

NEW HORIZONS IN PURIFICATION OF LIQUIDS: NOVEL COLLOIDAL AND INTERFACIAL STRATEGIES TO REMOVE HAZARDOUS MOLECULES, VIRUSES AND OTHER MICROORGANISMS FROM WATER OR BLOOD

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INTRODUCTION

Water is continually moving around, through, and above the earth as water vapor, liquid water, and ice. In fact, water is continually changing its form. The Earth is essentially a “closed system”, like a terrarium. That means that the Earth neither, as a whole, gains nor loses much matter, including water. This means that the same water that existed on Earth millions of year ago is still here. Thanks to the water cycle, the same water is continually being recycled all around the globe. It is entirely possible that the water you drank for lunch was once used by some other person for bathing.

The total volume of water on the earth is an abundant 326,000,000 cubic miles. Approximately 97% of that volume is made up of salt water, which cannot be directly

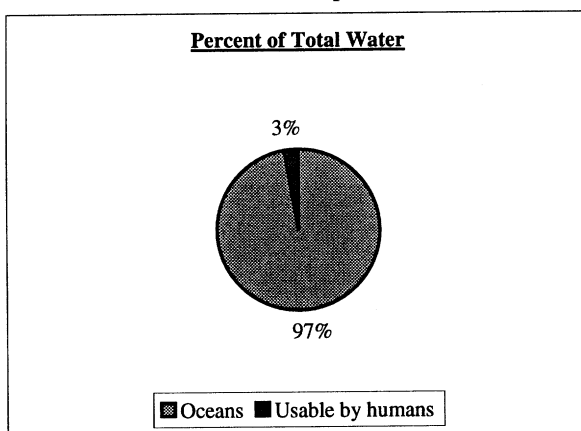


FIGURE 1. Earth's Water Supply

utilized by humanity. Of the 3% remaining, a little more than 2% is locked up as ice in the Polar regions. The remaining water (~0.63%) is found in lakes, rivers, in the atmosphere and underground as fresh water supporting all life forms on earth (See chart below), and much of it is contaminated with various disease-causing microorganisms. Therefore, the development of safe and efficient methodologies for the purification or recycling of water is of paramount importance.

According to a recent report by Johns Hopkins University, nearly half a billion people around the world face water shortages today. By 2025 the number will increase five-fold to 2.8 billion (35 percent of the world's projected total of 8 billion people). The world's population, now at nearly 6 billion, is increasing by about 80 million per year. As of 1995, 31 countries, with a combined population of 458 million, faced either water stress or water scarcity¹. Although the United States does not currently face critical shortages, there are problem areas:

1. Overall, ground water is being used 25 percent faster than it is being replenished. In particular, the Ogallala aquifer, which underlies parts of six states and irrigates 6 million hectares (14,826,300 acres), has been overexploited and, in some areas, half its available water has been withdrawn.
2. The Colorado River, which flows through several southwestern states, has fed agriculture and enabled rapid growth of desert cities such as Las Vegas, Phoenix, and San Diego. Demands have so drained the river that it no longer consistently reaches its mouth in Mexico's Gulf of California. The river's overuse has been a source of contention between the United States and Mexico.

Before water is available for human use, it undergoes purification treatment. This treatment consists of the removal of contaminants from the water in order to decrease the possibility of detrimental effects on humans and the rest of the ecosystem. The term "contaminant" is used here to refer to an undesirable constituent in the water or wastewater that may directly or indirectly affect human or environmental health. Many contaminants, including a wide variety of organic compounds, metals and microorganisms (bacteria, virus), are toxic to humans and other organisms.

The use of disinfectants as the final step in water treatment facilities has been quite useful in reducing the number of occurrences of waterborne disease outbreaks. However, it has disadvantages, including the harmful byproducts that may be released to the environment or consumed by humans².

CURRENT TECHNOLOGIES

The most widely used technology for removing turbidity and microbial contaminants from surface water supplies is conventional treatment. This treatment involves the pretreatment steps of chemical coagulation, rapid mixing, flocculation and sedimentation followed by filtration and disinfection (if necessary)³.

Some of the other current technologies that are used for water treatment are listed below:

1. Activated carbon adsorption
2. Ion-exchange resins
3. Diatomaceous earth and Sand Filters

Activated Carbon

Activated carbon has been used for many years in a number of industrial products and processes, and it has been utilized in water and wastewater treatment in both municipal and industrial settings⁴. Some potential drawbacks of using activated carbon include physical plugging, ash build-up, progressive loss of adsorptive capacity in the carbon particles after several cycles of regeneration, biological growth, septicity, and hydrogen sulfide production (which may contribute to corrosion of metals and damage concrete).

Ion Exchange

Ion exchange (use of resins) is a versatile separation process that has been commercially available for many years. Ion exchangers are materials that can exchange one ion for another, hold it temporarily, and release it to a regenerant solution. The major concern of using resins is the steady decline that is observed in resin-exchange capacity (resulting from improper backwash, channeling of the solution through furrows in the bed of the resin, incorrect chemical application, mechanical strain, and/or resin fouling)³.

Diatomaceous Earth

Diatomaceous earth is “basically the skeletal remains of algal forms known as diatoms, which contain siliceous materials⁵.” These deposits have a high void capacity, and therefore are characterized by a high permeability. Diatomaceous earth filtration units have been around since World War II and have been adapted for municipal water supplies. They are compact and may be portable. These systems are applicable for the direct treatment of surface waters for removal of relatively low levels of turbidity. Diatomite filters consist of a layer of diatomaceous earth about 1/8-inch thick that is supported on a septum or filter element³. This thin precoat layer is subject to cracking, so it must be supplemented by a continuous-body feed of diatomite to maintain the porosity of the filter cake. The use of diatomite filters is usually restricted to use in municipal applications because of the problems that are inherent in maintaining a perfect film of diatomaceous earth³.

