Antiviral Capabilities of a DABCO-hydrocarbon molecules

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Abstract

For many, getting sick after a long flight appears to be inevitable. As the cabin air is repeatedly circulated throughout the plane, airborne viruses, such as Influenza, can spread and infect many passengers. Although several air filtration systems attempt to eliminate the transmission of viral particles, the methods are not completely effective. A method to surpass the transmission and infectious activity of viruses is the usage of a DABCO-hydrocarbon chain (diazabicyclo- octane) attached to a cloth. DABCO is a nitrogen-based molecule that can be covalently bound to other molecules. DABCO is a nitrogen-based molecule that can be covalently bound to other molecules. The extra bond in the middle of the molecule attaches the two nitrogen molecules into a three dimensional formation, making DABCO positively charged. The experiment was specifically conducted to observe which length of hydrocarbon chains on the DABCO molecule would be most effective. The results were observed to be an example of a steric hindrance relationship between the DABCO and the phage. The shorter distance in the cloth may seem to be one charge, attracting less of the negative tail fibers of the bacteria whereas in the powder form, likely because the smaller chains can float freely and orient themselves to fit closely and attach to attach to more tail fibers as opposed to longer chained molecules. Ultimately, the antiviral abilities of the novel DABCO molecule can be used for military or pharmaceutical purposes as well as air filtration systems in airplanes.

Introduction

Viruses constantly pose harm onto humans' lives and the need to eradicate them is vital. A virus is a microscopic parasite capable of infecting almost all living organisms such as plants, animals, or bacteria. They are infectious particles that use the resources of the living host cells they target and take them for their own propagation. Although only 219 species are known to be able to infect humans, there are over 320,000 different types of viral species identified today (Woolhouse, 2012). Specifically, the notorious viruses such as Influenza or Measles are airborne viruses. Therefore, for many, getting sick after a long flight appears to be inevitable. This may be a direct result of the repeated circulation of cabin air on a flight that contains viral particles, which passengers are vulnerable to. Despite the air filtration techniques that have been created today, no antiviral methods have been perfected to completely eradicate viral transmission and infectivity. Several researchers have begun to study possible antiviral techniques that could be manufactured. One group of researchers has identified a protein that can eliminate viruses and aid the body in naturally targeting the virus based on genetic patterns (Mandal, 2019). However, as viruses maintain their harmful capabilities in society today, the need to create more effective antiviral techniques is a growing issue.

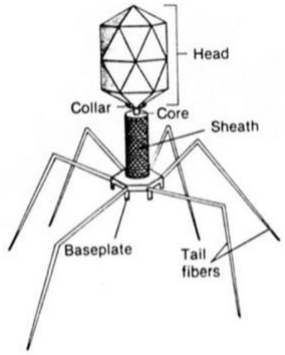

Figure 1: Structure of bacteriophage. Protein capsid (head) and negative tail fibers are shown.

Viruses can often be categorized into two groups: enveloped and non-enveloped. Enveloped viruses, such as HIV, are surrounded by an outer lipid membrane which enables them to infect target cells. The lipid membrane makes enveloped viruses harder to kill, but without the membrane, they are no longer pathogenic (Sakudo, 2011). On the other hand, non-enveloped viruses lack this outer membrane. They have a similar structure to enveloped viruses as they consist of a protein head or capsid that contains DNA or RNA. Non-enveloped viruses, such as T4 Bacteriophage, consist of DNA encapsulated in a capsid and has attached tail fibers (**Figure 1).** The specific virus bacteriophage is distinguished from other viruses as it infects only bacteria. Bacteriophage are one of the more common viruses, found very readily in the biosphere in freshwater and oceans, feces, sewage, soil, and many other environments (White, 2019). Despite their widespread abundance, these viruses are characterized by their high specificity to bacterial species along with their morphology and genome. For example, T4 bacteriophage is a specific virus in the family *Myoviridae* and is best known to kill the bacteria *E. coli*. Scientists are now beginning to look to bacteriophages as novel pharmaceuticals to destroy bacteria. The phage, which is usually about 90 nm x 200 nm, is assembled from three parts: the head, the tail, and the long tail fibers. The head, also known as the capsid, is a

protein that is responsible for holding the DNA of the virus. The phage also consists of 6 tails that are negatively charged and serve as sensors to target host cells (Yap, 2015). I chose to use bacteriophage in these studies as they are not pathogenic to humans. Other viruses that have been used in the past, such as Adenovirus and Influenza, are infectious and not a good way to learn and work out protocols.

