Novel DDBAC and TiO$_2$ Functionalized Hydrophobic Electrospun Nanofibrous Membranes for Virus Adsorption in Drinking Water Applications

by

Stéphane Liégey

A report submitted in conformity with the requirements for the degree of Master of Applied Science

Department of Chemical Engineering and Applied Chemistry

University of Toronto

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Abstract

Waterborne pathogens such as enteric viruses have been identified as one of the main threats to sources of drinking water and aquatic ecosystem in Canada and around the world. Current methods to address viral contamination of water all present high degrees of efficiency. However, they also pose significant drawbacks such as release of potentially toxic/carcinogenic byproducts into the environment, high operating cost, or large land footprint. Nanofibre polyvinylidene fluoride (PVDF) membranes functionalized with TiO$_2$ nanoparticles were developed. They were found to have a bacteriophage MS2 log removal value (LRV) of up to 1.2 likely through hydrophobic interactions and/or contact-inactivation with TiO$_2$. Similarly, nanofibrous PVDF membranes functionalized with dodecyldimethylbenzylammonium chloride (DDBAC) for its positive charge and electrostatic effect were found to have a consistent MS2 LRV of 2.4. These novel membranes could provide a cost effective alternative to other available membrane technologies.
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**Nomenclature**

AFM – Atomic force microscopy

AOP – Advanced oxidation process

BTEAC – Benzyltriethylammonium chloride

CCME - Canadian Council of Ministers of the Environment

CNT – Carbon nanotube

CT – (concentration x time)

DBP – Disinfection by-product

DDBAC – Dodecyldimethylbenzylammonium chloride

DLVO - Derjaguin, Landau, Verwey, and Overbeek

ENM – Electrospun nanofibrous membrane

FTIR-ATR – Fourier transform infrared spectroscopy-Attenuated total reflection

GAC – Granular activated carbon

HAA – Haloacetic acid

IEP – Isoelectric point

LRV – Log removal value

MAC – Maximum allowable concentration

MBR – Membrane bioreactor
MF – Microfiltration

MWCNT – Multi-walled carbon nanotube

NOM – Natural organic matter

OMF% - On-mass-fiber percentage

PBS – Phosphate-buffered saline

PFC - Perfluorocarbon

PFU – Plaque-forming unit

PTFE - Polytetrafluoroethylene

PVDF – Polyvinylidene fluoride

PZC – Point of zero charge

QAC – Quaternary ammonium compound

SABS – South African Bureau of Standards

SEM – Scanning electron microscopy

SRHA – Suwanee River humic acid

SRNOM – Suwanee River natural organic matter

SWCNT – Single-walled carbon nanotube

THM – Trihalomethane

TEM – Transmission electron microscopy
TMP – Transmembrane pressure

UF - Ultrafiltration

USEPA - United States Environmental Protection Agency

UV - Ultraviolet

WHO – World Health Organization

WSCP - poly [oxyethylene (dimethyliminio) ethylene - (dimethyliminio) ethylene dichloride]

XPS – X-ray photoelectron spectroscopy
1.0 Introduction

1.1 Background

Waterborne pathogens are a major concern in drinking water. They can be viral or bacterial in nature, and can be found in all water bodies as naturally occurring or be introduced due to nearby human activity (1-4). Environment Canada has listed them as one of the major threats to sources of drinking water and the aquatic ecosystem health, alongside twelve others such as endocrine disrupting substances, algal toxins, municipal wastewater effluents, and industrial wastewater discharges (5). The Canadian Council of Ministers of the Environment (CCME) listed them as an issue regarding their potential impact (as assessed by Health Canada) on aquatic life such as shell mollusks near wastewater discharge points, notably in Canada’s Far North where untreated effluents are released into natural wetland systems (6). These reports bring a focus on waterborne pathogens as a major public health and environmental concern.

Waterborne pathogens are often suspected to be responsible for isolated and occasional gastroenteritis cases, but more importantly, they have been known to be the cause of severe outbreaks that have impacted entire communities (2,7,8). Enteric viruses and bacteria are of particular concern due to their source and the illnesses associated with them. They are transmitted to the environment through feces, of human or animal origin, and can cause a wide range of health complications such as gastroenteritis (responsible for over 1.2 million deaths in 2014), conjunctivitis, and respiratory illnesses (7,8). Waterborne enteric viruses in particular, characterize
themselves by their low infective doses, strong environmental persistence, and widespread presence in the environment (1,4,9).

Causal links between viral infections and drinking water supplies over history have been predominantly established through epidemiological data rather than direct viral detection, due to the difficulties of viral detection with available technologies. This is why indicator organisms have been used as a reference when developing guidelines and standards for drinking water treatment. The acceptable risk level associated with drinking water is capped at one infection/10,000 consumers/years by the WHO and Health Canada (7,10). It must be noted that while standards are legally enforceable such as the ones set by the USEPA, guidelines generally are not, unless they are incorporated into provincial legislation or municipal permits as is the case in Canada. This complicates the task of having a consistent and safe drinking water supply across Canada, by muddying the boundaries between what should and has to be done to treat water for consumption purposes from a legal perspective. In industrialized nations, the consensus from experts is that waterborne pathogens are the single greatest risk in water consumption. Due to the wide variety in nature of waterborne pathogens and technical reasons, maximum allowable concentrations (MACs) are not established for the majority of them. This is why outcome-based standards have been established by the USEPA based on log removals or inactivation, and Canadian guidelines follow the same approach. In Ontario, Canadian guidelines are part of the provincial legislation and are thus enforceable. A minimum 4-log removal or inactivation of viruses must be obtained when the raw water supply is surface water or contaminated ground water. A
minimum 2-log removal or inactivation of viruses must be obtained when the raw water supply is ground water (10-14).

Current technologies to address viral contamination of water are of physical (removal) or chemical (disinfection) nature, in addition to ultraviolet light disinfection which relies on short-wavelength ultraviolet light. These technologies have proven to be effective to different degrees, but what they all have in common is that they present significant operational drawbacks which should be overcome or mitigated. (15,16)

Physical methods include coagulation processes and membrane filtration. The most common treatment process employed is conventional filtration which is conducted in three steps: coagulation/flocculation, sedimentation, and media filtration. Conventional filtration generally has limited effectiveness in removing viruses. Membrane filtration processes include membrane bioreactors (MBRs), ultrafiltration (UF) membranes, and microfiltration (MF) membranes. MBRs have significant operational challenges and are costly to maintain. UF membranes are relatively costly to operate and have operational challenges such as release of retained viruses across the membrane when transmembrane pressure drops (e.g., during periodical maintenance). MF membranes are generally used where a good coagulation process is employed as pretreatment.

Chemical methods include the addition of chlorinated compounds which can be highly effective but induce formation of toxic by-products (e.g. trihalomethanes) with negative environmental and public health impacts. Ozonation involves bubbling ozone into the water to inactivate pathogens. While ozonation is highly effective, it induces
formation of toxic by-products (e.g. organo-brominated compounds). It also is a complex system to operate and has high operating costs.

Ultraviolet (UV) light disinfection works through the strong germicidal and virucidal effect (i.e., inactivation of pathogens) of the UV rays. It presents high efficiencies, but faces significant operational challenges. In particular, particulate matter could shield pathogens from the disinfecting action of UV rays and decreasing UV disinfection efficiency. Also, certain viruses have a natural resistance to ultraviolet light through the process of photo-reactivation (17).

The environmental persistence of viruses is attributed to their ability to survive several weeks by adsorption to solid surfaces, which protects them from inactivating effects (e.g. steric effects). This adsorption is due to several factors. The nature of the virus has a strong influence on its adsorption behavior. The types of the proteins forming the viral capsid (the protein shell of a virus), and more specifically the amino acids constituting these, are responsible for the observed adsorptive interactions in a given environment.

The nature of a surface can promote viral adsorption based on its net surface charge. This indicates that electrostatic interactions are a significant adsorption mechanism for viruses. A surface with a high point of zero charge (i.e., the pH at which the electrical charge density on a surface is zero) will more easily adsorb negatively charged viruses due to its net positive charge at lower pH values (around pH 6) such as those of typical environmental waters. pH levels determine the net surface charge of a virus or particle based on its isoelectric point (i.e., the pH at which a molecule or particle carries no net electrical charge) or point of zero charge, respectively. The point of zero
charge and isoelectric point differ in the presence of specific adsorption (i.e. not H⁺/OH⁻ ions). Typical environmental waters pH of 6-7 means most viruses will be negatively charged (18). High ionic strength promotes viral adsorption by reducing the Debye length and thus the electrostatically repulsive forces between particles.

Finally, dissolved organic matter can provide additional binding sites for adsorption. In addition, hydrophobic interactions can also play a significant role in virus adsorption as the viral capsid tends to be hydrophobic, thus creating thermodynamically unfavorable interactions between polar water molecules and hydrophobic particles which seek to reduce these by agglomerating. (18-27)

Electrospun nanofibrous membranes (ENMs) offer desirable properties for virus adsorption. By functionalizing the nanofibers to promote virus removal by adsorption. Because no chemical is released in the water, there is no risk of by-product formation. In addition, the highly porous nature of ENMs with their interconnected pore structure could offer minimal resistance to flow, thus combining low energy requirements to operate these membranes with a high removal efficiency due to their very high surface area. This would make ENMs more cost-efficient than existing competing technologies.

Functionalizing ENMs with compounds that promote hydrophobic and electrostatic interactions could result in membrane systems with high removal efficiencies that meet the 4-log removal outlined in guidelines (18,26). The incorporation of virucidal and bactericidal agents could further enhance the membrane effectiveness by guaranteeing waterborne pathogens inactivation upon adsorption onto the nanofibers. Quaternary ammonium compounds have been shown to be excellent at promoting electrostatic interactions due to their positive net charge. They already enjoy
wide use in household cleaning products and the food service industry as disinfecting and sanitizing agents. One commonly used is dodecyldimethylbenzylammonium chloride (DDBAC), which could be incorporated for its properties in ENMs. Also, fluoropolymers such as polyvinylidene fluoride (PVDF) exhibit remarkable hydrophobic properties due to the high number of fluorine atoms per monomer (28-35). Finally, titanium dioxide is well known for its anti-viral and anti-bacterial properties (caused by photocatalytic reactions, and membrane disruption), and widely available; and silver-doped titanium dioxide in particular has been shown to excel in this domain by enhancing the photocatalytic properties of titanium dioxide (36-39).

1.2 Hypotheses

This project was guided by the following hypotheses:

- An ENM that promotes hydrophobic interactions in conjunction with a contact-inactivating virucidal nanoparticle such as TiO$_2$ could exhibit a high MS2 LRV through adsorption. This would be based on the hydrophobicity of MS2 capsids that would adsorb to the hydrophobic nanofibers surfaces and then be inactivated upon contact.

- An ENM that promotes electrostatic interactions in conjunction with a contact-inactivating virucidal chemical compound such as the quaternary ammonium salt DDBAC could exhibit a high MS2 LRV through adsorption. This would be based on the negatively-charged MS2 capsids that would adsorb onto the positively-charged nanofiber surfaces and then be inactivated upon contact.
2.0 Literature Review

2.1 Viruses in Water

Viruses are naturally occurring in the environment. They can be either airborne (e.g., droplets of pathogens expelled into the air from a body by coughing) or waterborne (e.g., fresh water contaminated by feces). They reproduce only by infecting host organisms. Once a virus infects the host, the DNA replication mechanism of the host’s cells is hijacked to replicate the genetic information of the virus, thus ultimately killing the infected cell. In this way, viruses tend to be highly specific to their target organism and thrive in areas densely populated by their target. Viruses enter the environment from a carrier host that may or may not be pathogenically affected by them (4,9). In addition to a high degree of persistence (viruses can survive for several weeks in a water environment (9)), they tend to have a low infective dose and a very high level of shedding following host infection.

Waterborne viruses can be found in nearly all water sources, including lakes, rivers, seawater, and drinking water. They are not only a direct potential threat to human through drinking water consumption, but also to aquatic ecosystems and indirectly to human through the consumption of contaminated organisms or their presence in recreational waters.

Viruses of interest in water include norovirus, rotavirus, sapovirus, astrovirus, adenovirus, enterovirus, and JC virus (40). Enteric viruses are of particular interest in water treatment. They are most often transmitted through direct human-to-human contact or the consumption of drinking water contaminated by infected feces. Infected feces typically contain $10^5$-$10^{13}$ viruses per gram of stool (9). Viruses such as hepatitis A
and the Norwalk virus have been known to be the cause of numerous outbreaks of waterborne diseases over the years (18). Enteric viruses are in particular associated with gastroenteritis (1.2 million deaths in 2012(7)), but also various other diseases such as conjunctivitis and respiratory illnesses.

Waterborne enteric viruses are a major public health concern due to their low infective doses, strong environmental persistence, and widespread presence in the environment. They have often been reported to be the sources of occasional severe outbreaks that have impacted entire communities (3) with significant adverse economic impacts. In fact, the World Bank estimates that lack of access to safe water across the globe is responsible for USD$260 billion in economic losses annually (2).

A study by Qiu et al. looked at the efficiency of human virus removal in a wastewater treatment plant in Edmonton, Canada (40). They collected samples after each of the five treatment steps (primary sedimentation, secondary treatment, membrane ultrafiltration, UV disinfection, and chlorination) over a duration of 16 months. They found a high prevalence of viruses in raw wastewater and different efficiencies in virus removal at various treatment steps. The efficiencies varied between 1.0 to 4.9 log reduction in virus count for UV and ultrafiltration treatments, respectively. This translated into viruses being detected in 95% of UV-treated wastewater effluents but near virus-free status for wastewater that had been treated by ultrafiltration (40). The results of this study demonstrate the prevalence of viral pathogens in raw as well as treated wastewater.

Grabow et al. looked at the presence of viruses in water samples from drinking water supplies in South Africa over a period of two years (41). They reported that 23%
of the 413 drinking water samples and 73% of the raw water samples contained viruses. In particular, 17% of the drinking water samples contained enteroviruses. Their findings also highlighted the large temporal variability in drinking water quality, and consequently the importance of finding better ways of assessing and achieving virus removal in water treatment plants (41).