Sand Filtration (slow and rapid)

There are two methods of sand filtration: slow and rapid. Slow sand filtration is effective in providing a safe, potable water supply, but its use has limitations. These filters must be operated at relatively slow rates of sand bed because the average

turbidity of filtered water is limited to 10 ppm with a maximum concentration of 30 ppm⁴. Within these limitations, there arises a need for a complementary means in addition to straining action to obtain adequate filtration of the turbidities, bacteria, and colors in the water. Therefore, adhesion of substances to the sand media becomes increasingly important, and that is why slow operation rates are recommended.

Rapid sand filtration occurs at rates that are about 40 times faster than slow sand filtration rates by using coarser sand. This methodology aims to reduce filter areas by increasing the filtration rate when treating turbid waters without subsurface clogging of beds. Rapid sand filters are vulnerable (due to the possibility of excessive negative head in the filter) to the development of air bubbles in the sand, which cause a decrease in the filter's porosity and may result in a loss of filter capacity or cause unequal rates of filtration⁴. Another drawback of rapid sand filter systems is that materials carried over from the flocculating or sedimentation basin are deposited on the surface of the sand and may form clumps that vary from pea size up to 1 or 2 in. or more in diameter. This could lead to cracking or clogging of a filter bed⁴.

Novel Colloidal and Interfacial Approaches

There have been many novel colloidal and interfacial approaches to the removal of contaminants from water supplies. Some of these approaches include coating various filter media (sand, diatomaceous earth, fabric, microporous membranes) with metal hydroxides², metal oxides^{2,3}, metal peroxides⁶, or cationic surfactants². These water treatment procedures are capable of removing bacteria, viruses, and molecules.

Bacteria are single-celled living organisms having an average size of approximately 1000 nm. Viruses are tiny geometric structures that can only reproduce inside a living cell, and range in size from 20 to 250 nanometers. Both bacteria and viruses have a negative surface charge in the pH range of natural waters⁷. Most filter media also have a negative surface charge in this pH range⁸. Therefore, one would expect it to be difficult to filter bacteria under these conditions due to Coulombic repulsion, unless the surface of filter media is somehow chemically altered to minimize repulsive interactions. It has been found that coatings of metallic hydroxides, oxides, or peroxides on diatomaceous earth or sand significantly increase the zeta potential of these media⁹. This increase in zeta potential facilitates a greater attraction between the bacteria and filter media (i.e. increases the filter media's adsorption affinity for microorganisms), thereby enhancing filtration efficiency.

Although electrostatic interaction has been reported to be the predominant factor in microbial adhesion to surfaces, external factors such as pH, temperature, and ionic strength have been shown to be important factors as well. Adsorption/adhesion of bacteria generally occurs around the pH range of 3 to 6. At neutral pH, the bacterial cells are negative, so one would expect that lowering the pH acts to decrease the repulsion between the bacteria and the filter surface².

Other approaches to enhancing filtration included impregnation of coal with metallic hydroxides¹⁰, addition of positive charges to silica using organosilane derivative¹¹, and incorporation of metallic hydroxides into and on the surfaces of solids using *in situ*

precipitation⁶. One of the initial approaches to water purification involved the use of metallic hydroxides flocs (ferric hydroxide and aluminum hydroxide)¹². Microorganisms were adsorbed by these flocs and removed from water by settling or filtration. The problem with this approach is that these systems are not very efficient for removing microorganisms from large volumes of water. If the filter's pores are large, it may not retain the flocs (i.e. poor adhesion of the flocs to the filter), and if they are small enough the filters clog rapidly and greatly restrict the flow of water. This necessitates frequent backwashing of the media.

Filtration of Nanoparticles Using Surfactant Coated Filters

The removal of particulate contaminants is very important in many industries, such as water reclamation facilities, water treatment during camping or traveling, microelectronics and pharmaceutical industries. Filtration of particulate contaminants is dependent on several factors including particle size and physicochemical properties of the particles and the filter media. Large particles can be filtered by entrapment mechanism. As the size of the particle decreases, however, particle removal becomes more difficult and thus the interaction between the particles and the filter media must be increased to enhance filtration efficiency.

Deposition of submicron particles onto filters has captured the interest of many investigators over the years.¹³⁻²⁴ The main focus of these researches has been on the interactions between the particles and the filters. The dominant factors in submicron particle adhesion/deposition are the surface interaction forces and Brownian motion.^{18,21,24} Onorato and Tien¹⁸ have found that favorable interactions between particles and the filter can significantly increase particle deposition. They reported a 10-fold increase in the deposition of negatively charged particles on a positively charged filter relative to a negatively charged filter. Chang and Vigneswaran²⁴ have shown that, by decreasing the electrostatic repulsion between the particle and the filter surface, particle attachment can be greatly enhanced. Harley²⁵ et al. studied the adsorption of small particles onto larger particles of opposite charge. Their results support the implication that long-range interactions play an important role in the concentration of particles adsorbed and the rate of adsorption.