A method to circumvent the infectious activity of viruses is the usage of a DABCO-hydrocarbon chain attached to a surface (cloth, paper, etc.). Past studies have shown that it is an effective method to reduce the infectious activity of viruses, specifically the T4 bacteriophage. Results have indicated that specific DABCO molecules **(Figure 2a)** attached to a cloth made of 100% cotton was effective in reducing the number of T4 viral plaques in *E. coli* (Stirling, 2015). The DABCO (1,4- diazabicyclo [2. 2. 2] octane) molecule has shown several antimicrobial characteristics when attached to a hydrocarbon chain. The DABCO molecule is a nitrogen-based molecule that is positively charged and can be covalently bound to other carbohydrates or even proteins (Engel, 2008). The extra bond in the middle of the molecule serves as an extra coordination bond that attaches the two nitrogen molecules in a three dimensional formation, allowing the nitrogen molecules to have four bonds and carry a positive charge. The negatively charged tails of the T4 bacteriophage are attracted to the positively charged DABCO molecule, creating a bond that would ultimately make the virus inactive or less infectious. Additionally, the DABCO's structure allows it to be combined with different carbon chains that vary in length or end moiety, changing the function of the DABCO molecule. For example, diDABCO molecules have been synthesized, creating two DABCO rings on each end of a hydrocarbon chain; this would enhance the overall charge of the molecule, making it more positive (see Figure 2a; one DABCO molecule has two positive charges, two DABCO's have 3 charges).

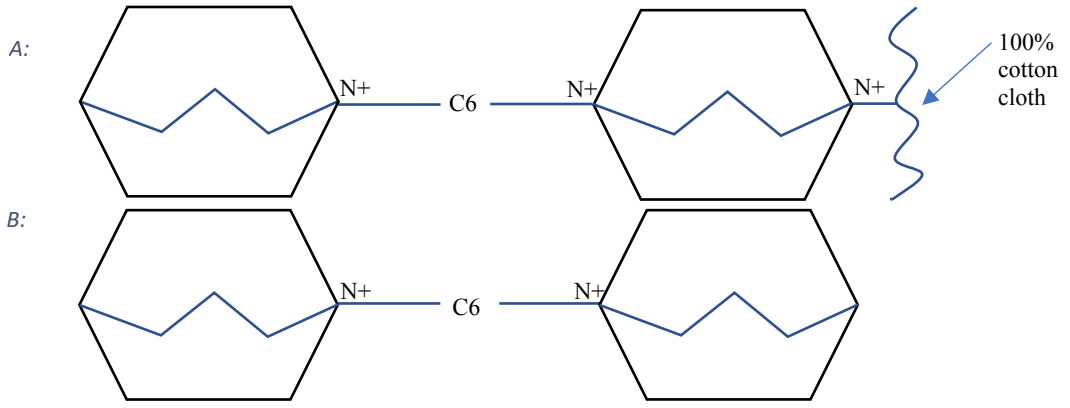

Figure 2: (A) Structure of diDABCO molecule with C6 hydrocarbon linker chain attached to cotton cloth. Molecule has +3 charge due to attachment to cloth that creates 4 bonds at nitrogen. (B) diDABCO-C6 molecule shown without cloth attachment (powder form). Without the cloth, the molecule now has a charge of +2.

Past results have also indicated that the usage of the DABCO molecule is much more effective than other antiviral treatments on the market today, such as the usage of QUATS (quaternary ammonium

compounds). QUATS are molecules commonly formulated into soaps due to their antibacterial and antiviral abilities. After comparing DABCO and QUATs, DABCO molecules proved to eliminate bacteria better, faster, and were more durable on the cloths. Therefore, as an antimicrobial treatment, DABCO was further explored as it was antibacterial, antifungal and antiviral. However, DABCO molecules unlike QUATs can be covalently attached to cloth in a more durable way that allows them to withstand 50 or more washes before loss of antimicrobial activity. The covalent attachment provides a distinct advantage over other antimicrobial cloths currently on the market.

Since the DABCO molecule forms 4 bonds around each nitrogen, each nitrogen carries a positive charge. Therefore, when the DABCO molecule is attached to cloth, it creates an additional charge on the DABCO-hydrocarbon molecule as compared to the same molecule not bound to cloth. From previous studies, it has been proposed that the virus's negatively charged tail fibers are attracted to the positive charges of the DABCO molecule. When binding occurs, the tail fibers of the virus, which attach to bacterial host cells, would no longer be effective as sensors, thus decreasing viral activity. It was hypothesized that if an electrostatic relationship exists between the DABCO molecule and T4 viral particles, then incubating diDABCO-C6 cloth with T4 bacteriophage would result in a decrease in viral plaques. Additionally, it was hypothesized that if shorter hydrocarbon chains permit more attachments to viral tails by dissolved DABCO molecules, then diDABCO-C3 will have the greatest percent reduction of viral plaques.

Methods

Virus Titer

In order to determine the ideal viral concentration for the *E. coli* strain used, I conducted a virus titer. Before beginning the titer, nine petri dishes filled with tryptic soy agar (TSA) were removed from 4ºC and allowed to come to room temperature. 200 µl of virus was serially diluted to 10^{-6} in SM+G.