### 2.2 Environmental Regulations and Environmental Assessment for Viruses

Despite a long history of associating viral infections with drinking water supplies, causal links between the two have predominantly been established based on epidemiological data, and not direct viral detection in drinking water supplies. This is simply due to the fact that most waterborne enteric viruses were not detectable by technologies available in the past. Because of this technological shortcoming in virus detection, legislation for drinking water has been based on indicator organisms (8). The current acceptable risk level when considering drinking water, as determined by the WHO, is one infection/10,000 consumers/year (7). In terms of disability-adjusted life year (DALY), the acceptable risk is capped at $10^{-6}$ DALY/person/year by the WHO and Health Canada (42). This is the benchmark from which the minimum 4-log removal target by water treatment plants was derived (42). This review will focus on drinking water quality standards and guidelines in Canada, and more specifically Ontario, and relevant comparisons to those in other legislations including the U.S., the European Union (E.U.), and Australia.

It should be noted that there is a subtle but important difference between standards and guidelines from a legal perspective. Standards are legally binding and
enforceable, which means not meeting them should result in actions being taken to ensure compliance. On the other hand, guidelines are voluntary targets that a given jurisdiction may aim for but not achieve, thus not meeting them may not necessarily result in any remedial actions. This is why standards tend to be expected to provide a better level of protection for public health and the environment. The U.S. and Europe use national and supranational standards, respectively, for drinking water quality. Indeed, in the E.U.’s case, all countries must meet the minimum standards as set by the E.U.’s Drinking water Directive. Meanwhile, Canada and Australia rely on weaker national guidelines that are not applied equally between provinces and states. This being said, the Guidelines for Canadian Drinking Water are legally binding in some provinces and municipalities, due to their incorporation into provincial regulations and operating permits for water treatment facilities respectively. Such cases mitigate the potential concerns resulting from the general non-enforceability of guidelines, but they do not address concerns of consistency, transparency, and enforcement. This means that while all provinces and territories and have drinking water regulations, they do all provide the same level of legal protection to drinking water consumers (10,11).

In industrialized nations, it is widely accepted by experts that the greatest water consumption risk stems from waterborne pathogens (i.e., viruses, bacteria, and protozoa). This is because they can have immediate and severe health effects, they are transferable from one human to another, and a single pathogen can be the source of an illness. On the other hand, chemicals tend to be a health concern when there is long-term exposure to them, and depending on their amounts present in the resulting drinking water. Water turbidity is an important parameter of concern when addressing
waterborne pathogens. It is a result of the presence of fine suspended matter such as clay or silt that can harbor pathogens, protect them from disinfection treatments, and interfere with their detection (10,11).

None of the nations reviewed in this section have set maximum allowable concentrations (MACs) for most waterborne pathogens due to technical reasons. In response to that, some have established outcome-based standards for drinking water treatment. For instance, the USEPA requires filtration (or an equally effective method such as UV or ozonation) in all water treatment plants that use surface water or groundwater contaminated by surface water as a source, with a 4-log removal result for viruses (13,14). In Canada, there are no outcome-based standards and only five provinces (Nova Scotia, Quebec, Ontario, Saskatchewan, and Alberta) require the filtration of surface water. In the E.U. and Australia, filtration is recommended but not legally required. All the jurisdiction reviewed have standards or guidelines for coliform bacteria and turbidity, and they all have the same MAC for *E. coli* and fecal coliform bacteria in drinking water systems set as none detectable per 100 mL. The Canadian guidelines apply this MAC if only one sample is taken monthly, but if multiple samples are taken then 10% of them can contain up to 10 total coliforms per 100 mL. Overall, the U.S. have the most rigorous standards for protecting public health and the environment from microbiological contaminants due to their outcome-based standards, while Canada has weaker guidelines with often no legal requirement for effective treatment (10,11).

In Ontario, the design of a drinking water disinfection and pre-disinfection treatment must be specific to the water source that will be used. In addition to being
characterized for its water quality parameters, the raw water supply should be assessed for its variability and vulnerability. From there, the appropriate CT tables should be used to determine disinfectant dosage if chemical disinfection applies. When the raw water supply is non-contaminated ground water, the treatment must at a minimum include disinfection and provide a 2-log removal or inactivation of viruses. On the other hand, when the raw water supply is surface water or surface water-contaminated ground water, the treatment process must at a minimum be as effective as a combination of chemically assisted filtration and disinfection processes, and provide a 4-log removal or inactivation of viruses, 3-log for *Giardia* cysts, and 2-log for *Cryptosporidium* oocysts. From these removal targets, at least 4-log removal or inactivation of viruses (0.5 log for *Giardia* cysts) must be obtained through disinfection (12).

### 2.3 Virus Adsorption

Virus response to changes in the environment (i.e., ionic strength, pH, temperature) has typically been characterized considering the virus as a single entity. In reality, it has been shown that viruses interact with suspended particles which form virus-particle complexes. A large body of research now exists on the interaction of viruses with solid surfaces (e.g. soil particles) in water and how that affects the virus response to environmental changes (18-21,24-27).

Having a better understanding of the mechanisms (i.e., hydrophobic and electrostatic interactions) that determine the kinetics of virus adsorption as well as the factors (i.e., virus and particle surface natures, and environmental parameters such as pH level, ionic strength and dissolved organic matter content) that influence them can enable the
scientific community to control virus behavior in the environment through changes in environmental properties. It can also enable the development of better technologies for virus removal.

2.3.1 DLVO Theory

Figure 2.3.1.1 Schematic representing the double layers about a virus and a solid (18)

Viruses tend to be negatively charged since their isoelectric point is usually lower than that of environmental waters (18,21,23). According to DLVO theory (Figure 2.3.1.1) -named after Boris Derjaguin, Lev Landau, Evert Verwey, and Theodoor Overbeek- a negatively charged virus (due to its surface ionizing residues) that is suspended in water is enveloped by a Stern layer composed of cations. As the distance from the virus surface gets larger, this layer transitions into a diffuse layer. This diffuse layer contains an excess of cations and a depletion of anions, as cations are attracted to the negatively charged
virus and anions are repulsed by it. The diffuse layer thickness depends on the ionic strength of the water. The higher the ionic strength, the more densely packed in ions the diffuse layer. This can accentuate electrostatic repulsion between two similarly charged surfaces or electrostatic attraction between two oppositely charged surfaces. The solid surface also presents a Stern layer followed by a diffuse layer, however its net surface charge depends on the nature of the material as well as the environmental conditions. This means that depending on surface charge, the electrostatic interaction between the virus and the solid can be either attractive or repulsive. It must also be noted that the ionizing residues and the surface hydroxyls have been speculated to be responsible for the net virus and surface charges, respectively (33).

The non-applicability of DLVO theory in unfavourable attachment conditions stems from its assumptions. DLVO assumes that the objects of interests are smooth bodies with ideal geometries and uniform properties. In reality, solid and virus particles are irregular with rough surfaces, and possess heterogeneous surface composition and charge (38).

2.3.2 Other Effects

2.3.2.1 Hydrophobic Effect

Hydrophobic interactions are not accounted for in DLVO theory (19,24,27). These interactions can play a dominant role in virus adsorption at higher pH values. This is because under this condition, most surfaces are negatively charged (the point of zero charge is in the 5-7 range) and most viruses are negatively charged too (the isoelectric point is low in the 3-5 range usually), which means there is a strong electrostatic repulsion (33) between surfaces and viruses. Hydrophobic interactions arise not from natural
attractive forces between hydrophobic particles. They are a result of the creation of a thermodynamically unfavorable interaction between hydrophobic substances and water. Thus the system tries to diminish its entropy and readjust itself through the agglomeration of hydrophobic particles.

### 2.3.2.2 Blocking

Blocking is the phenomenon that was not taken into account by the Langmuir adsorption model. It occurs when two virus particles with repulsive electrostatic interactions try to adsorb to adjacent sites. The first adsorbed virus particle will prevent another virus from adsorbing next to it due to the strong repulsion created by the overlap of their double layers. This phenomenon decreases the overall attachment rate to the surface. A low environmental ionic strength is often responsible for this, as the depletion of ions in the bulk water increases the size of the diffuse layer, and this increases the possibility of virus double layers overlapping and repulsing one another (20,27).

In conclusion, kinetic studies have been found to be preferable to equilibrium adsorption studies to better understand adsorption mechanisms in environmental waters. The DLVO theory has a strong case for being able to generally well predict virus adsorption behavior, but it faces certain shortcomings in the rarely encountered unfavorable attachment situations where adsorption is limited by virus mass transport to the surface. The hydrophobic effect and the blocking phenomenon are important interactions to consider in the virus adsorption model that are not part of the DLVO theory.
2.3.3 Environmental Parameters Affecting Virus Adsorption

2.3.3.1 Virus nature

The nature of a virus has a significant effect on its adsorption behavior in a defined set of environmental conditions. In a study by Gerba et al., it was found that viruses could be classified into two main groups based on their sorption behavior (18). Group I viruses tend to be more sensitive to pH and organic matter concentration changes due to their low isoelectric point, and their adsorption behavior can usually be predicted by DLVO theory. Group II viruses have higher isoelectric points, and their behavior is harder to predict and model (18,19,26).

2.3.3.2 Surface nature

In terms of surface nature, the most important parameter about it is its net surface charge. The higher the point of zero charge of a material, the better it is at adsorbing viruses since its consequently net positive charge will electrostatically attract the net negative charge of the virus. However, this effect is not always clear due to the high surface heterogeneity of particles in terms of composition and charge: some areas may concentrate one type of charge and thus contradict sorption predictions (18,19,26).

2.3.3.3 pH level

As previously mentioned, the pH level mainly determines the net surface charge of the virus and solid particles based on their isoelectric point (IEP) and point of zero charge (PZC), respectively. If the pH is above the respective IEP or PZC, the net surface charge of the particle is negative, and if it is below it the net surface charge is positive (18,19,26,27).
2.3.3.4 Ionic strength

As predicted by DLVO, high ionic strengths have been found to increase virus sticking efficiency by reducing the length of the diffusive layer which reduces electrostatic repulsions. There is a certain class of salts that can in addition play a role on hydrophobic interactions. These are called antichaotropic salts: they accentuate hydrophobic interactions by increasing the order in the water structure through the strengthening of hydrogen bonds between polar molecules (18,19,22,26).

2.3.3.5 Dissolved organic matter

Dissolved organic matter plays a complex role in affecting virus adsorption. It can disrupt hydrophobic interactions the same way surfactants would. It can also compete with viruses for binding sites. However, bonded organic matter can also increase virus adsorption by providing additional hydrophobic binding sites. The enhancing and attenuating effects of dissolved organic matter are suspected to be the main reason for large uncertainties in estimating the virus removal efficiencies (18,19,26).

In conclusion, virus and surface natures, pH level, ionic strength, and dissolved organic matter are important material and environmental parameters affecting virus adsorption. They must be considered when trying to elucidate the adsorption mechanisms in a defined environment. However, it is important to keep in mind that ultimately one must think in terms of the two main interactions that they directly affect: electrostatic and hydrophobic interactions (19).
2.4 Water treatment processes

Current virus removal and inactivation methods in water treatment applications are known to be effective, but they all present significant drawbacks that vary considerably in nature from one method to the other. It is important to note that none of these methods operate independently in a water treatment system, but they are rather integrated as part of a whole to reach the target 4.0-log credit. In all cases, it has been shown that the most substantial part of virus inactivation occurs during disinfection, and physical barriers tend to act mainly as “cleaners”: they remove substances that would otherwise interfere with the disinfection (16,43,44). This is why it is important to understand the relative importance of each barrier in a treatment system in order to optimize its performance for virus removal and inactivation.

In some systems, there can be several redundant barriers that ensure that if one fails then adequate treatment is still achieved. In others, the failure of a single barrier could lead to a waterborne disease outbreak. This depends on the margin of safety provided by the total log removal credits of the system: the higher it is above the minimum threshold, the higher the system’s capacity for redundancy.

Enteric viruses are difficult to remove from raw water due to their small size and relative ease of passage through filtration barriers, but disinfection technologies (chemicals or UV doses) can effectively inactivate viruses at relatively low dosages. In addition, source water quality should be characterized based on field water samples while taking into account seasonal conditions (e.g., spring runoff, storms). Treatment requirements can be met through a combination of physical and/or chemical treatment
steps. In this way, the virus log credits for separate barriers can be added up to determine the overall reduction for the treatment process (15,42).

Table 2.4.1 summarizes the log removal or inactivation of viruses by various treatment processes as reported in the literature.

Table 2.4.1 Log removal/inactivation of viruses by various treatment processes (45)

<table>
<thead>
<tr>
<th>Process</th>
<th>Removal/inactivation (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary treatment</td>
<td></td>
</tr>
<tr>
<td>Grit chamber</td>
<td>0-0.3</td>
</tr>
<tr>
<td>Fine screen</td>
<td>0.1-0.2</td>
</tr>
<tr>
<td>Secondary treatment</td>
<td></td>
</tr>
<tr>
<td>Activated sludge</td>
<td>0.7-2.9</td>
</tr>
<tr>
<td>Trickling filter</td>
<td>0.0.82</td>
</tr>
<tr>
<td>MBR</td>
<td>3.4-6.8</td>
</tr>
<tr>
<td>Tertiary/advanced treatment</td>
<td></td>
</tr>
<tr>
<td>Chemical coagulation-alum, iron salts</td>
<td>1-2.86</td>
</tr>
<tr>
<td>Microfiltration (0.1 μm)</td>
<td>0.2-5.1</td>
</tr>
<tr>
<td>Ultrafiltration (0.01 μm)</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Nanofiltration (0.001 μm)</td>
<td>&gt;5.4</td>
</tr>
<tr>
<td>Reverse osmosis (0.0001 μm)</td>
<td>&gt;6.5</td>
</tr>
<tr>
<td>Disinfection</td>
<td></td>
</tr>
<tr>
<td>Chlorination</td>
<td>0.81-2.8</td>
</tr>
<tr>
<td>Ozonation</td>
<td>0.24-6</td>
</tr>
<tr>
<td>UV radiation</td>
<td>1.43-6</td>
</tr>
</tbody>
</table>
2.4.1 Pre-treatment

Pre-treatment for drinking water processes generally receives no log removal or inactivation credits for viruses or other waterborne pathogens. However, some important considerations must be taken into account in that stage of the treatment. These include the need to prevent or minimize the proliferation of fouling organisms and potential external contaminations of the system, and the need to have a water quality suitable for the subsequent stages of the process (i.e., removal of large debris). (44, 45)

When pumping the raw water supply, this should be done directly from the source and sent to holding tanks through pipes. The containment infrastructure should be built of appropriate materials to minimize fouling and prevent accidental contamination of the supply. Screening of the raw water if it comes from a surface source is the first treatment step. Screen filters are used to remove large debris such as sticks, leaves, garbage, and other large elements that could severely interfere with the operation of the subsequent treatment units. Ground water generally does not require screening. Pre-chlorination is employed to minimize the fouling of pipes and operation units by microorganisms. However, this practice has been largely abandoned due to the issues associated with employing chlorine, especially when the raw water supply contains large amounts of organic matter. (45, 46)

2.4.2 Coagulation Processes

Conventional filtration provides three sequential stages including coagulation followed by flocculation, sedimentation, and then filtration. Alternatively, the sedimentation step can be skipped which is called direct filtration. The latter is given
only 1.0 log removal credit for viruses compared to 2.0 for most conventional filtration systems (see Table 2.4.2.1).