Conventional Filter

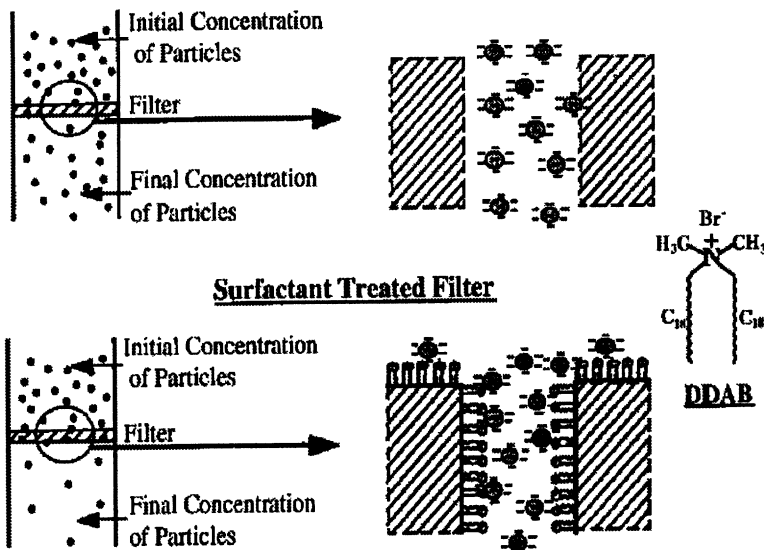


Figure 2. Schematic diagram illustrating particle capturing in conventional filter coated with a monomolecular layer of cationic surfactant

We plan to focus on the electrostatic attraction between the particles and the fibers of microporous polypropylene filters. We provide a unique method to lower the energy barrier between the particles and the filter surface and thus increase the deposition of these particles on the surface of the filter. Polypropylene is commonly used to make prefilters and filters because it is extremely inexpensive and very inert. Because most natural materials are negatively charged,¹⁵ we modified the microporous polypropylene filter with a monomolecular layer of cationic surfactant to give it a positive charge. The adsorption of negatively charged particles on the filters and the filtration efficiency of these surface modified filters were studied. This approach is useful not only for removing bacteria, viruses, or nanoparticles in waste water treatment, but can be equally useful in precious metal recovery processes (Figure 2).

Adsorption and Desorption of Surfactant from Filters
Microporous polypropylene filters were coated with a monomolecular layer of dimethyldioctadecylammonium bromide (DDAB) to give them a

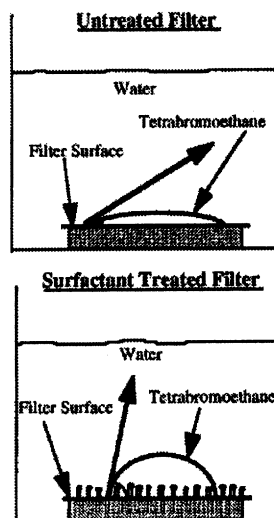


Figure 3. :Schematic diagram illustrating contact angle measurement for the microporous filters.

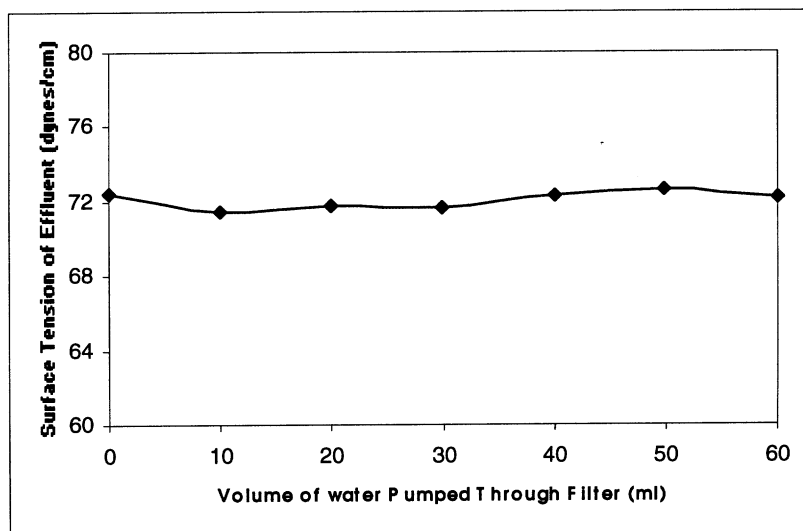


Figure 4. Surface tension of rinse water (rinse effluent) after it has been pumped through a 10 mM DDAB treated filters

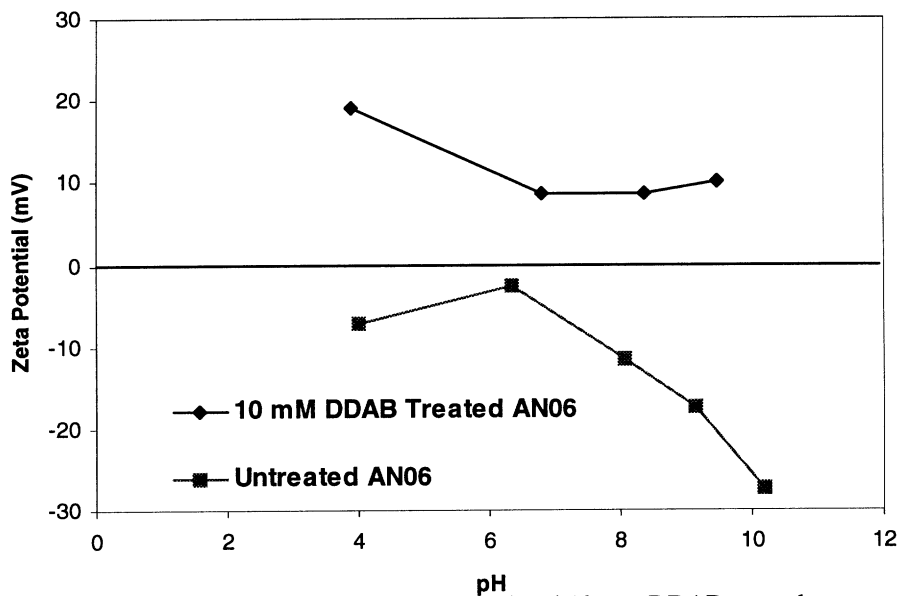
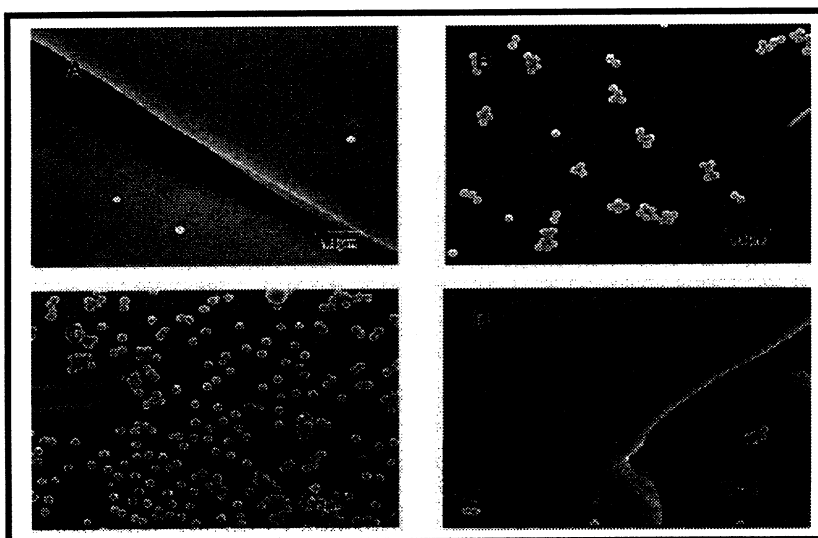