100 µl of each viral dilution was incubated with 100 µl of *E.coli*. at 1 x 10^5 CFU. SM+G (virus stabiliting media) only and *E.coli* only tubes were also made to serve as control groups. Once the *E.coli* and virus dilutions were mixed together, all tubes were incubated at 37ºC for 20 minutes.

During this time, soft agar was melted in a microwave. Then, 4 mL of soft agar was added to seven 15 mL snap cap tubes and placed in the water bath at approximately 55ºC to prevent solidification.

100 µl of each phage-bacteria mixture was added to individual snap cap tubes with soft agar. Then the mixtures were poured onto 10 cm agar containing petri dishes. The solutions were swirled around the plates to spread out the mixture and then left on the benchtop to solidify.

Once solidified, the plates were placed in a 37°C incubator overnight and the results (number of viral plaques present) were recorded the following day. The correct virus concentration used in the experiment was chosen by observing which plate had a countable number of plaque forming units (PFU), which was observed to be at 10^{-6}.

Plaque Assay

The plaque assay was conducted to observe which cloths appeared to be most efficient in changing the activity of the virus. After conducting several trial tests, the working dilution was chosen to be 10^{-6}. Dilutions were made in same procedure as used in the virus titer up to a dilution of 10^{-5}. Then, several microfuge tubes were prepared for each cloth (with a different DABCO-hydrocarbon) that was tested and 900 µl of SM+G was added to every tube, diluting the virus to 10^{-6}. 100 µl of the 10^{-6} virus dilution was then added to each new tube and pipetted up and down to mix.

The cloths chosen were cut into 2.25 cm^2 squares. Using sterile forceps, each cloth was placed in an individual microfuge tube containing the virus dilution at 10^{-6} and was placed at 4º Celsius and rocked overnight.

After incubation, 100 µl of the liquid was added to a new microfuge tube and combined with 100 µl of *E. coli*. The tubes were incubated for 20 minutes at 37°C, plated with soft agar and incubated overnight at 37ºC.

Results of the repeated plaque assays were observed the following day and ultimately helped me to decide which final cloth to use for experimentation.

Cloth Testing

The cloth test was conducted in the same procedure as the plaque assay. However, along with the liquid being plated with *E. coli*, the cloths that were incubated overnight in the virus liquid were also plated. Soft agar was melted and then 100 µl of *E. coli* was added directly to the soft agar in the snap cap tubes. Using sterilized forceps, the cloths were removed from the original microfuge tubes and laid across a 10 cm petri dish containing TSA. The soft agar was then poured directly over the cloth and swirled to evenly spread it out. The plates were incubated overnight at 37º C and examined for plaques the next day.

Wash Test

A wash test was then conducted to observe if washing the overnight cloths prior to plating would strip the virus off of them. The wash test was conducted in the same way as the cloth testing procedure. However, for each type of cloth, two cloths were placed in microfuge tubes. After rocking them overnight at 4°C, the cloths were removed from the tubes and placed on sterile aluminum foil. Two small petri dishes were filled with 6 ml of SM+G for the blank cloths and the diDABCO-C6 cloths. Cloths were washed with SM+G once or three times. For example, in the blank cloths, one cloth was quickly rinsed and then left to dry on sterile aluminum foil. The second blank cloth, identical to the first, was then quickly rinsed in the SM+G, then placed on a rocker in a dish containing a new 6 mL of SM+G. After 5 minutes, the cloth was then quickly rinsed again in a dish containing new 6 mL of SM+G and then left to dry on sterile aluminum foil. This process was repeated for the diDABCO-C6 cloth. After all four cloths were left to dry for 5 minutes, they were plated with soft agar mixed with 100 µl of *E. coli* again, similar to the cloth test procedure stated previously.

Detergents

SDS and TX-100 were made at 5% dilutions using SM+G. 2 grams of SDS were dissolved in 10 mL of SM+G in order to make the detergent at 20%. TX-100 was in liquid form and therefore 2 mL was mixed in 8 mL of SM+G. Three microfuge tubes were then filled with 675 ul of SM+G and 225 ul of 20% SDS to dilute the detergent to a 5% concentration. The same process was used to dilute the 20% TX-100. This resulted in a total of six microfuge tubes that would be allocated to the blank cloth, no cloth, and diDABCO-C6 cloth. The cloths were cut into 2.25 cm^2 sizes and placed into the microfuge tubes to be rocked overnight at 4^oC. The following day, the cloth was plated in the same manner as the cloth testing procedure above using soft agar. 100 ul of the liquid from each microfuge tube was also extracted and plated as well. The plates were then incubated overnight and results were observed and recorded the following day.

Powder Mixes and Test

18 different powders (DABCO starting materials) were tested during experimentation. Each powder differed in characteristics including chemical structure, solubility, consistency, size, etc. 0.1 grams of each powder was weighed using an electronic scale and weigh boat. They were then added to a microfuge tube containing 900 µl of SM+G, incubated and rocked overnight at 4°C. The liquid was plated the following day using soft agar combined with *E. coli*.