Coagulation is a critical step to ensure that particles are removed by the subsequent filtration. Adding a chemical coagulant to the influent water produces flocs that are agglomerations of suspended particles held together by adsorptive interactions. The particles can be inorganic such as clay and silt, or organic such as algae, bacteria, protozoa, viruses, and NOM. These flocs are then removed from the water using gravity sedimentation, a sludge blanket or dissolved air flotation. For the filtration stage, granular media filters tend to be the most commonly used as they have been traditionally used in water treatment and are relatively cheap and easy to maintain. They can be made from a variety of materials, including silica sand (slow/rapid sand filtration), and granular activated carbon (GAC). They are used to primarily remove natural organic matter and micropollutants (44). A study by Hijnen et al. determined the capacity of GAC filters to remove MS2 virus, E. coli, and protozoan cysts (C. parvum and G. lamblia) from water at a pilot plant in the Netherlands (47). They found that while for cysts the removal was significant (1.3-2.7 log) and for E. coli it was more limited (0.1-1.1 log), there was no detectable removal of MS2. In another study by Asami et al., the removal efficiency of indigenous viruses, pepper mild mottle virus (PMMoV) and JC polyomavirus (JC PyV), by coagulation-sedimentation (CS) followed by rapid-sand filtration (RSF) was assessed in a drinking water treatment plant in Bangkok, Thailand (48). The removal efficiency of PMMoV and JC PyV was considerably higher by CS than by RSF (1.61 log10 vs 0.78 log10 for PMMoV; and 1.70 log10 vs 0.59 log10 for JC PyV; CS and RSF, respectively). These studies show that limited virus removal occurs
during CS in conventional filtration, and that the filtration stage provides very limited to no detectable removal efficiencies depending on the virus being assessed. However, slow sand filtration systems can achieve high efficiencies of up to 98%, and even 3-log when a coagulant was employed prior to the filtration stage (45). Their downside is that they have a large land footprint, require regular daily maintenance, and large amounts of filter media and manual labor for cleaning (15,44).

Table 2.4.2.1 Virus physical removal credits for various treatment technologies (42)

<table>
<thead>
<tr>
<th>Treatment barrier</th>
<th>Virus removal credit (log$_{10}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional filtration</td>
<td>2.0</td>
</tr>
<tr>
<td>Direct filtration</td>
<td>1.0</td>
</tr>
<tr>
<td>Slow sand filtration</td>
<td>2.0</td>
</tr>
<tr>
<td>Diatomaceous earth filtration</td>
<td>1.0</td>
</tr>
<tr>
<td>Microfiltration</td>
<td>No credit</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>Demonstration and challenge testing; verified by direct integrity testing</td>
</tr>
<tr>
<td>Nanofiltration and reverse osmosis</td>
<td>Demonstration and challenge testing; verified by integrity testing</td>
</tr>
</tbody>
</table>

2.4.3 Disinfection

2.4.3.1 Chemical Disinfection

Chlorine, chloramine, chlorine dioxide, and ozone are common chemical disinfectants employed for treating drinking water. They are typically applied as a tertiary treatment, which means that it is performed after primary and secondary treatment processes which remove particles and organic matter. This ensures optimal
efficiency of the disinfectants, and minimizes the formation of disinfection by-products (DBPs). The efficacy of chemical disinfectants is predicted based on the residual concentration of disinfectant in the effluent, the temperature, the pH, and the contact time.

Chlorination is the most widespread method of disinfecting water supplies as it is the most accessible one: chlorine compounds are cheap and widely available. It is also the oldest water disinfection method employed at the industrial scale, being implemented and permanently used since the turn of the 20th century (46). It is widely used across North America, but in Europe the trend has been to phase out this treatment technology for safer ones (16).

Chlorine kills pathogens through the oxidation of organic molecules. However, for its significant advantages, chlorination also presents important drawbacks. Chlorine can react with naturally occurring organic matter present in the water, resulting in the formation of suspected toxic/carcinogenic DBPs such as trihalomethanes (THMs) and haloacetic acids (HAAs) (42,45). Their formation should be minimized as much as possible in a given treatment without compromising the disinfection effectiveness. Also, the handling of a strong oxidizing agent like chlorine presents significant safety concerns for the operators at water treatment plants. Despite this, the WHO has stated that the health risks posed by DBPs are outweighed by the risks associated with inadequate disinfection of drinking water supplies (15). In a study by Dryden et al., it was shown that chlorination without pre-treatment of the effluent resulted in a 4.3 to 4.5 log inactivation of viruses with a chlorine residual of 4 mg/L (43). With a full tertiary treatment system, log removals of over 5 were reliably achieved (43).
Ozonation is an advanced oxidation process (AOP) quite prevalent in Europe and increasingly popular in North America. It consists of bubbling ozone through water, which will damage the genetic material of viruses and bacteria, and break down all other harmful organic substances. The disadvantages of ozonation are that it is an energy-intensive process, this method leaves no residual ozone in the treated water to prevent its contamination (like chlorination does) as it travels through the distribution network, and it can produce DBPs that are only partially degraded in such a way that in some cases they are more toxic or carcinogenic than the parent substance. In a study by Dryden et al., it was found that a 10mg/L ozonation dose resulted in 5.2-5.4 virus log removals (43). This result is only slightly better than what is obtained through chlorination, meaning the two methods are comparable in terms of their efficiencies, but ozonation produces fewer disinfection by-products (16,43).

The CT concept corresponds to the product of C (the residual concentration of disinfectant in mg/L) and T (the disinfectant contact time, in minutes) (42). It is a measure of the efficacy of chemical disinfectants. As it can be observed from the CT values in Table 2.4.3.1 ozone is the most effective disinfectant, followed by free chlorine, chlorine dioxide, and finally chloramine which is relatively inefficient compared to the other chemicals. However, free chlorine provides the advantage of a secondary disinfectant residual over ozone (it decays rapidly after being applied), thus allowing water to remain potable for longer periods of time following treatment. Hepatitis A virus has been shown to be overall the most resistant enteric virus to chlorine dioxide and ozone. It is also more resistant to free chlorine than rotavirus and adenovirus (40). On the other hand, the resistances of coxsackievirus B5 and poliovirus seems to vary
significantly, thus further research is needed to assess this. This is why hepatitis A virus has been used as the reference for virus disinfection targets (10,12,42).

Table 2.4.3.1 Comparison of CT values for 99% (2-log) inactivation of selected enteric viruses by various disinfectants at 5-15°C (42)

<table>
<thead>
<tr>
<th>Virus</th>
<th>CT values for 99.99% (4-log) inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free chlorine (Cl₂) pH 6-7</td>
</tr>
<tr>
<td>Poliovirus 1</td>
<td>1.1-6</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>0.01-0.05</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>0.7-1.18</td>
</tr>
<tr>
<td>Coxsackievirus B5</td>
<td>1.7-12</td>
</tr>
<tr>
<td>Adenovirus 40</td>
<td>0.02-2.4</td>
</tr>
</tbody>
</table>

**2.4.3.2 Ultraviolet (UV) Light Disinfection**

UV light is an AOP and an alternative method to the use of chemicals for disinfection. The UV dose applied to a system, also called fluence, is expressed in mJ/cm². UV light has been shown to be highly effective at inactivating viruses when applied after particle removal barriers (e.g. filtration systems) in order to avoid shielding effects by suspended macroparticles and allow better penetration of the light through the water (42). Turbidity has been demonstrated (for most particles) to have no significant effect on the UV-dose response of microorganisms up to a critical value of 10 nephelometric turbidity units. However, humic acid particles and coagulants in particular
have been shown to have significant negative effects on UV disinfection efficacy (42,45).

For most enteric viruses, a fluence of 15-30 mJ/cm\(^2\) is sufficient to reach a 4-log inactivation target in buffered demand-free water. However, adenoviruses are notoriously more resistant to UV light. A relatively high dosage of approximately 186 mJ/cm\(^2\) is needed to reach this efficiency in the same conditions. While the mechanisms for this higher resistance are not fully understood, it appears that double-stranded DNA viruses such as adenoviruses are more resistant to UV radiation than single-stranded RNA viruses such as hepatitis A viruses. This resistance may be a result of natural properties of the viruses, or of the ability of viruses to repair UV-induced damage to their genetic structure with the help of host cell enzymes through the process known as photo reactivation (17,42,45).

A UV dose of 40 mJ/cm\(^2\) is typically applied for most water supply systems in Canada as it is sufficient for most enteric viruses (42). In most cases, a multi-disinfectant strategy is preferred. UV systems are often combined with chemical disinfection (e.g. chlorine) or physical removal barriers in order to meet the minimum 4.0-log credit requirements, in particular when there is a concern with the more resistant adenoviruses.

2.5 Membrane Filtration

Conventional membrane processes in water treatment are classified according to their pore size. Microfiltration membranes (0.1-10 microns pore size, 15-60 psi operating pressure) are capable of removing suspended particles and bacteria.
Ultrafiltration membranes (0.01-0.1 microns pore size, 30-100 psi operating pressure) can remove macromolecules and most viruses. Nanofiltration membranes (1-10 nm pore size, 90-150 psi operating pressure) are typically used for removal of divalent ions. Reverse osmosis membranes (0.1-1 nm pore size, up to 1200 psi operating pressure) remove monovalent ions. The smaller the pore size, the higher the required operating pressure for the membrane is.

Microfiltration (MF) membranes receive no virus removal credit (see Table 2.4.2.1) as their pore size (0.1-10 microns) is too large to retain any viruses. Nonetheless, some studies have shown that 4-log removal rates can be obtained if MF is preceded by coagulation (direct filtration), in which case the system could be eligible for virus removal credit (42,49).

Ultrafiltration (UF) membranes typically have pore sizes around 0.01 micron and operate by size exclusion. UF can efficiently remove nearly all viruses from water due to the smallest known viruses being around 0.02 microns in size (50). While water treated by UF can be considered to be safe for consumption in terms of pathogenic virus presence, these membranes must be preceded by additional treatment steps (e.g., pre-chlorination) to ensure their operational reliability (43,44,51). These pre-treatments help balance the water pH which can significantly affect the membrane performance, and remove other contaminants which may cause accelerated and severe fouling of the UF membrane.

The virus removal credit given to a UF membrane must be obtained by demonstration of its capacities by challenge testing and direct integrity testing (see Table 2.4.2.1). Despite the efficiency of UF membranes in virus removal, they are not
widely used at the industrial scale in water treatment to this effect due to their high operating pressures, which entail high energy requirement and consequently high operating costs.

Membrane bioreactors (MBRs), an integration of activated sludge and membrane filtration, have been shown to be able to remove viruses by mechanisms of virus attachment to mixed solids particles, interception by the membrane and membrane cake layer, and inactivation by enzymatic breakdown. Depending on the operating conditions and the nature of the virus, log removals between 3.0 and higher than 6.0 were demonstrated. However, MBR systems present many significant challenges including need for aeration and are relatively costly (45,52).

2.6 Electrospun Nanofibrous Membranes (ENMs)

Electrospinning of polymer solutions is a well-known conventional method of producing nanofibers, which are of great interest due to their applicability in a wide range of fields. They generate great interest in environmental applications such as filtration and catalysis, as well as in optical applications such as photocatalytic devices and colorimetric sensors (53). In particular, electrospun nanofibrous membranes (ENMs) are one of the new frontiers in water treatment research due to their unique properties and the potential advantages (e.g., higher surface area, lower TMP, interconnected pore structure, potential for functionalization) they offer over conventional membranes and other water-purifying technologies (53,54).

As it can be seen from figure 2.6.1, an ENM is formed by the random deposition of nanofibers on a collector plate. The polymer solution consists of a high molecular weight polymer dissolved in a volatile and conductive solvent. As it is subjected to a
high voltage, a drop forms at the tip of the syringe containing the solution. This drop forms what is called a Taylor cone. From the tip of this cone, a jet of polymer solution is drawn by the electrostatic forces acting between the collector plate and the solution. As this jet travels through the air, it elongates into nanofibers and the solvent evaporates. The nearly-dry nanofibers deposited on the collector plate forms a non-woven structure, whose thickness can be controlled simply by the duration of the electrospinning process.

Figure 2.6.1: Electrospinning process and setup
Compared to conventional asymmetric membranes produced by the Loeb-Sourirajan phase inversion method, ENMs possess very high specific surface areas and porosities that can exceed 90% due to the interconnected nature of their uniform pores (55). This in turn allows ENMs to have much higher water fluxes, which can be sustained due to their excellent mechanical properties, and thus they would require considerably lower operating pressures to process equivalent amounts of water. This could make ENMs more cost-efficient to operate than conventional membranes. In addition, their high surface to volume ratio makes them good candidates for applications as filters or adsorbents.