Figure 5. Zeta potential of untreated and 10 mM DDAB treated filters as measured by streaming potential method.

positively charged surface. DDAB is chosen because of the two hydrocarbon chains that can hydrophobically bond to the surface of the polypropylene and make this surfactant



- A. Untreated Filter and 197 nm negatively charged particles
- B. Untreated Filter and 200 nm positively charged particles
- C. 10 mM DDAB treated filter and negatively charged particles
- D. 10 mM DDAB treated filter and 200 nm positively charged particles

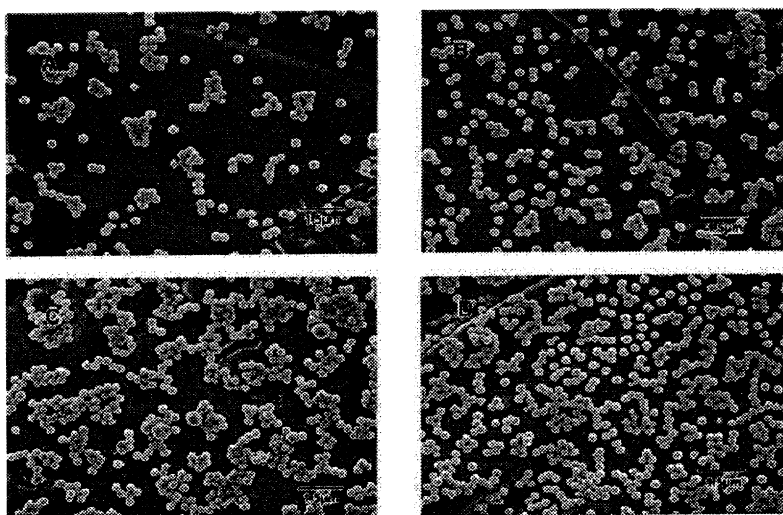
Figure 6. (A, B, C, D) Particle adsorption on filter surfaces at pH = 4.0 and 0.012 wt% solids particle concentration.

insoluble in water. Contact angle is used as a measure of the amount of DDAB adsorption at the surface of the filters. For contact angle measurements, carbon tetrachloride could not be used because it is too hydrophobic and immediately gets absorbed into the fibers upon contact. Therefore, liquid 1,1,2,2-tetrabromoethane (TBE) was used for contact angle measurements. High contact angle is an indication that the surface is hydrophilic or low contact angle indicates that the surface is hydrophobic (Figure 3). The average contact angle for the untreated filters is approximately 25° for water/TBE system on polypropylene filter surface. The standard method for the treatment of filters utilized 10 mM DDAB.

The adsorption strength of the cationic surfactant was tested by flushing the treated filters with different amounts of water and then measuring the contact angles. The amount of water pumped through the filters does not have a significant effect on the contact angle, which indicates that the surfactant is not desorbing. The effluents were collected for surface tension measurements (Figure 4). The results indicate that the surfactant is not desorbing as detected by surface tension measurements. From previous experiments, we have shown that 2.5×10^{-5} mol/L surfactant in water decreased the surface tension of water from 72.4 to 43.9 dynes/cm. Therefore, surface tension method should be able to detect DDAB in the range of micromoles per liter of water.

The zeta potential of the untreated filters indicates that the filters are slightly negatively charged while the surfactant treated filters exhibited a positive charge for the whole pH range (Figure 5). Since the untreated filters are slightly negatively charged, it is expected that negatively charged particles will not adsorb on the surface due to the electrostatic repulsion but positively charged particles will adsorb due to the electrostatic attraction. Figure 6A and 6B show adsorption of negatively and positively charged particles (200nm) on uncoated filter surface. Figure 6C and 6D show the adsorption of the same particles as 6A and 6B on DDAB coated filter surfaces

Particle adsorption is also a function of the suspension concentration. By increasing the suspension concentration, more particles will diffuse to the surface of the filter and adsorb on the surface. Effects of concentrations from 0.020 to 0.050 wt% solids are shown in (Figure 7), which illustrates that the number of particles adsorbed on the filter surface increases with their concentration in the aqueous phase.



Adsorption on 10mM DDAB treated AN06 filters at pH 4.0 :

- (A) 0.020 wt% solids; (B) 0.030 wt% solids;
(C) 0.040 wt% solids; (D) 0.020 wt% solids

Figure 7. Effect of suspension concentration on particle adsorption on treated filters.