Results

diDABCO-C6 chain attached to cloth results in decreased activity of T4 bacteriophage

Several tests were conducted using the diDABCO-C6 cloth. A total of five trials of the plaque assay were completed over the course of five different days. For each trial, three cloths were used: diDABCO-C6 cloth, blank cloth, and no cloth (virus only).

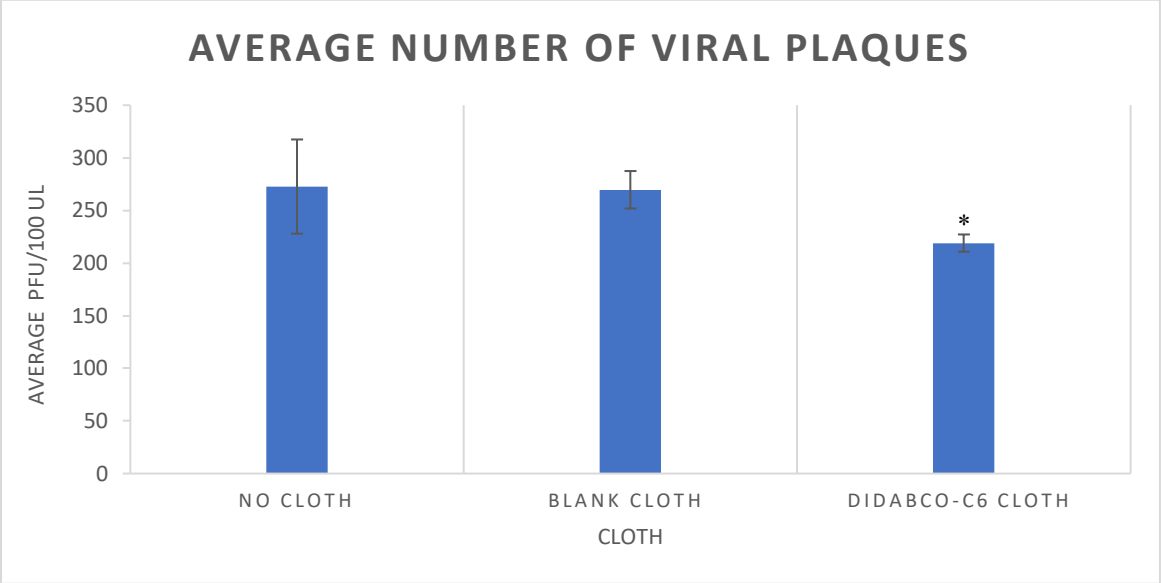

***Figure 2**: Average number of viral plaques in C6 cloth, Blank cloth, and No cloth (Virus only) at 10^6 with 100 ul of E.coli. * indicates where the data is statistically significant in comparison to blank cloth when p<0.01.*

Figure 3 shows that the petri dishes with a viral concentration at 10^{-6} and no cloth had the greatest number of viral plaques, with an average of 272.7 plaques (n= 5). This value was similar to the blank cloth which had an average number of plaques of 269.7 (n= 5) whereas the C6 cloth had an average of 219 plaques (n=5). A Welch's ANOVA test was conducted using the above data to analyze the statistical significance of the data with unequal variances. The Welch test indicated that the data was statistically significant, where $p<0.01$. Following the Welch test, the Games-Howell post hoc test was then used to determine the specific relationship that was statistically significant. The test indicated that the data was statistically significant between the blank cloth and C6 cloth, where $p<0.01$. Although the no cloth had a greater number of average PFU and would be predicted to be statistically significant as well, the high variance of the no cloth did not result in a p-value less than 0.05.

	1 Wash	3 Washes	% Change
Blank cloth	61 plaques	10 plaques	83.6%
diDABCO-C6 cloth	96 plaques	24 plaques	75%

Table 1: Plaque Forming units of Blank cloth and diDABCO-C6 cloth after wash test

Three Washes of Blank and diDABCO-C6 cloth results in decline in viral plaques

After conducting the wash test, the results indicated that rinsing the DABCO-conjugated cloths after virus incubation multiple times did impact the number of plaque forming units (PFU) associated with the cloths. Results (**Table 1)** indicate that washing the cloth reduced the PFU associated with the cloth. It was shown that as the washes increased, the number of PFU's decreased suggesting that each wash strips some virus off the cloth. More specifically when comparing one vs three washes, the number of plaques associated with the blank cloth decreased by about 84% after the third wash and 75% for the diDABCO-C6 cloth. This illustrates how some of the virus particles stick to the blank cloth nonspecifically, in the absence or presence of the diDABCO-C6 molecule.

SDS and TX-100 detergents show significant reduction of viral plaques in blank cloth and diDABCO-C6 cloth compared to no cloth (V. only).