The versatility of ENMs stems from the ability to control a wide range of process and solution parameters that can affect the morphology and properties of the nanofibers, thus making them highly tailorable to specific applications. Parameters such as the polymer nature (hydrophobic or hydrophilic), the conductivity of the solution, the nature of the solvent, the method of functionalization (pre-treatment of the solution or post-treatment of the nanofibers), and the electrospinning conditions can be modified to obtain the desired product (54). This makes ENMs an excellent candidate to attempt removing viruses from water by adsorption.

2.7 Functionalizing compounds: Quaternary Ammonium Compounds (QACs), Titanium Dioxide, and Perfluorocarbons

Quaternary ammonium compounds are widely used as disinfectants due to their excellent antibacterial and antiviral properties (29). They are membrane-active agents that disrupt the cytoplasmic membranes of bacteria and the lipids of virus capsids.
Selecting the right QAC for the particular pathogen of interest is of great importance. The general chemical structure of a QAC consists in a nitrogen atom covalently bonded to four R-groups which may be the same, different, or even connected among themselves (see Figure 2.7.1). Hence, a QAC is always positively charged independently of the environmental pH. QACs can possess a wide variety of chemical structures, which has allowed them to be used more effectively over the years in a wide variety of applications, including against viruses containing no lipids (e.g., noroviruses) (29). There is concern that their overuse could induce antibiotic resistance in viruses and/or bacteria, but this must be balanced with the public health benefits of using these compounds. Presently, there appears to be no serious reason to restrict the usage of these compounds (29).

![Chemical structure of a Quaternary Ammonium Cation](image)

**Figure 2.7.1** Quaternary ammonium cation

QACs have been found to be particularly effective at preventing biofouling. They disrupt adsorbed bacteria on surfaces by direct contact-killing, thus preventing them from forming biofilms. Similarly, their positive charge attracts negatively-charged viruses, leading to their adsorption and subsequent deactivation.
Studies have been performed investigating the impact of a wide range of QACs such as dodecyldimethylbenzylammonium chloride (DDBAC), poly [oxyethylene (dimethyliminio) ethylene -(dimethyliminio)ethylene dichloride] (WSCP), and benzyltriethylammonium chloride (BTEAC) on biofouling prevention and virus removal. In a study by Zhang et al., they investigated the impact of a PVDF microporous membrane surface-functionalized with DDBAC on biofouling (35). It was found that DDBAC was not only able to drive surface segregation with a high structural stability, but it also had a prolonged sustained anti-biofouling effect even after a dead bacterial layer had formed on the surface. This was hypothesized and confirmed to be due to indirect contact-killing effect of QACs, which in addition retarded biofilm growth (35).

Similarly, Daels et al. tested the anti-biofouling effectiveness of WSCP used as a functionalizing agent in an ENM. The prepared ENM had 5 wt% WSCP. It was shown to be very efficient with a bacterial log removal value of 5.2. Kim et al. tested the effectiveness of BTEAC incorporated into an ENM also for the same effect, and obtained 3-log removals of filtered bacteria (30). Limited number of research that has been done on applying QACs for virus removal has shown them to be efficient in this respect (31,32). Mi et al. investigated the use of water-stable quaternized hydrophilic chitosan nanofibers for virus adsorption (31). They found high log removal values between 3.3 and 4.2 depending on the virus tested, with the sole contributing factor to the adsorption being the positively-charged ENM surface. However, chitosan nanofibers swell in contact with water, and thus present a challenge for prolonged use in water in environments where it is critical such as a wastewater treatment plant. Sato et al. used positively charged nanofibers to attempt virus removal, but instead of using QACs they
embedded positively-charged cellulose nanofibers into the ENM (32). The results were a 6 log removal value of viruses. This further indicates the importance of electrostatic interactions in virus adsorption to solid surfaces in water. However, the process of infusing cellulose nanofibers into an electrospun scaffold results in a lengthy and complex ENM fabrication procedure.

Virucidal agents that can deactivate viruses through other means than adsorption have shown promise too in water treatment applications. Titanium dioxide and silver are the most promising compounds to that effect (36,39). These agents can serve a dual purpose as they possess bactericidal properties as well (34,37). Titanium dioxide can adsorb viruses, but it also exhibits photocatalytic activity when exposed to light. This translates into the release of hydroxide free radicals that can damage the virus genetic material, thus inactivating them. Silver ions have been shown to disrupt bacterial cell membranes, while silver nanoparticles bind viruses and damage their genetic material.

Silver and titanium dioxide nanoparticles have been tested independently or in conjunction with their virucidal and bactericidal effects in water treatment by several research groups. Zhang et al. prepared ENMs functionalized with silver nanoparticles for antimicrobial properties, and obtained up to 7 log removals after 30 mins of contact time (34). On the other hand, Nguyen et al. developed a microporous membrane coated with silver and titanium dioxide nanoparticles (37). The silver acted as an antimicrobial agent while the titanium dioxide as a silver-regenerating agent by decomposing organic matter accumulated on the membrane surface. A 67-72% recovery of the initial water flux was obtained by this Ag/TiO₂ coated membrane, compared to only 33% for the
control membrane. These results indicate the potential of these agents not only as bactericidal but also as flux loss mitigation compounds due to their self-cleaning properties when used together.

Xiang et al. investigated the use of titanium dioxide nanoparticles on flat membranes for virus removal in drinking water (36). It was found that equilibrium adsorption was reached in 60 mins only, and that high removal efficiencies of 3.88-6.40 log removal could be obtained. Liga et al. tested silver doped titanium dioxide nanoparticles for virus removal in drinking water treatment (39). They found that this doping resulted in a more than 5-fold increase in virus inactivation rates compared to the base titanium dioxide material or leached silver ions. Although the leaching of silver ions from the doped product certainly contributed to the increased inactivation rate, the conclusion was that nanosilver particles increased the photocatalytic activity of the titanium dioxide, thus resulting in a significantly larger release of hydroxyl free radicals.

Perfluorocarbons (PFCs) are organofluorine compounds that contain only carbon and fluorine. Due to the very electronegative nature of the fluorine atom, its presence in PFCs can lend them hydrophobic or superhydrophobic properties depending on the number of hydrogen atoms substituted by fluorine in the monomer unit. This means that under environmental water pHs, the hydrophobic effect of such compounds could be very useful for promoting virus adsorption to the membrane surface (32,35,36).
2.8 Gaps in Literature

In addition to the predicted benefits of ENMs over current technologies, the novelty and contribution of this research would be a virus removal membrane that attempts to combine multiple mechanisms that promote virus adsorption and inactivation: electrostatic interactions, hydrophobic effect, and virucidal properties. No published research to date has attempted for the purpose of removing enteric viruses to make use of all these interactions/properties in one integrative system such as ENMs that have the potential to be a game-changer in the water treatment industry.
3.0 Materials and Methods

3.1 ENM Preparation

The polyvinylidene fluoride (PVDF) powder (Mw>550,000) was obtained from Arkema (Kynar® 761); the TiO$_2$ nanoparticles were purchased (P25, ~25 nm diameter) from Degussa; the N,N-dimethylacetamide (DMAC) solution, the acetone solution, and the dodecyl dimethyl benzyl ammonium chloride (DDBAC) powder were purchased from Sigma-Aldrich.

ENMs were produced by electrospinning polymer solutions in an electrospinning machine (see Figure 3.1.1). Unless stated otherwise, these solutions consisted of PVDF as the base polymer in a 1:1 V/V DMAC/acetone solution. Based on desired solution concentration (12.5 w/v% unless stated otherwise), the appropriate amount of PVDF powder was dissolved in the solvent mixture by leaving the solution in a 70°C oven overnight after stirring it to disperse the powder.

For the TiO$_2$ polymer solution, TiO$_2$ nanoparticles were then added to a pre-determined on-mass-fiber % (OMF%) in desired amounts (2.5, 5, and 10 OMF%), and first stirred manually into the solution. OMF% is the theoretical wt% of functionalizing compound that would be present in a dried ENM assuming a perfectly dispersed or mixed solution (see Appendix – Section A). Then, the solution was sonicated in an ultrasonic tubular reactor (M/N UTR-600, Advanced Sonic Processing Systems) for 1 hour, and left to rest for 5 minutes. This was repeated 3 consecutive times. For the DDBAC polymer solution, the desired amount of DDBAC was stirred in the polymer solution, and the solution was left in the oven at 70°C for 2 hours.
Figure 3.1.1 shows a picture of the electrospinning machine employed and Figure 3.1.2 its schematic. The electrospinning setup was within an air-tight enclosure in the machine. A syringe-filled solution, after being placed on the syringe pump, was connected via a tube to a needle tip resting on a platform. This platform could be manually moved on an axis that is parallel to the length of the rotating collector drum. The rotating collector drum was wrapped in aluminum foil, on which the electrospun nanofibers were collected. Applied voltage and syringe pump speed (for the solution flowrate) could be adjusted on the control panel.

The solution was then inserted in a 30 mL plastic syringe to which was attached an 18 gauge needle tip. The syringe was placed horizontally on the syringe pump as can be seen in Figure 3.1.1. The syringe pump was calibrated to dispense the optimum volumetric flowrate of polymer solution (typically 2 mL/hr). The applied voltage was typically set at 25 kV, however, it was occasionally adjusted to achieve a more stable electrospinning operation. The distance between the syringe needle tip and the collector plate was set at a fixed distance of 20cm. The applied voltage was adjusted until a Taylor cone was formed at the tip of the needle. Under the optimum conditions polymer filaments are drawn and elongated across the electric filed and whip through the air before being collected and dried on the aluminium foil.

After about the desired amount of polymer solution had been spun (typically after ~9-10 hours of collection time), the resulting ENM was carefully peeled from the foil. In order to minimize the residual surface charge potential on the prepared ENM and to ensure its dryness, it was left air-drying for at least 24 hours before use (56). The edges of the ENM which presented a notably non-uniform thickness were trimmed.
Figure 3.1.1 Picture of the electrospinning machine used for ENM preparation

Figure 3.1.2 Schematic of the electrospinning machine used for ENM preparation
3.2 Membrane Characterization

The ENMs were examined using a field emission scanning electron microscope (Hitachi, SU-8230) for their morphology. Samples were coated with carbon prior to analysis and were imaged at a voltage of 1.5 kV, and a sample-to-detector distance of \( \sim 3.00 \text{mm} \pm 0.3 \) to adjust image quality for the depth of the sample. Nanofiber diameters were obtained from SEM images using ImageJ software.

Transmission electron microscopy was used to study and confirm the adsorption of MS2 bacteriophage to nanofiber walls. For this, the ENMs underwent a fixation and embedding procedure. Primary fixation of MS2 was performed on the sample, followed by primary washing, a secondary wash, staining with uranyl acetate, a third wash, and finally a series of dehydrations in ethanol before the sample was fixed in Spurr resin and cut with a microtome. Samples prepared for SEM followed a similar procedure however up to the stage of MS2 staining with uranyl acetate.

Membrane thickness was determined with a precision micrometer (Testing Systems Inc., Model No. 49-61).

X-ray Photoelectron Spectroscopy (Thermo Scientific, K-Alpha Compact XPS) was performed on all TiO\(_2\)-ENMs and DDBAC-ENMs tested to assess the relative amount of active functionalizing agent present at the surface of the sample. The surface mass \% for TiO\(_2\)/DDBAC was calculated (see Appendix – Section A) from the surface atomic \% raw data (see Appendix – Section C). It was obtained by dividing the relative mass of the functionalizing compound (TiO\(_2\) or DDBAC) by the total relative mass of all
the atoms detected. The relative mass of a given atom given by multiplying its surface atomic% by its molecular weight.

Thermogravimetric analysis (TGA Q500, TA Instruments, USA) was performed on the samples to determine whether the nanofibers contained or not the theoretical amount of TiO$_2$ they should (assuming a near-perfect dispersion of TiO$_2$ in solution was obtained from sonication). The heating was performed in steps of 10°C/min in a N$_2$ environment, up to a maximum temperature of 800°C, well above the decomposition temperature of pure PVDF.

Polymer solutions conductivities were measured with an electrical conductivity meter (Thermo Scientific Orion 0136SO Intrinsically Safe Portable Conductivity Meter).

BET (Quantachrome, Autosorb-1) measurements were performed on tested ENM samples using a degassing temperature of 100°C. It should be noted that BET measurements could not be obtained for DDBAC-ENMs due to the low melting point (~38°C as determined in-lab) of DDBAC. This low melting point prevented proper degassing of the sample and thus BET could not provide reliable measurements.

### 3.3 Virus Propagation and Enumeration Protocol

To prepare the primary virus stocks, MS2 virus (ATCC® 15597-B1™) and *E. coli* C-3000 (ATCC® 15597) pure freeze-dried strains were ordered from the American Type Culture Collection (ATCC). Glycerol stocks (1:4 V/V glycerol/distilled water) were prepared for both MS2 and *E. coli* and stored in a freezer at -80°C. From these stocks, substocks for monthly and weekly usage were prepared in order to avoid contamination of the primary stock using the following procedure. *E. coli* was incubated in a nutrient-
rich media such as LB broth and incubated at 37°C and 120 rpm shaking for 12-16 hours to multiply exponentially. Pre-melted semi-solid agar at ~55°C was inoculated with 0.1mL of E. coli broth and 0.1mL of MS2 stock, and was poured onto a solid agar plate. The plate was left to incubate at 37°C for at least 6 hours in order to allow the MS2 to propagate, following which 4-5mL of phosphate-buffered saline (PBS) was added to it. It was then wrapped in parafilm and left in the fridge at 4°C overnight, after which the viruses would have leached from the agar into the PBS. The PBS was poured into a syringe and filtered through a 0.22μm filter, from which the final virus titer was obtained.