Filtration Efficiency of DDAB Treated Filters

Filtration efficiency or percent removal is significantly enhanced with DDAB treatment. Each of the data points plotted in Figure 8 is an average of two runs. For the untreated filters, the filtration efficiency ranges from 5% to 10%, but after the filters were treated with 10 mM DDAB, filtration efficiency increased to 50% or 60% for the lower initial concentration range. The increase in capture efficiency is mainly due to the electrostatic attraction between the negatively charged particles and the positively charged polar head of the surfactant molecules on the filter surface. Since the average pore size is 0.6

•m, the increase in capture efficiency is partly due to the smaller pore size in certain regions of the filters due to surfactant adsorption in clusters. However, by examining the

results for the filtration at pH 10.0 (Figure 8), it is evident that the increase due to entrapment is not very significant since the filtration efficiencies of the treated and untreated filters are very similar.

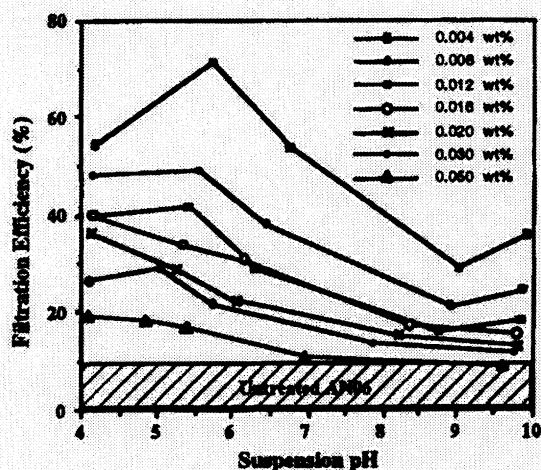


Figure 8. Capture efficiency of 10mM DDAB treated (0.6 mm) filters at various initial concentrations of 60 nm and different suspension pH values.

If entrapment is the main mechanism, then filtration efficiency should not be dependent on the pH of the suspension unless pH-induced flocculation is occurring. However, if pH-induced flocculation is taking place, then the capture efficiency of the untreated filters should increase as pH is lowered. This was not observed experimentally. In addition, quasi-elastic light scattering measurements showed that the average particle size did not increase with decreasing suspension pH, indicating an absence of flocculation at lower pH.

Since the particles are more negatively charged at higher pH values (due to a higher degree of ionization), higher

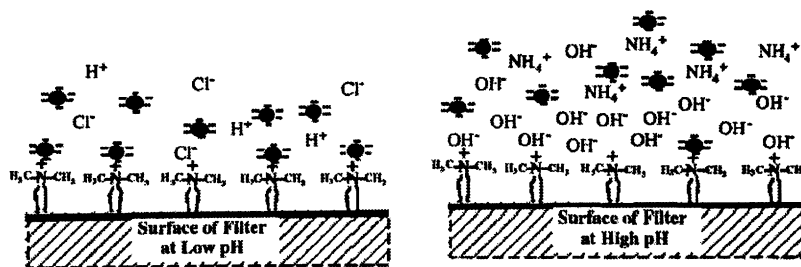


Figure 9. Schematic illustration of the mechanism of competitive adsorption at low and high suspension pH values.

filtration efficiency is expected at the higher pH range. However, the results show the opposite trend. Capture efficiency is higher at the lower pH range. This trend could be due to one of the following two effects: either the surfactant is desorbing at the higher pH range or competitive ion adsorption is taking place (Figure 9).

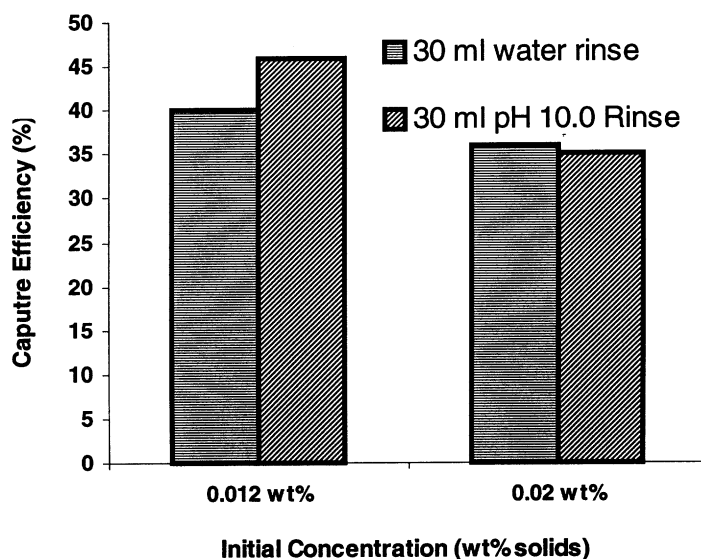


Figure 10. Comparison of capture efficiency of water rinsed vs. pH 10 solution rinsed 10 mM DDAB treated filters.

To determine if the surfactant is desorbing, the treated filters were flushed with pH 10.0 solution. The effluents were collected for surface tension measurements. If the DDAB is desorbing, then the surface tension of the effluents should be lower than that of the pure water due to the surfactants at the surface. Results indicate that there was no change in surface tension of the effluents. Further proof that the surfactants are not desorbing is offered by contact angle measurements. Even with 200 mL of pH 10.0 solution flush, the average contact angle of the surface, $70.7 \pm 12.7^\circ$, remains approximately the same as that for those that were flushed with 30 mL distilled water. Finally, the filters were rinsed with 30 mL of pH 10.0 solution, followed by 20 mL of distilled water, and then used to filter particles at pH 4.0. Results, as shown in Figure 10, indicate that the filters are just as effective as those that were rinsed with distilled water, which indicates that the surfactant is not desorbing at the higher pH values.