The 5% SDS and 5% TX-100 detergents allowed the potential electrostatic relationship between the DABCO and the virus to be observed. Since the cloth itself and the liquid from the microfuge tubes were plated separately, two statistical analyses were conducted for the detergents. The liquid extraction determined how effectively the virus was attached to the cloth after the addition of the detergents. Using the Welch's ANOVA, the results indicated that the data was statistically significant, where $p<0.005$, indicating that both detergents had an impact on the number of viral plaques present (**Figure 4a**). Furthermore, the results from the extracted liquid also indicated that the data was significant, where $p<0.05$ (**Figure 4b**). After comparing the cloth itself and the liquid extraction results, there did not seem to be a drastic difference between the number of viral plaques. However, when comparing these results with previous results from the cloth test (Figure 3), it can be concluded that the detergents interfere with the effectiveness of the cloths.

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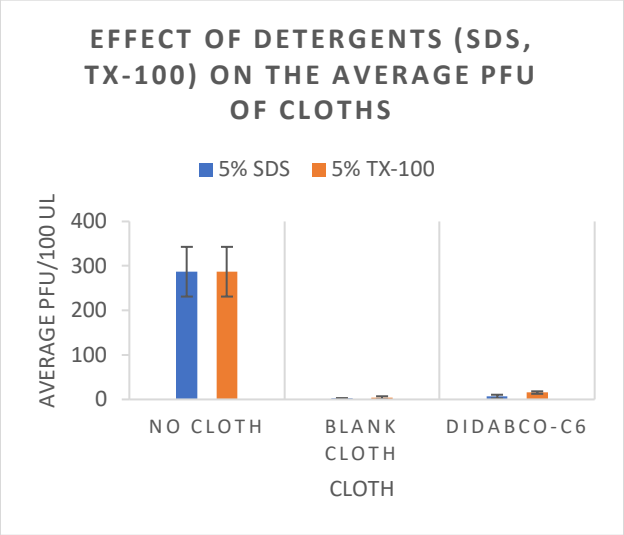

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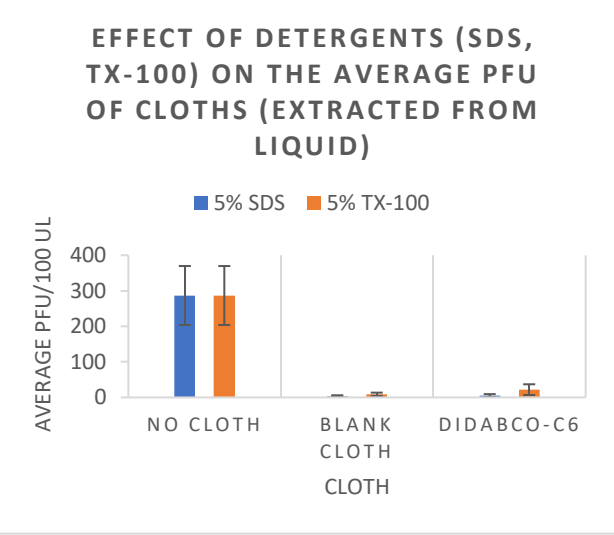

Figure 4: (A) Detergents (SDS and TX-100) exposed to no cloth, blank cloth, and diDABCO-C6 cloth. Results indicate that p<0.005 when comparing blank cloth and diDABCO-C6 cloth to no cloth.

Free DABCO molecules show antiviral effects where shorter hydrocarbon chains have increased PFU

After unsuccessfully trying to strip the virus off the cloths using SDS and NaCl (results not shown), we decided to examine the interaction of the virus with various DABCO molecules before attachment to cloth. This allowed observations to be made about the effectiveness of the cloth itself and how sufficient the original solutions of the DABCO molecules were at inactivating or decreasing viral activity. Four DABCO molecules were utilized in this experiment: DABCO-C3, diDABCO-C9, diDABCO-C3, diDABCO-C6. Due to DABCO only starting material being unattainable, he DABCO-C3 molecule simulated a DABCO only molecule because the short three carbon chain was small enough to be comparable to no chain at all.