The following enumeration protocol was used to quantify the amount of viruses, in plaque forming units per mL of solution (PFU/mL). A plaque forming unit represents a viable virus, thus non-viable viruses were not detected (see figure 3.3.1). First, an E.coli broth was prepared overnight using the procedure described earlier and serial dilutions of the sample of interest were prepared. Pre-melted semi-solid agar at ~55°C was inoculated with 0.1mL of E.coli broth and 0.1mL of the desired dilution, poured onto a solid agar plate, and left overnight to incubate at 37°C. For each dilution tested, efforts were made to prepare triplicate samples. The agar plates where the virus dilutions used were too low would have been clear and transparent, while plates where the virus dilutions used were too high presented a bacterial lawn. The right dilution for plate count was considered when individual plaques were readily identifiable (i.e., one plaque corresponds to one PFU). These plates typically contained a 30-300 plaques (see Figure 3.3.1). It should be noted that days 2 and 3 of the propagation could be
overlapped with days 1 and 2 respectively of the enumeration in order to save time, resulting in a 4-5 day propagation-enumeration joint protocol.

![Reference pure agar plate and agar plate presenting a bacterial lawn with circular transparent plaques]

**Figure 3.3.1 Reference pure agar plate (left) and agar plate presenting a bacterial lawn with circular transparent plaques (right)**

### 3.4 Membrane Filtration Experiments

The membrane testing set up (Figure 3.4.1) included a digital peristaltic pump (Masterflex® L/S digital pump), 1/6” anti-protein-adhesion tubing (Masterflex, Canada), and a 47mm polycarbonate in-line filter holder from Pall Laboratory to house ENMs (Figure 3.4.2).

The setup also consisted of a feed tank filled with distilled water spiked with a concentrated virus stock to the desired concentration (10^6-10^7 PFU/mL). The feed tank virus suspension was pumped through at flow rates of 4.5, 10, or 15 mL/min.
The transmembrane pressure was determined using two pressure gauges installed before and after the membrane unit.

Figure 3.4.1 Schematic of the ENM-testing setup

Figure 3.4.2 Picture of the in-line filter holder used as a support for ENM membranes, closed (left) and open (right)
The setup used for testing the performance of commercial NF membranes (Figure 3.4.4) included a stainless-steel membrane cell (CF042SS-FO, Sterlitech Co., US). This system is rated for 1000 psi and provides an active membrane area of 42 cm². The cell consisted of a membrane support plate for high pressure filtration applications, a feed spacer, and two O-rings for adequate sealing. The commercial NF membrane was a DK series membrane obtained from GE water (now Suez) and was made of Polyamide. Its molecular weight cut-off was 150-300 Da, with a nominal flux of 22 gfd at 100 psi. The pump was obtained from MTH Pumps (T41B AB). The digital pressure gauge (DPG9030-SK, Omega) was rated for 5,000 psi. The feed tank was filled with distilled water spiked with a concentrated virus stock to the desired concentration ($10^6$-$10^7$ PFU/mL). Since a TMP of at least 120 psi is required to properly operate this NF membrane (equivalent to a NF membrane), the valve downstream of the cell was shut off, and the valve upstream of the cell was carefully adjusted until a
reading of 120 psi was obtained. The upstream valve was then opened carefully while ensuring a TMP of close to 120 psi was maintained.

![Figure 3.4.4 Schematic of the commercial membrane-testing setup](image)

**3.5 Polysaccharide Nanoparticles Challenge Test**

Orange-colored polysaccharide nanoparticles (NPs) prepared in our lab were employed to assess the performance of ENMs. The polysaccharide NPs were made of glycogen anhydroglucose units (AGUs), and had carboxyl functional groups to which fluoresceinamine dyes were attached. They were about 60nm in size (hydrodynamic diameter) after fluorescent labeling.

The same setup as in Figure 3.4.1 was used in these experiments. One liter of pure Milli-Q water was spiked with 1 mL of 100 ppm polysaccharide NPs solution, and used as feed. Once the feed had been filtered through the ENMs, the membranes were removed from the in-line holders and inspected for orange patterns on both the feed and permeate sides.
3.6 DDBAC Leaching from ENM

A calibration curve to establish the relationship between the concentrations of DDBAC in Milli-Q water and the corresponding solution conductivities was established with an electrical conductivity meter (Thermo Orion). Pure Milli-Q water was filtered through the membrane at a flowrate of 4.5 mL/min in 100 mL increment using the setup shown in Figure 3.4.1. At the end of each 100 mL of water filtered, a conductivity reading on the permeate solution was taken. This procedure was repeated until no detectable change in conductivity could be noted over three consecutive measurements.

3.7 Multi-stage filtration

The multi-stage filtration setup (Figure 3.7.1) included a digital peristaltic pump (Masterflex® L/S digital pump), 1/6” anti-protein-adhesion tubing (Masterflex, Canada), and 47mm polycarbonate in-line filter holders from Pall Laboratory to house ENMs (Figure 3.4.2).

The setup also consisted of a feed tank filled with distilled water, which was pumped through at flow rates of 4.5 mL/min. The membrane units were placed in series and the system operated, first with a total of 2, then 3, and finally four ENMs. At every instance, the total pressure drop ΔP was recorded with the use of the pressure gauges.
Figure 3.7.1 Schematic of the multi-stage filtration setup

3.8 Statistical analysis

All MS2 inactivation/removal efficiency experiments were performed in triplicates. All reported error and error bars represent the standard deviation from the mean. The statistical significance of the results obtained was tested using unpaired t-tests at a confidence level $\alpha$ of 95%. The skewness of nanofiber diameter distributions was confirmed by normal Q-Q plots (see Appendix – Figure A).
4.0 Results and Discussions

It should be noted that since the virus propagation and enumeration protocols only allowed the detection and quantification of viable MS2 bacteriophages, it was not known whether the ENM only removed viruses by adsorption, or perhaps inactivated MS2 by contact-killing as well resulting in inactivated MS2 in the permeate.

Physical removal adsorption could be proved by SEM and TEM. However, while very likely to occur, inactivation could not be proved or disproved. To make the prose more legible, “removal efficiency” will be used in the discussions.

4.1 Development and Testing of a TiO₂-Functionalized PVDF ENM

4.1.1 Membrane Characterization

4.1.1.1 Impact of Polymer Solution Concentrations on Nanofiber Diameter

Nanofiber diameter of ENMs prepared with various PVDF concentrations are provided in Table 4.1.1.1. The electrospinning parameters were subsequently optimized to those outlined in section 3.1 by iteration, and applied to the production of all subsequent ENMs. Also, at least 20 nanofibers were measured for each ENM described in this section and the average was reported together with the standard deviation.

It was found that the more viscous the polymer solution, the higher the average fiber diameter), and the larger the fiber diameter distribution.
Table 4.1.1.1 Nanofiber diameter of prepared ENMs in nm (% are in wt%) as measured using SEM. Electrospinning condition: voltage of 15 kV, a tip-to-collector distance of 20 cm, and a feed rate of 3 mL/hr.

<table>
<thead>
<tr>
<th>Prepared ENMs</th>
<th>Nanofiber diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average (nm)</td>
</tr>
<tr>
<td>Pure PVDF (12.5%)</td>
<td>706</td>
</tr>
<tr>
<td>Pure PVDF (15%)</td>
<td>980</td>
</tr>
<tr>
<td>Pure PVDF (17.5%)</td>
<td>1290</td>
</tr>
<tr>
<td>Pure PVDF (20%)</td>
<td>1620</td>
</tr>
</tbody>
</table>

It should be noted that an ideal adsorption ENM should have an average nanofiber diameter below 1000nm and preferably as small as possible to maximize the available specific surface area. In addition, nanofibers should ideally have a more uniform diameter to ensure better removal efficiency and reproducibility. Subsequently, new membranes with and without TiO$_2$ were prepared using 12.5% PVDF solution under optimum conditions and were examined in the remainder of this study.

4.1.1.2 SEM Images of prepared ENMs

SEM images of ENMs prepared subsequently were used to assess their morphology. Figure 4.1.1.2.1 shows that pure PVDF nanofibers possess less beads with a more uniform fiber diameter, in contrast with Figure 4.1.1.2.2 which shows that the 2.5 OMF% nanofibers present more beads and also some fibers were more noticeably thicker than the others. The beads are suspected to be caused by localized agglomerations of TiO$_2$ nanoparticles into clumps in the polymer solution, that when spun, result in the polymer encapsulating said clumps into a bead.
Figure 4.1.1.2.1 FE-SEM image of a pure 12.5 wt% PVDF ENM (x5,000)

Figure 4.1.1.2.2 FE-SEM image of a 2.5 OMF% TiO$_2$-ENM (x5,000)
4.1.1.3 Nanofiber Diameter Analysis

The mean nanofiber diameters of 12.5 wt% pure PVDF, 2.5 OMF%, 5 OMF% and 10 OMF% TiO$_2$-ENMs were 539 ± 108, 600 ± 168, 636 ± 174, and 689 ± 217 nm respectively. The distributions were determined to be in the range of 200-800 nm, 100-1000 nm, 200-1100 nm, and 300-1300 nm respectively as seen in Figure 4.1.1.3.1. As shown in the Figure, with increasing TiO$_2$ content, the distribution curves of the fiber diameter became wider and flatter. The increase in fiber diameter from 12.5 wt% pure PVDF to 2.5 OMF% (p=0.0332<.05, t=2.16), and to 10 OMF% TiO$_2$-ENM (p=0.0240<.05, t=2.29) suggested that a balance should be found between the removal efficiency and the nanofiber diameter (i.e. specific surface area) of the ENMs. In addition, the wide diameter distribution observed after adding nanoparticles was also undesirable in terms of producing uniform membranes with reproducible performance. The distribution curves for 2.5 OMF%, 5 OMF%, and 10 OMF% TiO$_2$-ENMs were more heavily left-skewed with tails at higher nanofiber diameters, compared to the pure PVDF. It is known that a membrane can only be as efficient as its weakest spot. This further supports the notion that with the presence of sections with significantly larger than average nanofiber diameters on the ENM, these might pose a threat to its efficiency. This is because larger nanofiber diameters entail larger pores, which reduces the available surface area and probability of contact between the ENM and the adsorbate. In the end, adding nanoparticles to an ENM might enhance the adsorptive properties of individual nanofibers and removal efficiency of an ENM as a whole, but could come at the cost of a reduced reliability for the overall membrane.
Figure 4.1.1.3.1 Distribution of nanofiber diameters for TiO$_2$-ENMs with different TiO$_2$ concentrations (n=50)

The TiO$_2$-ENMs prepared were all of similar thickness. For 2.5 OMF%, 5 OMF%, and 10 OMF% ENMs, the corresponding thicknesses were 137.7 ± 7.7, 137.0 ± 7.9, and 137.4 ± 5.2 μm respectively.

4.1.1.4 Transmembrane Pressure

The transmembrane pressure (TMP) recorded for TiO$_2$-ENMs was less than the minimum detection threshold of 0.5 psi. This was despite the hydrophobic nature of fluoropolymer PVDF that would suggest a larger TMP to push water through the membrane. This was consistent across variations in permeate flux (156, 346, and 519 LMH), membrane thickness, and TiO$_2$ concentration, indicating that the TMP was
negligible for prepared TiO$_2$-ENMs. This observation suggests the potential of ENMs as a low-energy and thus low-cost alternative to conventional NF membranes for virus removal.

4.1.1.5 SEM and TEM images of Adsorbed MS2 Viruses

Figure 4.1.1.5.1 shows stained MS2 viruses (white) adsorbed onto 2.5 OMF% TiO$_2$-ENM nanofibers following a virus filtration test. Considering the small size of MS2 (~25 nm), it is evident that they were adsorbed as clumps onto the surface of nanofibers. This is further evident from the TEM image in figure 4.1.1.5.2 that shows a viral aggregate attached to the nanofiber surface.

Figure 4.1.1.5.1 FE-SEM image of a 2.5 OMF% TiO$_2$-ENM with MS2 bacteriophage adsorbed after filtration (x5,000)
4.1.1.6 TiO$_2$ Dispersion

Figure 4.1.1.6.1 shows that pure PVDF starts decomposing at around 420°C, leaving a residue of 31.6% of its initial mass, which forms the baseline for further thermogravimetric analysis (TGA) results. In TiO$_2$-ENMs, the higher the TiO$_2$ content, the lower the PVDF decomposition temperature was. This is in accordance with findings that TiO$_2$ reduces the thermal stability of PVDF at high temperatures (57). From the difference between the baseline and the final residual amounts for TiO$_2$-ENMs, it was found that the 2.5 OMF% ENM contained 0.3 wt% TiO$_2$, the 5 OMF% ENM contained 1.9% TiO$_2$, and the 10 OMF% ENM contained 7.7% TiO$_2$. According to these results, the estimated amount of TiO$_2$ was 2~3% less than the nominal TiO$_2$ content of the composite membrane. A similar trends can be deduced from the study of Yang et al.
where a lower TiO$_2$ nano-tube could be estimated from the TGA analysis of a PVA/nt-TiO$_2$/PSSA membrane (58). This discrepancy is likely due to the inadequacy of TGA analysis for the accurate measurement of ash content. In addition, the difficulty in obtaining a well-dispersed TiO$_2$ suspension in a polymer solution, TiO$_2$ agglomeration and hence settling during the electrospinning process could also contribute to this observation.

The TiO$_2$ surface mass % was also calculated from the atomic % data obtained by XPS analysis (see Appendix – Section A and C). The estimated TiO$_2$ contents were 0.58%, 0.88%, and 2.47% for 2.5, 5, and 10 OMF% TiO$_2$-ENMs, respectively. This could be partially explained by the TGA results that pointed to the non-uniform dispersion of TiO$_2$ in polymer solutions, resulting in fibers with a potentially high variation in TiO$_2$ content. In addition, it can be deduced that TiO$_2$ nanoparticles did not properly migrate to the fiber surface during the electrospinning process.
4.1.1.7 Specific Surface Area

Assuming smooth, cylindrical nanofibers, the estimated SSA values could be calculated as $4.17 \pm 0.87 \text{ m}^2/\text{g}$ for pure PVDF ENMs, $3.62 \pm 1.05 \text{ m}^2/\text{g}$ for 2.5 OMF% TiO$_2$-ENM, $3.23 \pm 1.06 \text{ m}^2/\text{g}$ for 5 OMF% TiO$_2$-ENM, and $2.87 \pm 1.10 \text{ m}^2/\text{g}$ for 10 OMF% TiO$_2$-ENM (see Appendix – Section A). The considerable decrease in available surface area for adsorption is due to the increase in the mean fiber diameter because of TiO$_2$ addition. For the 2.5 OMF% TiO$_2$-ENM, this calculation result was compared to BET data (see Appendix-Figure B). The BET plot had a positive constant $c$, its slope was continuously positive, and it exhibited a fairly linear behaviour over the range of...
pressures exerted on the sample, indicating the reliability and accuracy of the obtained BET SSA of 3.76 m$^2$/g. This result was very close to the theoretical calculation, thus confirming the theoretical expression developed to be a good approximation for the SSA of a smooth nanofiber.