Since the surfactants are not desorbing, the only other explanation is that the hydroxide ions are competing with the negatively charged particles for the positively charged surfactant sites. At pH 10.0, for example, because of the high number of hydroxide ions and because of their higher mobility due to their smaller size, they can diffuse to the surface of the filter much faster than the large negatively charged particles. The adsorption of the hydroxide ions shields the charge of the surfactants from the particles, and therefore, the particles are not adsorbing to the surface of the coated filters at high pH (≈ 10) (Figure 9).

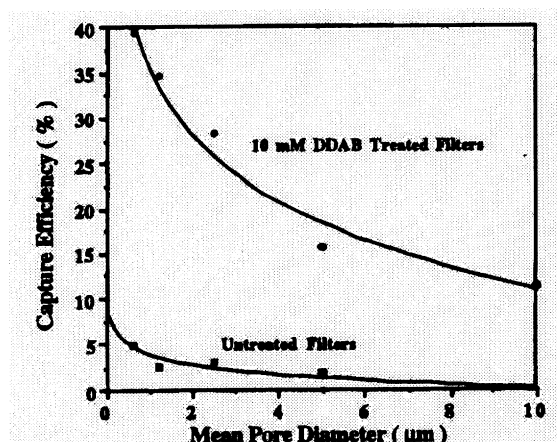


Figure 11. Effect of mean pore diameter on filtration efficiency of filters for 60 nm nanoparticles at pH 4.0 and initial concentration of 0.012 wt% solids.

Effect of Pore Diameter

The capture efficiency of filters with different mean pore diameters was also studied at pH 4.0. Both untreated filters and surfactant treated filters show a sharp decrease in filtration efficiency as the pore diameter of the filters increases (Figure 11). However, for the 10 mM DDAB treated filters, the capture efficiency remains much higher than for the untreated filters. The decrease in capture efficiency as pore diameter increases is expected, as the electrostatic attraction between the particles and filter surface is only effective within a certain distance. If the particles and surface are too far apart, each will not feel the presence of the other. When the pore sizes are too large, the particles do not have enough time to diffuse to the surface and no interaction takes place. Thus, the particles will pass straight through the filters.

Effects of Multiple Layers of Filters

The polypropylene filters used for these experiments are very thin (150 μm). For this type of filter to be effective in any application, multiple layers of filters must be used. With just four thin filters, more than 95% of the particles can be removed from the solution. As the number of filters increases, the increase in capture efficiency levels off (Figure 12).

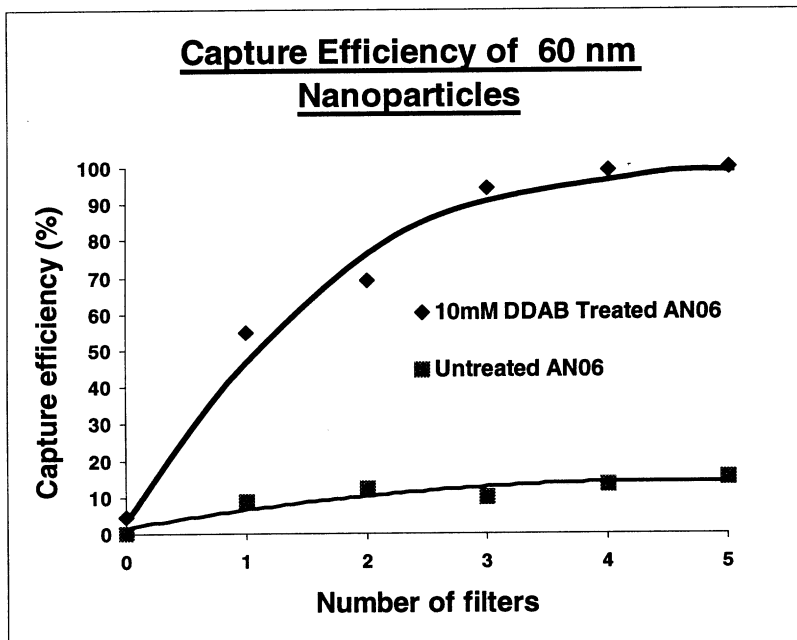


Figure 12. Effect of multiple filters on capture efficiency at pH 4.0 and initial particle concentration of 0.012 wt% solids.

Coating of Fabrics

The many versatile uses of polyester yarn (e.g. clothing, tire cord thread, conveyer belts, waterproof canvas, fishing nets), its availability, and its tenacity led to the incorporation of microdenier polyester textile fabric as a filtration medium for removal of nanoparticles and biological particles from water². The textured and woven surface of polyester increases the tortuosity of the flow path, thereby increasing the probability of particle collision with the filter surface. The fabric was treated with DDAB surfactant to enhance its filtration capabilities and make it more wettable. SEM images of treated and untreated fabric samples are shown below (Figure 13 A, B, C, D) at low and high magnification.

Zeta potential measurements revealed that the untreated fabric was negatively charged in the pH range of 4 to 9; while the surfactant treated fabric remained positively charged throughout this pH range. It was also shown that surfactant treatment leads to a decrease in pressure drop across the polyester fabric filters relative to untreated filters during water filtration. This is due to the increased wettability of the fiber surface by adsorption of DDAB. Low pressure drop is desirable because less energy would be required to pass the water through the filters. Kang et al. also found that increasing the number of filter layers served to greatly increase filtration efficiency for removal of bacteria (*E. coli* and *S. aureus*) (Figure 14).



Figure 13. Scanning electron micrograph of polyester textile fabric.