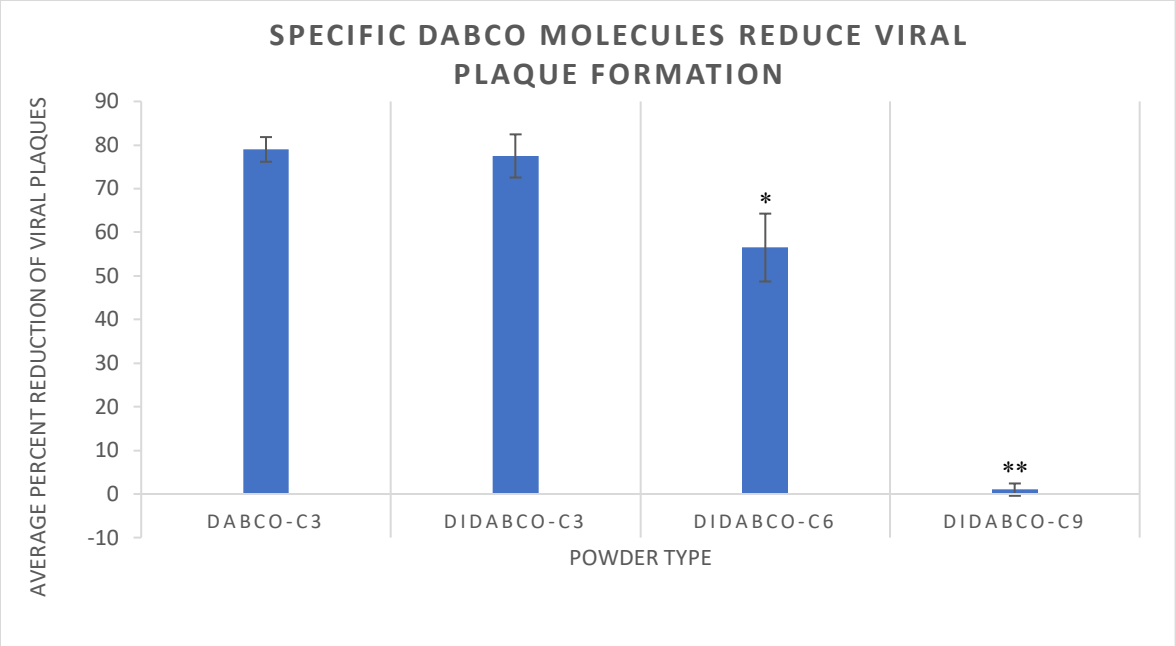

***Figure 5**: Average Percent Reduction of Viral plaques by four different powder mediums: DABCO-C3, diDABCO-C9, diDABCO-C3, diDABCO-C6. * indicates that p<0.05 when compared to DABCO-C3. ** indicates that p<0.01 when compared to DABCO-C3.*

An ANOVA single factor test was also conducted to analyze the statistical significance of the antiviral data of the DABCO molecules as well. The averages (n=2) of each of the DABCO molecules were calculated, measuring the average percent reduction of viral plaques. The diDABCO-C9 molecule showed little reduction in viral plaques (**Figure 5**) while DABCO-C3 and diDABCO-C3 proved to be much more effective at reducing viral plaques. Aside from the C3 molecules, the diDABCO-C6 was seen to have a high percent reduction greater than 50%. Furthermore, the results of the Welch's ANOVA test then indicated that the data was statistically significant, where $p<0.01$. The results indicate that the data is extremely significant and that specific DABCO molecules have a significant impact on reducing the number of viral plaques observed on the agar dish.

Discussion

The objective of the experiment was to observe the antiviral capabilities of a diazabicyclo-octane cloth. The results of the experiment described herein support the hypothesis that if an electrostatic relationship exists between DABCO modified cloth and viral particles, then incubating these cloths with T4 bacteriophage would result in a decrease in viral plaques.

The results of the experiment indicate that the diDABCO-C6 molecule when attached to a cloth surface does cause a decline in the number of viral plaques on *E. coli* plates. After statistical analysis was conducted, the tests indicated that the reduction in plaques seen with the diDABCO-C6 cloth was statistically significant when compared to no cloth (the virus only group). The blank cloth with no DABCO molecule showed some decrease in observed viral plaques, however, the difference was not significant. The data suggests that the virus may stick nonspecifically to the 100% cotton cloth (blank cloth). Therefore, blank cloth does not serve as a sufficient antiviral method while the diDABCO-C6 cloth appeared to be more effective.

These results showed that the diDABCO-C6 cloth reduced viral activity but they did not fully address a possible electrostatic interaction. I tried to disrupt the interaction between cloth and virus using a charged detergent (SDS) in comparison to an uncharged detergent (TX-100). The incorporation of the detergents indicates that the effectiveness of the DABCO molecule is inhibited when exposed to both SDS and TX-100 (**Figure 4a and 4b**). SDS is a negatively charged detergent and thus would interfere with the virus' ability to attach to the positively charged diDABCO-C6 molecule. TX-100, however, also showed a decline in PFU of the virus, although it has no charge. Consequently, the hypothesis was not supported because the relationship between the virus and the DABCO is not strictly electrostatic. Since the TX-100 influenced average PFU, the relationship can more likely be categorized as hydrophobic as both SDS and TX-100 are detergents.

Finally, I chose to address the direct interaction between the viral particles and various DABCO conjugated molecules (powder form) before attachment to cloth. The powder mediums of the DABCO molecules also served as an additional control to the original experiment, allowing observations to be made about whether the cloth was influencing the number of viral plaques. It is clear that there was some interaction between the DABCO molecule and the virus. These results gave insight into how effectively the DABCO molecules with varying hydrocarbon lengths can inhibit viral activity.

