acid + TPP (E)	0.239*	7
Citric acid + NaOH (F)	0.0957*	7

The NaOH chitosan films, the citric acid + TPP chitosan films, and the citric acid + NaOH chitosan films had the best performance

Tensile Strength

Film Type	Average Tensile Strength (N)	n	p-value
Control	13.85	5	-
TPP	17.07	5	0.051
NaOH	23.53	5	0.00024*
CA	18.26	5	0.017*
CA + TPP	18.42	5	0.014*
CA + NaOH	26.87	5	0.00022*

There was no significant difference between the tensile strength of the control chitosan film and the tensile strength of the TPP chitosan film. There was a significant difference between the tensile strength of the control chitosan film and the rest of the films. All films were compared to the control chitosan film. An asterisk is shown next to the p-values with a significant difference.

Antimicrobial Tests

Day 1

Film Type	Control	TPP	NaOH	CA	CA + TPP	CA + NaOH
Average Zones of Inhibition (mm)	0.73	1	1.07	0.9	0.9	0.93
t-test result p-values		0.196	0.113	0.415	0.415	0.310

Zones of inhibition were present for all films and there was no significant difference in the size of the zone for each film when compared to the control.

Day 2

Film Type	Control	TPP	NaOH	CA	CA + TPP	CA + NaOH
Average Zones of Inhibition (mm)	0.73	0.92	0.85	0.8	1.03	0.8
t-test result p-values		0.637	0.663	0.766	0.184	0.929

Zones of inhibition were present for all films and there was no significant difference in the size of the zone for each film when compared to the control.

Day 3

Film Type	Control	TPP	NaOH	CA	CA + TPP	CA + NaOH
Average zones of Inhibition (mm)	0.73	0.77	0.92	0.76	1.03	0.8
t-test result p-values		0.876	0.430	0.876	0.151	0.922

Zones of inhibition were present for all films and there was no significant difference in the size of the zone for each film when compared to the control.

Recommendations:

- In the future, we would also create a plastic film using a combination of citric acid, sodium hydroxide, and tripolyphosphate to see if this would be an even stronger prototype.
- Further testing such as pH testing and thermal stability testing will be carried out.
- We would also vary the amounts of cross-linker used in the films to see if adding more would strengthen the chitosan plastic.
- We would also like to do further research into the chemical makeup of chitosan and our biodegradable films.
- We would also like to carry out further research into potential cross-linkers that we may use.

Conclusion:

- We can conclude that our CA + NaOH chitosan film exhibited the most promising results.
- These films had the best performance in both the water and saline tests. They had the least increase in mass out of all the biodegradable films in each test.
- They had the strongest average tensile strength of 26.87 N compared to the control chitosan film which had an average tensile strength of 13.85 N. They had a 93% increase in tensile strength.
- Our NaOH chitosan films and our CA + TPP chitosan films also exhibited promising results.
- They also performed exceptionally in the water and saline tests. Their average mass increase had no significant difference with the average mass increase of the CA + NaOH chitosan film.
- The CA + TPP films had an average tensile strength of 18.42 N and the NaOH chitosan film had an average tensile strength of 23.53 N compared to the control chitosan film which had an average tensile strength of 13.85 N: 33% and 70% increase in tensile strength respectively.
- The antimicrobial tests showed that the size of the zones of inhibition for each biodegradable film ranged from 0 mm to 2 mm.
- There was no significant difference between the size of the zones of inhibition between all of the films created, with the p-values of all the films compared to the controls greater than the critical value of 0.05.
- The p-values ranged from 0.113 - 0.929.
- Therefore, it can be concluded that adapting the control chitosan film with CA, TPP, and NaOH had no effect on the antimicrobial properties of the chitosan films.
- Thus, crosslinking and alkali treatment are viable options for adapting chitosan films as they preserve the antimicrobial effects of the chitosan.

With future experimentation and further research, we believe that our CA + NaOH chitosan films, our CA + TPP chitosan films, and our NaOH chitosan films have great potential as biodegradable alternatives. The control chitosan films, the TPP chitosan films, and the CA chitosan films may be better suited as packaging of disposable tools as they don't need to be as strong.