4.1.2 Membrane Virus Removal Efficiency Experiments

4.1.2.1 Impact of Permeate Flux and TiO$_2$ OMF%

The removal efficiency of a pure PVDF ENM was determined to be 57.7 ± 2.8% at a flowrate of 4.5 mL/h (156 LMH).

Figure 4.1.2.1.1 shows the impact of TiO$_2$ OMF% on MS2 removal efficiency. Overall, regardless of the flowrate, the ENM removal efficiency increased with increasing TiO$_2$ OMF%. This trend held for 4.5 mL/min, 10 mL/min, and 15 mL/min (156 LMH, 346 LMH, and 519 LMH, respectively), but seemed to be more pronounced at 4.5 mL/min where the virus removal efficiency reached a maximum of 86.4% at 10 OMF% TiO$_2$. This is in accordance with expectations, as a lower flowrate promotes a higher contact time between the feed and the ENM and consequently higher removal efficiency. The removal efficiency for a pure PVDF ENM at a flowrate of 4.5 mL/min was 57.7 ± 2.8%. The mechanism of virus removal for this membrane could be attributed to the hydrophobic interactions between the nanofiber surface and the MS2 bacteriophage capsid.

Figure 4.1.2.1.2 is a rearrangement of the data shown in previous figure. It shows that for any given OMF%, the ENM removal efficiency decreased with increasing
flowrate. It must be noted however that there is overlap between the standard deviations of the different results, and that the removal efficiencies are all very close (p>.05): from 74.4 ± 5.0% at 2.5 OMF% and 15mL/min (worst-case tested parameters for adsorption) to 86.4 ± 4.2 % for 10 OMF% at 4.5 mL/min (best-case tested parameters for adsorption).

Figure 4.1.2.1.1 MS2 removal efficiency of TiO\textsubscript{2}-ENMs at different TiO\textsubscript{2} concentrations, and tested at different filtration flowrates
While it was observed that addition of TiO$_2$ improved adsorption properties of the ENMs ($p<.001$), increasing the TiO$_2$ concentration above 2.5% did not show any significant improvement in removal efficiency, regardless of the flowrate. At 4.5 mL/min, the improvement in the removal efficiency due to TiO$_2$ addition was 49.7% (from 57.7% for pure PVDF to 86.4% for 10 OMF%). The results obtained so far were using membranes with the same thickness ($\sim 137$ $\mu$m $\pm$ 10%).
4.1.2.2 Impact of Thickness

As shown in figure 4.1.2.2.1, there was found to be no significant correlation or difference in removal efficiency when increasing the membrane thickness from 68.8 ± 2.3, to 100.5 ± 3.1, and finally 195.5 ± 0.7 μm. The efficiency stays constant at around 80%. It was suspected this result could be due to a problem with the membrane uniformity, as the efficiency of a membrane is only as good as the efficiency of the largest hole in it.

4.1.3 Polysaccharide Nanoparticles Challenge Test

To examine the uniformity of membrane, a particle challenge test with orange-staining polysaccharide nanoparticles was performed on TiO$_2$-ENMs. If the ENM had a uniform structure, it would have been expected that the feed would flow through the
entire surface of the membrane at the same flowrate, thus creating a uniform shade of orange on the membrane surface. However, Figure 4.1.3.1 shows well-defined small areas of the membrane in were disproportionately stained compared to the rest of the ENM surface. The stained areas likely experienced a higher local flux, and hence creating a channeling effect that would compromise the membrane performance.

Figure 4.1.3.1 TiO\textsubscript{2}-ENM feed side after filtration with polysaccharide nanoparticles

SEM analysis of the stained area and non-stained area of a 2.5 OMF\% TiO\textsubscript{2}-ENM was performed as shown in Figure 4.1.3.2. In image A, the contrast between the dark stained area of the ENM and the white non-stained area of the ENM can be observed. Also, not visible to the naked eye, is the presence of uniformly dispersed clusters of nanoparticles on the non-stained area. Image B enhances the border portion of the membrane. The brighter left side of the image is marked “1” and the darker right side is marked “2”. The two portions were enhanced again and are shown at 10 µm scale at the bottom of the Figure. As shown in image 1, the non-stained area is relatively clean.
with some nanoparticles attached to the fibers. Image 2 shows the stained area of ENM pores which is clogged and the nanofibers enveloped in thick layers of nanoparticles. The above findings confirmed the existence of a channeling effect, likely due to localised lower fibre density resulting in larger pore size in those areas, thus explaining the surprising findings of section 4.1.2.2 that the membrane thickness had no impact on removal efficiency.
Figure 4.1.3.2 SEM images taken at different magnifications of a 2.5 OMF% TiO2-ENM after filtration with polysaccharide nanoparticles. A: x15; B: x250; 1 (clean area), 2 (stained area): x5,000
4.1.4 Improved Membrane Efficiency Experiments

An attempt to improve the uniformity and morphology of the prepared TiO$_2$-ENMs was made. During the electrospinning process, the syringe tip needle was moved every 30 mins in 5 cm increments from the edge of the drum along its length. The resulting membrane was expected to be more uniform due to a more even distribution of deposited nanofibers along the length of the rotating collector drum. Removal efficiency results showed a significant improvement ($p=0.0053<0.05$, $t=5.5154$) from 79.4 ± 2.6% to 93.8 ± 3.7% for 2.5 OMF% TiO$_2$-ENM. It is important to note that the improved results were obtained using a thinner membrane (82.8 ± 1.3 μm). It is proposed that the mechanism for the increased efficiency in MS2 removal of an ENM by the addition of TiO2 nanoparticles is a combination of:

- nanofiber surface roughness created by the nanoparticles increased local surface area and the probability of a MS2 bacteriophage sticking.
- adsorption of MS2 by nanoparticles.
- partial contact-killing of MS2 after adsorption through capsid and potentially RNA disruption and damage.
- limited release of hydroxyl free radicals that contributed to damaging and inactivating MS2 bacteriophages.
Figure 4.1.4.1 MS2 removal efficiency of the improved 2.5 OMF% TiO$_2$-ENM compared to the first 2.5 OMF% TiO$_2$-ENM and the control

The improved performance was confirmed by performing a polysaccharide nanoparticles challenge test on the improved membrane. Figure 4.1.4.2 shows that the stained area is considerably less pronounced and smaller on the improved ENM, proving that this ENM is more uniform and that the channeling effect was responsible for a significant loss in removal efficiency. With a LRV of up to 1.2 for a single TiO$_2$-ENM, in theory a filtration in several stages could significantly improve LRV to around 2.4 with 2 stages for example. The very low TMP exhibited by the prepared ENMs could further enhance the viability of such a multi-stage setup (e.g. <1 psi TMP for 2 stages).
Figure 4.1.4.2 Improved TiO$_2$-ENM after filtration with polysaccharide nanoparticles

4.2 Development and testing of a DDBAC-Functionalized PVDF ENM

4.2.1 Membrane Characterization

4.2.1.1 SEM Images of Prepared ENMs

SEM images of prepared DDBAC-ENMs were taken to assess their morphology. Figure 4.2.1.1.1 shows DDBAC-ENM nanofibers are smooth and seemingly uniform in diameter. This is also noted from the narrow distribution of the nanofiber diameter as seen in Figure 4.2.1.2.1. The SEM also shows very thin and web-shaped fibers that are usually formed when polymer solutions with high conductivity are electrospun at high voltage (31). It is suggested that these webs contribute to the MS2 removal efficiency of the DDBAC-ENM by providing additional ENM surface area for adsorption. They could also act as a secondary sieve for smaller particles that could pass through larger pores. The thickness of the DDBAC-ENMs examined in this study was 83.0 ±7.6 μm that was slightly larger than that of the pure PVDF membranes (77.9 ± 2.1 μm).
4.2.1.2 Nanofiber Diameter Analysis

The mean nanofibers diameter was determined by SEM to be 191 ± 76 nm. The distribution of nanofiber diameters was between 100 to 300 nm with a slight positive skewness (see Appendix – Figure A), compared to 200 to 800 nm for a pure PVDF ENM as seen in Figure 4.2.1.2.1. The narrow distribution compared to pure PVDF ENMs as well as the significant decrease (p<0.0001, t=18.6334) in mean nanofiber diameter from 539 ± 108 nm to 191 ± 76 nm are expected to result in improvement in the removal efficiency of the membrane due to higher SSA and a more uniform and pore structure compared to pure PVDF. This effect can be attributed to the large increase in the polymer solution conductivity from 10.4 μS/cm for the pure PVDF solution to 0.314 mS/cm for the DDBAC-PVDF solution, a 30 fold increase. It is known
that in electrospinning, the higher the solution conductivity, the thinner the fibers, and the narrower the distribution of nanofiber diameters (55).

![Figure 4.2.1.2.1 Distribution of nanofiber diameters for a 5 OMF% DDBAC-ENM (n=50)](image)

**4.2.1.3 Transmembrane Pressure**

The transmembrane pressure (TMP) recorded for DDBAC-ENMs was below the scale of the pressure gauges (0.5 psi). The negligible TMP was due to the high porosity and larger pore size of ENMs in comparison with conventional membranes. The
hydrophilicity of the DDBAC-ENMs was expected to contribute to and enhance the water permeability of the membrane.

4.2.1.4 SEM and TEM images of Adsorbed MS2 Viruses

Figure 4.2.1.4.1 shows MS2 viruses adsorbed onto DDBAC-ENM nanofibers. The same clumping phenomenon and uniform distribution over the nanofiber surface can be observed as for MS2 adsorption onto TiO$_2$-ENM nanofibers. The clumping adsorption behaviour of MS2 can be observed in the TEM cross-section image of the nanofiber (Figure 4.2.1.4.2). It is more pronounced for DDBAC-functionalized nanofibers than for TiO$_2$-functionalized ones, pointing to a higher MS2 adsorption capacity for the former.

![Figure 4.2.1.4.1 FE-SEM of a 5 OMF% DDBAC-ENM with MS2 bacteriophage adsorbed after filtration (x5,000)](image)
4.2.1.6 Specific Surface Area (Model)

As mentioned in section 3.2, the melting point of DDBAC is too low to perform the degassing step on a DDBAC-ENM sample for the BET procedure, thus the SSA estimate was calculated assuming smooth nanofibers. This approximation seems reasonable from the nanofiber morphology in the SEM image (see Figure 4.2.1.2). The calculated SSA was found to be $12.07 \pm 5.69 \text{ m}^2/\text{g}$. 

Figure 4.2.1.4.2 TEM image of a 5 OMF% DDBAC-ENM nanofiber cross-section with MS2 bacteriophages adsorbed (x100,000)
4.2.2 Membrane Virus Removal Efficiency Experiments

The removal efficiency of 5 OMF% DDBAC-ENMs was tested and found to correspond to a 2.9 ± 0.1 LRV. This was compared to the removal efficiency of a pure PVDF membrane at 0.4 ± 0.01 LRV (57.7 ± 2.8%). The removal efficiency of the DDBAC-ENM was significantly higher than that of the pure PVDF (p<0.0001, t=38.6834). The DDBAC-ENM likely exhibited high MS2 LRV partly due to the electrostatic interactions (the positively-charged quaternary ammonium compound interacted with the negatively-charged capsid amino acids) which are longer range and stronger in magnitude than hydrophobic interactions. Another likely important factor is the higher uniformity of the DDBAC-ENM nanofibers compared to those of pure PVDF ENMs, as well as their smaller average diameter which leads to a higher SSA.

Figure 4.2.2.1 Comparison of MS2 removal efficiency of 5 OMF% DDBAC-ENM compared to pure PVDF membranes
4.2.3 Polysaccharide Nanoparticles Challenge Test

A polysaccharide nanoparticle challenge test, performed on DDBAC-ENMs in the same manner as conducted for TiO$_2$-ENMs in section 4.1.3. The results were a membrane with a faint orange taint uniformly distributed over the surface of the ENM. This indicated that contrary to the TiO$_2$-ENMs, there was no important channeling effect that compromised the integrity and efficiency of the membrane. This can be attributed to the demonstrated lower average fiber diameter, and much more narrow distribution in fiber diameter of the DDBAC-ENMs, resulting in an ENM with a uniform structure and morphology across its surface and depth. In addition, the webs observed by SEM (Figure 4.2.1.1.1) could have also been a contributing factor. Figure 4.2.3.2 shows a higher resolution image of the sample of the DDBAC-ENM shown in Figure 4.2.3.1. The nanoparticles are clearly uniformly distributed over the entire surface of the ENM, thus confirming the previous observations.