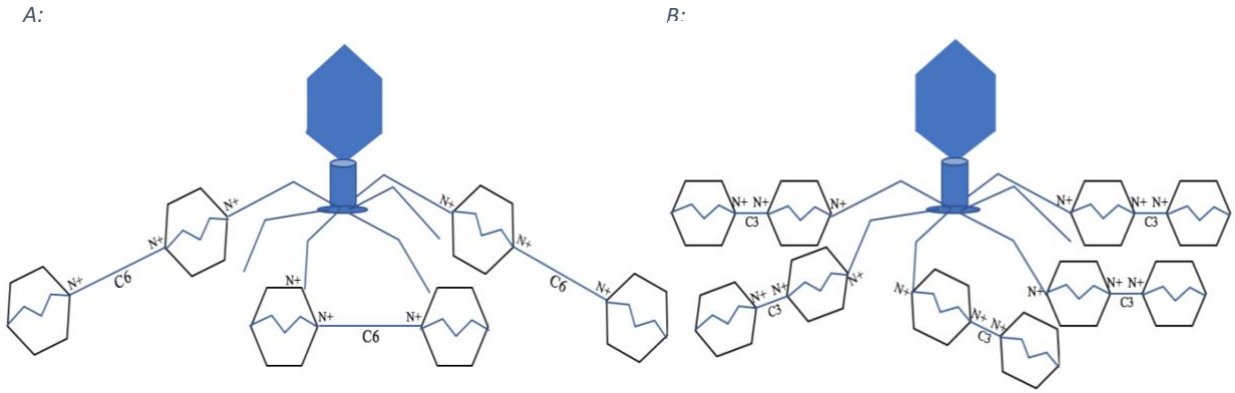

Figure 6: (A) T4 Bacteriophage binding with diDABCO-C6 molecules in form of powders that have been dissolved in liquid medium. (Illustrates 3 possible tail fibers being attached to DABCO molecule). (B) T4 Bacteriophage binding with diDABCO-C3 molecules in form of powders that have been dissolved in liquid medium. (Illustrates 5 possible tail fibers being attached to DABCO molecule)

As shown by the data (**Figure 5),** the shorter chains of DABCO molecules (C3) were seen to be more effective than longer chain (C9) DABCO molecules. This could be due to steric hindrance, as the molecules in the form of powder were able to coat the negative tail fibers of the T4 bacteriophage in shorter chains **(Figure 6a).** Longer chains would not be able to coat the tail fibers as effectively as the chains between the DABCO molecules are too long, like the diDABCO-C9, so some tail fibers on the bacteriophage would not be coated and would still be able to attach to *E. coli* resulting in an infection **(Figure 6b).** On cloth, however, the shorter DABCO chains were not shown to be as effective in decreasing viral activity (previous studies). The close proximity of the DABCO moiety on each short DABCO molecule (diDABCO-C3) may present as one positive charge. As a result, the molecule only attaches to one tail fiber at a time on the bacteriophage **(Figure 7),** leaving other tail fibers to attach to a bacterium resulting in an infection. Longer chains that separate the DABCO molecules spread out the positive charges, allowing for a stronger attraction and interaction between the DABCO-conjugated cloth and virus **(Figure 7).** This would bind more tail fibers and hinder the virus's ability to interact with bacteria. Steric effects determine the ability and activity of a drug and how it will interact with its target bio-molecules. Due to the molecular structures of the varying DABCO molecules and their organization on cloth or if they are free in the environment, some prove to be more effective than others when targeting T4 bacteriophage.

The DABCO-C3 powder was used to simulate a DABCO only molecule in the "powder" experiments; its structure is almost identical to a "DABCO-only" sample, excluding the short hydrocarbon tail (C3) and an extra charge due to that hydrocarbon chain. DABCO-only molecules when attached to cloth are expected to have little to no plaque reduction when exposed to *E. coli,* with some

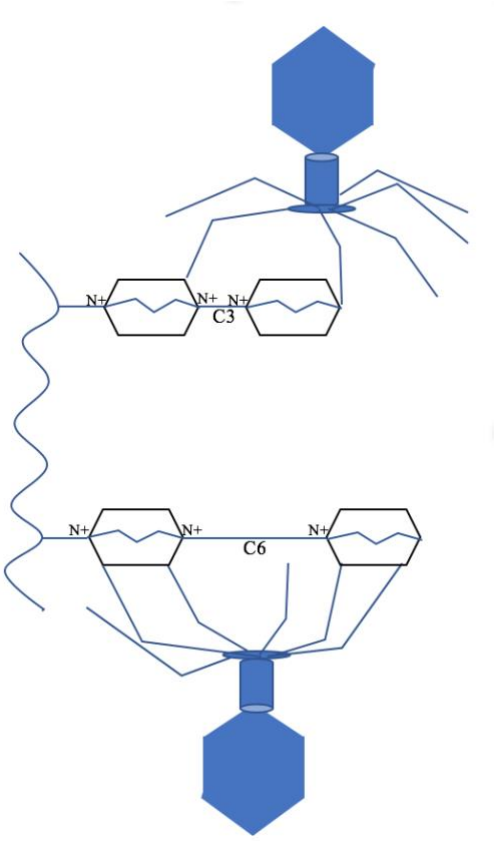

Figure 7: T4 Bacteriophage binding to diDABCO-C3 and diDABCO-C6 molecules attached to cloth. (diDABCO-C6 shown to be more effective at attaching to phage).

plaque forming units present from nonspecific interactions. However, upon examining the data repeatedly, this did not occur in the powder. The DABCO-C3 powder showed plaque reduction, which was not predicted. Therefore, these molecules behave differently when free in solution compared to organized (bound) to cloth. The change on the DABCO-C3 makes it behave more like the diDABCO-C3 instead of a DABCO only in the free form. On a cloth, the DABCO-C3 still simulates a DABCO only.