- A. Untreated fabric - woven pattern (low magnification)
- B. Untreated fabric - individual fibers of woven fabric (high magnification)
- C. 10 mM DDAB treated fabric - surfactant coating on woven pattern (low magnification)
- D. 10 mM DDAB treated fabric - surfactant coating on individual fibers of woven fabric (high magnification)

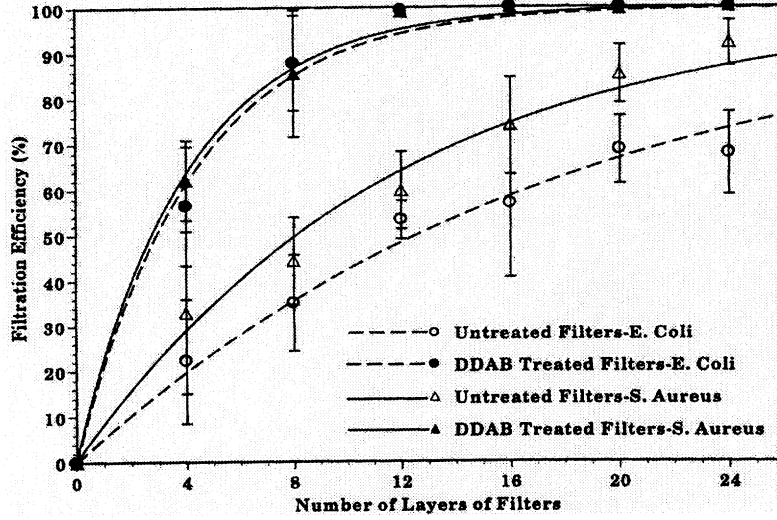


Figure 14. Filtration results of the polyester fabric filters for the removal of *E. coli* and *S. aureus* at pH 7.0.

Experiments involving filtration of bacteriophages yielded some interesting results, which added to the validity of the proposed role of electrostatic interaction in increasing filtration efficiency. The phages and their respective hosts were MS2 (*Escherichia coli* C-3000), Φ X-174 (*Escherichia coli* KC), and PRD-1 (*Salmonella typhimurium*), and their characteristics are given below:

TABLE 1: Characteristics and Properties of Bacteriophages.

Phage	Host	Size (nm)	Structure	Isoelectric	Capture efficiency for 12 filters coated with DDAB (%)
MS2	<i>E. coli</i> C-300	24	Icosahedral	3.9	95
PRD-1	<i>S. typhimurium</i>	63	Icosahedral	4.2	80
Φ X-174	<i>E. coli</i> KC	27	Icosahedral	6.6	20

Filtration results showed that the surfactant treated filters were not as effective at removing Φ X-174 as they were at removing MS2 and PRD-1. This result suggested that there is a strong correlation between the isoelectric point (IEP), the pH where the zeta potential is zero, and the filtration efficiency. When operating at a pH of 7.0, phages MS2 and PRD-1 have more of a negative charge than Φ X-174 (i.e. since the IEP of Φ X-174 is 6.6, it is only slightly negative at neutral pH). The filtration efficiency correlated directly with this, as MS2 had the highest removal levels, followed by PRD-1, with Φ X-174 having the lowest efficiency. The results of this study provided

conclusive evidence that the increase in filtration efficiency of surfactant treated fabric filters is mainly due to Coulombic attraction between the negatively charged bacteria or viruses and the positively charged surfactants on the filter surface².

Kang et al. then extended their study of fabric filters, and instead of DDAB coating, performed experiments with iron oxide coatings.² There are sixteen different forms of iron oxides, so in these tests, the form of the iron oxides was controlled to allow for the determination of the effects that each form has on filtration efficiencies. Three forms of the iron oxides (goethite, haematite, and magnetite) were coated on the textile fabric. Identification of the forms was confirmed by visual observation of the colors and by X-ray diffraction analysis of the precipitates formed under identical reaction conditions without the fabric².

The zeta potentials of the iron oxide coated filters, although negative, were still less negative than the zeta potentials of the uncoated filters. The coatings substantially increased wettability, which resulted in a reduction in the pressure drop across the filters during filtration. In the filtration of bacteria, *S. aureus* and *E. coli*, the iron oxide coated filters showed some improvement in filtration efficiency (as measured by the filter coefficients). Compared to uncoated filters, the improvements in filtration efficiencies of the goethite, haematite, and magnetite coated filters were 22%, 60% and 53%, respectively for *S. aureus* and 36%, 45% and 51%, respectively for *E. coli*².

In the filtration of bacteriophages, goethite had the highest filtration efficiency for removal of phages MS2 and PRD-1, and haematite and magnetite were partially effective in the removal of these phages. Phage ΦX-174, which is one of the most difficult phages to remove because of its poor adhesion to surfaces, had a very strong affinity for the iron oxide surfaces, having improvements in filtration coefficients (relative to uncoated fabric filters) of 985%, 1,035% and 406% for goethite, haematite, and magnetite, respectively².

Since filtration efficiencies for iron oxide coated fabric filters were not as high as those of surfactant treated fabric filters, attempts were made to increase the efficiency by coating the filters with mixtures of iron and aluminum oxides of varying ratios. These mixtures would serve to make the surface of the filter more electropositive, thereby enhancing electrostatic interaction between the filters and the bacteria (or viruses). These experiments were performed using the goethite iron oxide form. The zeta potentials of the mixed oxide surfaces were positive throughout the acidic pH range, unlike that of the iron oxide surfaces. It was found that the mixed oxides were better than the iron oxides in the filtration of the bacteria and the phages, including the ΦX-174².