Figure 4.2.3.1 DDBAC-ENM feed side after filtration with polysaccharide nanoparticles
4.2.4 DDBAC Leaching from ENM

Since DDBAC is a very water-soluble quaternary ammonium compound, the stability of the embedment of DDBAC at the nanofiber surface was tested. After being repeatedly washed with water, DDBAC-ENMs that exhibited at first use a LRV of 2.9 ± 0.1 maintained a LRV of the same magnitude at 2.4 ± 0.2 (see Figure 4.2.4.1). This indicated that the DDBAC-ENMs could reliably maintain their removal efficiency after repeated use.
Figure 4.2.4.1 Comparison of the LRV of a new 5 OMF% DDBAC-ENM (first 0.1L filtered) with the same 5 OMF% DDBAC-ENM after several usages (5 filtrations of 0.1L)

The leaching of DDBAC was also quantified by correlating the conductivity of the permeate with the DDBAC concentration in solution. First, it was established that permeate conductivity did not change over time for a filtration with a pure PVDF membrane. Then, a calibration curve of solution conductivity as a function of DDBAC concentration in MilliQ water was established as shown in Figure 4.2.4.2. Due to the range of tested DDBAC concentrations being at low molar concentrations (<<0.1 mol/L), a linear function could be established (at higher molar concentrations this ideal behaviour does not apply).
Figure 4.2.4.2 Calibration curve to establish the relationship between the DDBAC concentration in Milli-Q water and the solution conductivity (μS/cm).

\[ y = 0.85x + 0.66 \]
\[ R^2 = 0.9876 \]

Figure 4.2.4.3 shows the quantitative release of DDBAC as well as the relative cumulative release of DDBAC from the DDBAC-ENM for every 100 mL filtered through the ENM. The highest amount of DDBAC released was close to 8.00 mg after the first 100 mL filtered, on the second pass a significantly lower amount of close to 1.00 mg was released, and on the next three subsequent passes the amounts leached, if any, were undetectable as the conductivity readings were below the detection limit of the conductivity meter (0.1 μS/cm). Thus, the amounts leached after the first 300 mL filtered can be considered negligible, and the total amount of DDBAC at the nanofibers surface...
stabilized. The total relative amount of DDBAC lost by the ENM was found to be 46.6%, with 89% of that amount leaching in the first 100 mL filtered.

![Graph](image)

Figure 4.2.4.3 Left axis: Cumulative release of DDBAC (in % of total DDBAC on ENM) from a 5 OMF% DDBAC-ENM as a function of the water volume filtered. Right axis: Total release of DDBAC from a 5 OMF% DDBAC-ENM as a function of the water volume filtered.

Assuming a uniform distribution of DDBAC in the nanofiber, and all DDBAC molecules being optimally oriented at the nanofiber surface (see Appendix A – Figure A1), it was found that 3.8% of the total amount of DDBAC molecules should be exposed at the surface of the nanofiber. This means that this was the maximum amount that
should theoretically be able to leach from an ENM. However, as can be seen in Figure 4.2.4.3, 46.6% of the total DDBAC amount was leached (over 12x more).

On the other hand, Figure 4.2.5.1 indicates that as per XPS analysis, the mass % at the surface for DDBAC-ENMs is 5.35% at 5 OMF% (1.1x more): the measured concentration is close to the nominal concentration. In addition, still with the previously stated assumptions, 19.9% of the total amount of DDBAC molecules should have been detected by XPS analysis near the surface based on the sample penetration depth of X-rays (see Appendix A), which was still considerably lower than the detected 46.6% of DDBAC leached.

Based on these results, it was proposed that the reason the total amount of DDBAC leached was over 12 times higher than the theoretical amount that should have, was due to the presence of a strong DDBAC concentration gradient from the nanofiber surface to its center. The DDBAC strongly segregated to the surface during the electrospinning process as a result of its amphiphilic nature: the hydrophobic tail sought contact with the polymeric nanofiber while the positively-charged hydrophilic head sought contact with the air and the charged ENM surface (as a result of the electric field exerted on the polymer solution jet). This would also explain why XPS analysis, which has a depth of analysis about 5.4 times larger than the length of the DDBAC alkane chain, did not detect this sharp concentration gradient: its depth of analysis gives a surface mass% that averages the gradient concentrations close to OMF%. This notion seems to be further reinforced by the observed trend that at lower nominal concentrations such as 1 OMF% and 2.5 OMF% (see Figure 4.2.5.1), the corresponding surface mass % are increasingly higher by factors of 2.8 and 1.6,
respectively (compared to only 1.1 for 5 OMF%). The lower the nominal concentration, the more pronounced the concentration gradient seems to be in the fiber.

### 4.2.5 Surface Segregation of DDBAC as a Function of its Concentration

To determine the extent of the surface-segregating properties of DDBAC under electrospinning conditions compared to TiO$_2$, the surface mass % as calculated from the surface atomic % by XPS (see Appendix – Section A) was determined for a range of DDBAC OMF% from 1.0 OMF% to 10.0 OMF%. Figure 4.2.5.1 shows that the measured DDBAC concentration was quite close to the nominal DDBAC loading in the fiber ($R^2=0.80$) from 5 OMF% upward. The OMF% could be optimized to obtain a surface mass % high enough to maintain a good LRV while minimizing the amount of DDBAC used, thus trying to obtain the highest LRV/OMF% ratio possible. This could be done by testing the LRV of DDBAC-ENMs that vary in OMF% by small increments (e.g. 0.5 OMF%). In addition, Figure 4.2.5.1 suggests that DDBAC is more concentrated in the surface than the bulk of the fibers particularly at low OMF%, which in fact, is a favorable property when cost of material is concerned.
Figure 4.2.5.1 Surface concentration of DDBAC on prepared DDBAC-nanofibers (expressed as mass %) as a function of OMF%.

4.3 Testing of Commercial NF Membrane

As an adsorptive membrane, prepared ENMs showed to have low TMPs (<0.5 psi) due to their interconnected pore structure and high porosity. This presents a major improvement over commercial membranes which require operating pressures 2-3 orders of magnitude higher. If conventional membranes are employed, NF membranes can remove nearly all viruses from water due to their pore size being just below the size of nearly all viruses. MF membranes have a pore size too large, UF membranes can remove most viruses, and RO membranes have a pore size much smaller than required for the removal of viruses. This is why a commercial NF membrane was prepared to compare its performance to prepared ENMs.
The commercial NF membrane exhibited a MS2 LRV of 5.6 ± 0.9. As expected, LRV for the commercial membrane was significantly larger than for pure PVDF adsorptive ENMs (by a factor of 15) and comparable to the LRV reported in the literature for tight UF/NF membranes (Figure 4.3.2). Interestingly, the developed DDBAC-ENM had a LRV comparable to the reported LRV of MF commercial membrane systems rated for virus removal as seen from Figure 4.3.2 (59), while operating at a considerably lower TMP (>0.5 psi against 30 psi, respectively).

Figure 4.3.1 MS2 removal efficiency of commercial NF membranes compared to pure PVDF ENMs
4.4 Pressure Drop in ENM Multi-Stage Filtration

It can be seen that as the number of ENMs placed in series increases, the average increase in pressure drop $\Delta P$ is 0.45 psi per ENM added, up to a value of 1.7 psi for 4 ENMs in series (see Table 4.4.1). This is considerably lower than the operating pressure requirements of conventional membranes such as UF (30-100 psi) and NF (90-150 psi). These results support the proposition that ENMs, especially in a multi-stage filtration configuration, could potentially be an economically viable alternative to conventional membranes in terms of their lower energy requirements (due to significantly lower operating pressures) while maintaining high LRVs (at least 2.4 LRV).
Table 4.4.1 Pressure drops across ENMs placed in series

<table>
<thead>
<tr>
<th>Number of ENMs placed in series</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure drop $\Delta P$ (in psi)</td>
<td>&lt;0.5</td>
<td>0.8</td>
<td>1.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>
5.0 Conclusions

The objectives of this study were to develop a new generation of membranes for virus removal in drinking water applications through novel ENMs that could:

1. Exhibit MS2 LRVs comparable to (if not higher than) conventional MF, UF, and NF membranes used for enteric virus removal in drinking water applications.

2. Have the potential to be more cost-effective than conventional MF, UF, and NF membranes used for enteric virus removal in drinking water applications through careful tailoring of their morphological and surface properties.

ENMs using the hydrophobic fluoropolymer PVDF as a base were developed, and functionalized with TiO$_2$ nanoparticles, or DDBAC, a quaternary ammonium cation. The purpose was to take advantage of different properties of the additives to promote adsorption and inactivation of MS2 viruses upon contact. Specifically, the hydrophobic interactions and the virucidal effect of TiO$_2$, and electrostatic interactions and the virucidal effect of DDBAC were of interest.

The TiO$_2$-ENMs exhibited hydrophobic surface properties, a relatively large average fiber diameter and a large distribution of fiber diameters, as well as a poor uniformity in the distribution of TiO$_2$ nanoparticles in the nanofibers. The latter was due to their tendency to agglomerate, the difficulty in breaking up these clumps, and their poor surface segregating capability. They also exhibited a very low TMP (<0.5 psi), a key advantage compared to conventional membranes which require high operating pressures of up to 120 psi for NF membranes.
This ultimately resulted in ENMs that could achieve a reasonable LRV of 1.2 (see Figure 5.1), but still not high enough to be competitive with commercial membranes. However, it was hypothesized that a multi-stage filtration approach with ENMs placed in series could result in systems that surpass a 4 LRV and are competitive with commercial membranes. This is because high pressure requirements are the largest overall operating expense for commercial membranes: with their significantly lower operating pressures, ENMs would be expected to see a significantly lower operating cost. This was found to be promising as a multi-stage filtration approach resulted in a total pressure drop of only 1.7 psi for 4 ENMs placed in series.

The DDBAC-ENMs exhibited a hydrophilic surface while maintaining a hydrophobic bulk, a low average fiber diameter and a small distribution of fiber diameters, and a great surface functionalization. The latter was due to the excellent surface segregating and self-orienting capability of DDBAC, in turn due to the combination of its amphiphilic nature with the base PVDF polymer used. Like the TiO$_2$-ENMs, these ENMs had very low TMPs (<0.5 psi). The removal efficiency of the DDBAC-ENMs was found to be relatively high at a LRV of 2.4. Although up to 46.6% of the surface DDBAC was washed away in the first 200 mL filtered, this efficiency was shown to be maintained and stable even after prolonged use of the membrane.

While the DDBAC-ENM cannot achieve LRVs as high as a commercial NF membrane, it is comparable in efficiency to MF membranes rated for enteric virus removal. Compared to the developed TiO$_2$-ENM, it exhibited significantly higher LRV (p<.0001), and more desirable morphological properties for MS2 adsorption and membrane reliability (higher surface area, more uniform structure). Also, the stronger
effect of electrostatic interactions over hydrophobic interactions to promote adsorption and remove/inactivate MS2 bacteriophage was clearly demonstrated as well through the removal efficiencies in increasing order of pure PVDF ENM, TiO\textsubscript{2}-ENM, and DDBAC-ENM (see Figures 5.1 and 5.2).

In conclusion, the DDBAC-ENM showed the best results compared to TiO\textsubscript{2}-ENMs in terms of fulfilling the objectives of this study by having a competitive MS2 LRV, and being potentially more cost-efficient than MF, UF, and NF membranes due to its very low TMP. In addition, as far as the author is aware, it is the first ENM developed for the purpose of enteric virus removal in drinking water applications that promotes electrostatic interactions through the relatively simple procedure of blending a widely-available and already commercially used functionalizing agent (DDBAC). This is in opposition to synthesizing new molecules that would require regulatory approval, or doing time-consuming and expensive chemical surface modifications.

Table 5.1 Properties and Performances of Prepared ENMs

<table>
<thead>
<tr>
<th>Prepared ENMs</th>
<th>Removal Efficiency (LRV)</th>
<th>ENM Thickness (nm)</th>
<th>ENM Permeate Flux/Flowrate (LMH/mL/min)</th>
<th>TMP (psi)</th>
<th>Avg. fiber D (nm)</th>
<th>SSA Estimate (m\textsuperscript{2}/g)</th>
<th>BET SSA (m\textsuperscript{2}/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PVDF</td>
<td>0.4 ± 0.01</td>
<td>77.9 ± 2.1</td>
<td>156 / 4.5</td>
<td>&lt;0.5</td>
<td>539 ± 108</td>
<td>4.17 ± 0.87</td>
<td>-</td>
</tr>
<tr>
<td>2.5 OMF% TiO\textsubscript{2}-ENM</td>
<td>1.2 ± 0.02</td>
<td>82.8 ± 1.3</td>
<td>&lt;0.5</td>
<td>600 ± 168</td>
<td>3.62 ± 1.05</td>
<td>191 ± 76</td>
<td>12.07 ± 5.69</td>
</tr>
<tr>
<td>5 OMF% QAC-ENM</td>
<td>2.4 ± 0.2</td>
<td>80.2 ± 0.5</td>
<td>156 / 4.5</td>
<td>&lt;0.5</td>
<td>191 ± 76</td>
<td>12.07 ± 5.69</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Figure 5.1 Comparison of the MS2 removal efficiency of different 2.5 OMF% TiO$_2$-ENMs to pure PVDF ENMs

Figure 5.2 Comparison of the LRV of the DDBAC-ENM (post-leaching) to a NF membrane
6.0 Future Work & Recommendations

In this study, a functionalized DDBAC-ENM with high MS2 LRV value that shows potential as a replacement for conventional membranes in particular. However, all the removal efficiency tests were performed in Milli-Q water. It would be interesting to assess the MS2 removal efficiency under conditions that directly mimic surface water conditions. The water pH, dissolved organic matter content, ionic strength, and temperature could be varied to get a full understanding of the potential of the ENM. For instance, it could help broaden the use of the DDBAC-ENM to portable drinking water filters for people with no or limited access to clean water. In addition, the extent of fouling of the membrane over prolonged exposure (several days, or weeks) to feed water should be assessed, including ENM regeneration methods. This is an important limitation of conventional membranes, where ENMs could perform better due to their interconnected pore structure, thus giving them an edge in applicability. Finally, due to the strong surface segregating capability of DDBAC in PVDF nanofibers, an optimal MS2 LRV to DDBAC OMF% ratio should be investigated. Finding that point would enhance not only the economic viability of eventually producing functionalized ENMs at an industrial scale, but also their cost-effectiveness compared to conventional membranes. The removal efficiency of TiO₂-ENMs could be attempted to be improved by doping the TiO₂ with silver (39), or by constructing a cell in which the TiO₂-ENM would be submitted to UV-light to unlock its photoactive virucidal properties. Finally, preliminary tests with dendrimers have shown promising results as a tool for inactivating viruses, but further research would be required to find an efficient way of recovering them for re-use (see Appendix – Section B).
7.0 References


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Figure A. Normal Q-Q plot of nanofiber diameters for PVDF membranes with varying concentrations of functionalizing agents (TiO$_2$ or DDBAC)
Figure B. BET plot for a 2.5 OMF% TiO$_2$-ENM sample
Section A – Calculations

• Calculation of OMF%:

For a given prepared polymer solution, assuming it is homogeneously mixed, the OMF% is:

$$OMF\% = \frac{m_a}{m_a + m_p}$$

With $m_a$ the amount of functionalizing agent added to the solution, and $m_p$ the amount of base polymer added to the solution.