Contrary to my prediction, the DABCO-C3 powder **(Figure 5)** had a very high percent reduction in viral plaques (79%), indicating that it did not act similarly to a DABCO-only molecule when in "free" form. Furthermore, the diDABCO-C3 molecule had approximately the same average percent reduction of viral plaques as the DABCO-C3 with 77.5% plaque reduction. In spite of the +3 charge of the diDABCO-C3 molecule, the average percent reduction of plaques remained roughly equivalent to the diDABCO-C3 (+4 charge), indicating that charge may be an important factor in the interaction between DABCO-molecules and virus. However, the plaque reduction data was also found to be statistically significant between the diDABCO-C3 and diDABCO-C6 molecules, indicating that the different spacing between the molecules affects the activity of the viruses. This result suggests that there may be other factors, such as steric effects, affecting the virus in addition to electrostatic interactions. This data supports the idea that steric hindrance is occurring in the free form of DABCO molecules because the shorter chains are able to attach to the virus tail fibers more readily and prevent infection. This was not evident in the diDABCO-C9 chain, as it had almost 0% reduction. Due to the long hydrocarbon chain that separates the DABCO molecules, it may block many of the viral tail fibers from binding with additional DABCO molecules but still allow them to interact with bacteria. Since the powders are dissolved in liquid, the DABCO molecules are free floating particles that are capable of changing their orientation to bind more efficiently. The diDABCO-C9 molecules may block tail fibers whereas the smaller molecules, such as diDABCO-C3 or diDABCO-C6 allow for additional DABCO molecules to attach to the negatively charged tail fibers **(Figures 6a and 6b).**

After conducting my experiment, I recognized some limitations. One limitation of the experiment was that the sample size for the powder experiment was small, where n=2. Due to time constraints, more trials were not able to be conducted. Consequently, the data is not as credible for the powders as there was a minimal number of repetitions.

Another limitation that was recognized was when I conducted the wash test and embedded the cloths in TSA agar. After incubating the cloth and virus overnight, the cloth still had some viral mixture on it that was not completely rinsed off. Consequently, there were several blobs (large clear areas) of viral plaques (no *E. coli*) that were uncountable and were therefore not included into the number of viral plaques observed. As a result, the percent reduction of viral plaques may have been more accurate if the "blobs" had been accounted for.

In the future, the experiment could be expanded by using additional viruses and testing their activity after treatment with the DABCO-antiviral cloth and free DABCO powder methods. Viruses such as Adenovirus or Influenza can be utilized to observe how effective are these molecules. Since the "powders" have not been studied previous to this project, it is important to begin to understand which method may be more effective with viruses. This would allow the interactions between the DABCO molecules and the viruses to be studied more in depth and help to clarify if the relationship is electrostatic, hydrophobic, steric or something else.

In order to minimize the burden of viral diseases, the need for virology and microbiology studies is increasingly important in society today. Viruses exist in all shapes and forms and affect every part of the world. Ebola was seen to have a detrimental impact on the population in West Africa. Chikungunya spread through the Caribbean and the United States decimating populations (Imperiale, 2015). Influenza is an annual problem, resulting in hundreds of thousands of hospitalizations and tens of thousands of deaths world-wide. The best hope of preventing or minimizing the impacts of these viruses and others is research. In the past, viruses such as polio or HIV used to be extremely dangerous and deemed deadly to any human exposed to them. Overtime, however, viral infections have become much more manageable and even preventable due to medical advancements and intense scientific research.

While antiviral methods do exist today, there is a greater need for less expensive solutions. The DABCO cloth method could prove to be an alternative antiviral method that could be applicable to all viruses. As new viruses are constantly merging in other organisms or in new forms/strains, it is important to have a feasible antiviral method to prevent infections.

The DABCO cloth utilized in this experiment has been tested by the US Army for medical purposes (bandages in the field) and also apparel (protection from biowarfare). Additionally, the cloth could be installed in air filtration systems, especially in airplanes, to minimize the concentration of viruses that are present in the recirculated air to which all passengers are exposed. On the other hand, the

free DABCO molecules could be transformed into potential pharmaceuticals in the future. This would allow for an internal method of limiting the activity of viruses within humans. As viruses continue to approach the epicenter today, it is becoming more important to find more feasible and economic methods to eradicate them.

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