• Calculation of theoretical ENM surface area based on average fiber diameter

Assuming the ENM can be modeled as a single long cylindrical nanofiber of diameter d, and that the ends of the cylinder are of negligible surface area due to the length l of the cylinder, we have:

1. $$SA = \frac{2\pi d l}{m_s} = \frac{\pi d l}{m_s}$$

With SA the surface area of the cylinder, d the average diameter of the nanofibers in an ENM as determined by SEM, l the length of the cylinder, and $m_s$ the mass of the 2.5 OMF% TiO$_2$ sample used with $m_s=26.2$ mg. The density of the sample $\rho_f$ is determined such as $\rho_f = 2.5\%\rho_{TiO_2} + 97.5\%\rho_{PVDF} = 1.84 \text{ g/cm}^3$ ($\rho_{TiO_2}=4.23 \text{ g/cm}^3$; $\rho_{PVDF}=1.84 \text{ g/cm}^3$).

The unknown $l$ is determined from the volume of the cylinder such as:

2. $$V = \pi \left(\frac{d}{2}\right)^2 l \leftrightarrow \frac{m_s}{\rho_f} = \pi \left(\frac{d}{2}\right)^2 l \leftrightarrow l = \frac{m_s}{\rho_f \pi \left(\frac{d}{2}\right)^2}$$
Plugging equation 2 back into equation 1, one obtains the final expression for the theoretical ENM surface area:

3. \[ SA = \frac{4}{\rho_f d} \]

- Calculation of surface mass % of functionalizing compound in the nanofibers by XPS

\[
\text{Mass} \% = \frac{\text{at}\%_M (MW_M + \sum_{k=1}^{n} \frac{c_k}{C_M} MW_k)}{\text{at}\%_M MW_M + \sum_{k=1}^{n} (\text{at}\%_k MW_k)}
\]

With at% the surface atomic percentage as determined by XPS (see Appendix – Section C), MW the molecular weight of a given atom, c the number of a given atom in a molecule of the functionalizing compound, subscript M the single atom that distinguishes the functionalizing compound from the rest of the compounds present in the ENM, subscript k another atom present other than atom labeled M.

- Calculation of log removal value (LRV)

\[
LRV = log_{10} \left( \frac{C_f}{C_p} \right)
\]

With \( C_f \) the bacteriophage concentration in the feed, and \( C_p \) the bacteriophage concentration in the permeate, as determined by the virus propagation and enumeration protocol.

- Calculation of theoretical amount of DDBAC at the nanofiber surface under optimal DDBAC molecule orientation
V1 the volume of the whole nanofiber cross-section of radius $r_1 = 95.5$ nm; V2 the nanofiber volume occupied by the DDBAC alkane chains of DDBAC molecules optimally oriented at the surface (aromatic ring exposed at the surface, and alkane chain embedded inside the nanofiber) with $r_2 = r_1 - (\# \text{ of carbon atoms in DDBAC alkane chain}) \times (\text{carbon-carbon bond length in alkane chain}) = r_1 - 12 \times (0.154 \text{ nm}) = 93.7 \text{ nm}$; and V3 the nanofiber volume that is penetrated by XPS rays with $r_3 = r_1 - (\text{Penetration depth of XPS rays}) = r_1 - 10 \text{ nm} = 85.5 \text{ nm}$. We have:

\[
\begin{align*}
V_1 &= \pi r_1^2 = 28652 \text{ nm}^3 \\
V_2 &= \pi (r_1^2 - r_2^2) = 1098 \text{ nm}^3 \\
V_3 &= \pi (r_1^2 - r_3^2) = 5686 \text{ nm}^3
\end{align*}
\]

Assuming a uniform distribution of DDBAC in the nanofiber, and all DDBAC molecules being optimally oriented at the nanofiber surface, we have:

\[
\begin{align*}
\% \text{ of Total DDBAC molecules exposed at the surface} &= V_2/V_1 = 3.8% \\
\% \text{ of Total DDBAC molecules detected by XPS analysis} &= V_3/V_1 = 19.9%
\end{align*}
\]
Figure A1. Schematic of nanofiber cross-section indicating the radius of the nanofiber ($r_1$), the distance from the nanofiber center to its depth at which the hydrocarbon tails of the DDBAC reach when optimally oriented at the surface ($r_2$), and the distance from the nanofiber center to its depth at which XPS rays reach ($r_3$).

Section B - A Potential Promising Virucidal Compound: Dendrimers

Dendrimers are polymers with a branching, tree-like structure (see Figure B1). They are symmetric around a core, and have a spherical 3-D morphology. The ends of its branches can be functionalized with molecules or groups of interest for specific applications. In addition to their functional groups, they are also classified by generation. A generation represents a branching cycle, which can be controlled during synthesis. At generation 0, the core, four functional groups are present on the molecule. With every additional generation, their number doubles due to the branching nature.

Testing of dendrimers for a variety of applications is still in its infancy, and its main research focus is into biomedical applications as a drug-delivery system. The
application of dendrimers as a virus-removal tool in water is as of the time of this writing and to the best of my knowledge, an unexplored application.

Figure B1. Schematic 2-D representation of a dendrimer

**B-1.0 Materials and Methods**

**B-1.1 Dendrimer Suspension Preparation**

Generation 0 (G-0) and generation 3 (G-3) 20 wt% polyamidoamine (PAMAM) dendrimer suspensions in methanol were purchased from Sigma-Aldrich. After measuring the volume of the desired amount of solution, the methanol was evaporated under a continuous stream of pure nitrogen gas for 48 hours. Following this, the dendrimers were re-suspended by the addition of Milli-Q water to the desired concentration.
**B-1.2 Virucidal Effect Experiment**

Table B-1.2.1. Experimental parameters and inactivation results of dendrimers suspended with MS2 virus at different dendrimer concentrations from low to high, and different dendrimer generations (V: MS2 virus; C: concentration; D: PAMAM dendrimer)

<table>
<thead>
<tr>
<th>V stock C (PFU/mL)</th>
<th>Gen 0 - Low</th>
<th>Gen 0 - High</th>
<th>Gen 0 - Very High</th>
<th>Gen 3 - Low</th>
<th>Gen 3 - High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment volume (mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C of V (PFU/mL)</td>
<td>3.80E+11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount of V (PFU)</td>
<td>1.90E+12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount of D (g)</td>
<td>0.002</td>
<td>0.02</td>
<td>1</td>
<td>0.02675</td>
<td>0.2675</td>
</tr>
<tr>
<td>C of D (g/L)</td>
<td>0.4</td>
<td>4</td>
<td>200</td>
<td>5.35</td>
<td>53.5</td>
</tr>
<tr>
<td>D:V ratio</td>
<td>1.23E+06</td>
<td>1.23E+07</td>
<td>6.13E+08</td>
<td>1.23E+06</td>
<td>1.23E+07</td>
</tr>
<tr>
<td>Amount of methanol D solution (g)</td>
<td>0.01</td>
<td>0.1</td>
<td>5</td>
<td>0.13375</td>
<td>1.3375</td>
</tr>
<tr>
<td>Amount of methanol D solution (μL)</td>
<td>12</td>
<td>116</td>
<td>5794</td>
<td>155</td>
<td>1550</td>
</tr>
<tr>
<td>Amount of virus stock (mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount of MilliQ water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual initial C (PFU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.43E+10</td>
</tr>
<tr>
<td>Final C (PFU/mL)</td>
<td>1.35E+11</td>
<td>1.14E+10</td>
<td>1.91E+05</td>
<td>4.50E+08</td>
<td>4.23E+08</td>
</tr>
<tr>
<td>Inactivation rate</td>
<td>-109.84%</td>
<td>82.28%</td>
<td>100.00%</td>
<td>99.30%</td>
<td>99.34%</td>
</tr>
<tr>
<td>LRV</td>
<td>-0.32</td>
<td>0.75</td>
<td>5.53</td>
<td>2.16</td>
<td>2.18</td>
</tr>
</tbody>
</table>

The amount of virus stock added to the re-suspended dendrimer solution was determined based on the desired dendrimer to virus ratio. This ratio was incrementally increased by an order of magnitude (see B-1.2.1). The dendrimer-MS2 suspension was kept sterile overnight for 16 hours with a foam stopper and shaking at 80 rpm to avoid potential settling of either the virus or dendrimer. The amount of viable MS2 viruses left in suspension was determined with the same methodology as detailed in section 3.3. No replicates were performed for this preliminary experiment.
Figure B-2.1: Inactivation efficiency of different dendrimer-MS2 virus solutions varying in dendrimer generation (G-0; G-3) and in dendrimer concentration (low to high).

As can be seen from Figure B-2.1, at the relatively lowest D:V ratio tested for G-0 dendrimers, there was no apparent MS2 inactivation at a LRV of -0.32. The negative LRV can be attributed to variability in the virus enumeration protocol, as it would not be possible for there to spontaneous generation of MS2 viruses in the prepared suspension. As the D:V ratio is increased, there seems to be a marginal improvement in MS2 inactivation to a LRV of 0.75. Finally, as the D:V ratio is again increased, the LRV significantly increases to 5.53. For G-3 dendrimers, the LRVs are significantly higher at 2.16 and 2.18 than for G-0 dendrimers at the same low and high D:V ratios,
respectively. In addition, the LRV between low and high D:V ratios seem to remain constant.

The following was hypothesized from these results:

- G-0 dendrimers at low concentration do not have enough surface groups (4 in total) and size (15 Å diameter compared to 270 Å for MS2) to significantly block MS2 surface proteins that allow it to bind to E.Coli receptors; to produce an overall strong enough electrostatic interaction to denature the virus RNA. As the dendrimer concentration increases, these effects are mitigated, thus increasing the LRV.

- G-3 dendrimers have a higher LRV than G-0 dendrimers at the same D:V ratios due to the larger number of surface groups (32 in total) and their larger size (36 Å). There is no change in LRV between low and high concentrations, indicating the presence of a plateau: beyond a critical D:V ratio, further addition of dendrimers to a suspension does not improve the LRV as the MS2 surface is already saturated with electrostatically bonded dendrimers.

**B-3.0 Conclusions**

Preliminary tests on PAMAM dendrimers have shown promising results in terms of their ability to inactivate viruses in pure water suspensions at levels above the required LRV of 2 by the MOECC (12). Further experiments would need to be carried out to test the hypotheses outlined in the results discussion. In addition, due to the relatively high cost of dendrimers, a system to desorb dendrimers from viruses and recover them for re-use could be envisioned. This would entail experiments to determine whether the virus inactivation process is irreversible or reversible once
dendrimers are desorbed. Finally, the encapsulation of virucidal compounds in the dendrimers could be a viable option to further enhance their virus inactivation efficiency.

**Section C – Raw XPS Data**

Table C.1 XPS Elemental ID and Qualification for pure PVDF ENM

<table>
<thead>
<tr>
<th>Name</th>
<th>Peak BE (eV)</th>
<th>FWHM eV</th>
<th>Area (P) CPS.eV</th>
<th>At. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1s</td>
<td>287.34</td>
<td>1.80</td>
<td>260733.70</td>
<td>54.20</td>
</tr>
<tr>
<td>F1s</td>
<td>688.66</td>
<td>2.01</td>
<td>754384.11</td>
<td>45.20</td>
</tr>
<tr>
<td>O1s</td>
<td>533.18</td>
<td>3.28</td>
<td>7420.81</td>
<td>0.60</td>
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</table>

Table C.2 XPS Elemental ID and Qualification for 2.5OMF% TiO2-ENM

<table>
<thead>
<tr>
<th>Name</th>
<th>Peak BE (eV)</th>
<th>FWHM eV</th>
<th>Area (P) CPS.eV</th>
<th>At. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti2p</td>
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<td>3534.65</td>
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</tr>
<tr>
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<tr>
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<td>1.72</td>
<td>8971.63</td>
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Table C.3 XPS Elemental ID and Qualification for 5 OMF% TiO2-ENM

<table>
<thead>
<tr>
<th>Name</th>
<th>Peak BE (eV)</th>
<th>FWHM eV</th>
<th>Area (P) CPS.eV</th>
<th>At. %</th>
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</thead>
<tbody>
<tr>
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Table C.4 XPS Elemental ID and Qualification for 10 OMF% TiO$_2$-ENM

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<th>Name</th>
<th>Peak BE (eV)</th>
<th>FWHM eV</th>
<th>Area (P) CPS.eV</th>
<th>At. %</th>
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</thead>
<tbody>
<tr>
<td>C1s</td>
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Table C.5 XPS Elemental ID and Qualification for 1 OMF% DDBAC-ENM

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<th>Name</th>
<th>Peak BE (eV)</th>
<th>FWHM eV</th>
<th>Area (P) CPS.eV</th>
<th>At. %</th>
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Table C.6 XPS Elemental ID and Qualification for 2.5 OMF% DDBAC-ENM

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<th>Peak BE (eV)</th>
<th>FWHM eV</th>
<th>Area (P) CPS.eV</th>
<th>At. %</th>
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</thead>
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Table C.7 XPS Elemental ID and Qualification for 5 OMF% DDBAC-ENM

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<th>Name</th>
<th>Peak BE (eV)</th>
<th>FWHM eV</th>
<th>Area (P) CPS.eV</th>
<th>At. %</th>
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<tbody>
<tr>
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Table C.8 XPS Elemental ID and Qualification for 7.5 OMF% DDBAC-ENM

<table>
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<tr>
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Table C.9 XPS Elemental ID and Qualification for 10 OMF% DDBAC-ENM

<table>
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<th>Name</th>
<th>Peak BE (eV)</th>
<th>FWHM eV</th>
<th>Area (P) CPS.eV</th>
<th>At. %</th>
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