Adsorption of Microorganisms to Coated Diatomaceous Earth particles

Coated Sand and Diatomaceous Earth

Farrah *et al.*^{26,27} have contributed substantially to the field of knowledge in this area. Their first efforts were focused on the adsorption of viruses by diatomaceous earth coated with metallic oxides²⁶, peroxides²⁶ and hydroxides¹². Diatomaceous earth was saturated with solutions of aluminum chloride, ferric chloride, calcium chloride, magnesium chloride, or manganese chloride, followed by treatment with deionized water, hydrogen peroxide (for the magnesium oxide sample), or ammonium hydroxide. Studies were performed around pH 7 to 9 to quantify the adsorption of phage MS2, Poliovirus 1 (P1), Coxsackievirus B5 (CB5), and echovirus 5 (E5) onto diatomaceous earth alone and diatomaceous earth in combination with filters. Since metal oxides are slightly soluble in water and are likely to contaminate filtered water with ions or increase the pH of filtered water to objectionable levels (due to formation of metallic hydroxides), the reported results reflect the use of diatomaceous earth coated with manganese oxide or ferric oxide, which are relatively insoluble²⁶. The adsorption results for the diatomaceous earth and filter system with coatings of metallic oxides or peroxides are given in graphical form below (Figure 15) (Note: the adsorptions of CB5 and E5 were negligible, and as such, are not shown):

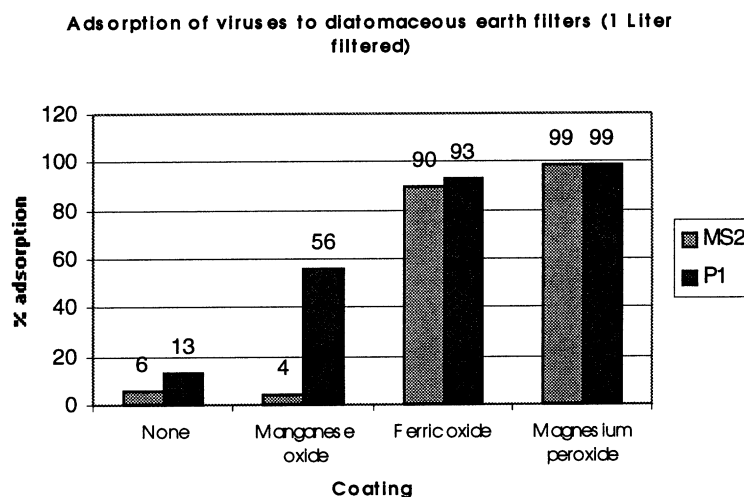


Figure 15. Dechlorinated tapwater with added viruses was passed through two 47-mm filters in tandem that contained 3.5 g of diatomaceous earth (pH 6.9).²⁶ Values represent the average and range of duplicate determinations.

Farrah *et al.*²⁷ found that using the coated diatomaceous earth in combination with filters serves to increase the filtration capacity relative to coated diatomaceous earth alone. It was later discovered (in experiments with metallic hydroxide coatings) that the problems of poor floc adhesion to the filters and clogging of the filters could be eliminated by modifying the coating method (by *in situ* precipitation of individual metallic salts or combinations of two metallic salts)²⁷. This modification led to incorporation of precipitate within the pores, and greatly increased the concentration of

metals on the diatomaceous earth. Varying the salts for coating led to an increase in the zeta potential of the diatomaceous earth, which directly reflected the adsorption affinity of the viruses to the filter media (i.e. the more electropositive the zeta potential, the greater the adsorption efficiency will be)²⁷. It also became evident that combinations of metallic salts (e.g. mixing ferric chloride with aluminum, calcium, and magnesium chloride) provided coatings that significantly increased filtration efficiency relative to coatings with single salts (See Table 2 below).²⁷

TABLE 2. Percentage of virus adsorption and correlation between zeta potential and virus adsorption by modified diatomaceous earth^a

Treatment of diatomaceous earth ^b	Zeta potential (mV)	% Virus adsorption in:			
		Buffer		Trickling filter effluent	
		MS2	P1	Indigenous phage	P1
None	-71	1 ± 2	47 ± 7	0 ± 0	45 ± 11
Rinsed with ammonium hydroxide	-66	4 ± 4	63 ± 11	0 ± 0	36 ± 14
1 M AlCl ₃	-22	>99	99 ± 2	>99	98 ± 3
1 M CaCl ₂	-55	4 ± 4	68 ± 4	3 ± 5	30 ± 3
1 M MgCl ₂	-21	86 ± 6	>99	46 ± 3	83 ± 14
1 M FeCl ₃	-48	98 ± 4	99 ± 2	92 ± 6	93 ± 5
1 M FeCl ₃ + 1 M AlCl ₃	-37	92 ± 10	>99	97 ± 5	>99
1 M FeCl ₃ + 1 M CaCl ₂	-12	>99	>99	71 ± 10	82 ± 11
1 M FeCl ₃ + 1 M MgCl ₂	-24	>99	>99	58 ± 22	78 ± 3
<i>r</i> ^c		0.857	0.873	0.679	0.726
<i>R</i> ^{2c}		0.734	0.762	0.462	0.527

^a Adsorption of viruses in buffer (pH 8.0) and in trickling filter effluent (pH 7.8) was compared to the zeta potential of diatomaceous earth at pH 8.0. Samples (100 ml) with approximately 10⁵ PFU of poliovirus 1 (P 1) or coliphage MS2 per ml or 10² PFU of indigenous phages per ml were passed through 0.5 g of diatomaceous earth in 25-mm holders. Values represent the mean ± standard deviation of triplicate determinations.

^b Diatomaceous earth was modified by using the indicated salt solutions with *in situ* precipitation as described in the text. Samples of

untreated diatomaceous earth and diatomaceous earth rinsed with 3 M ammonium hydroxide served as control samples.

^c Correlation between zeta potential and percent adsorption.

Eventually, Farrah et al. decided to expand their studies to consider coating sand with metallic hydroxides.²⁸ The coating increased the concentration of metals associated with diatomaceous earth and sand particles, their zeta potential, and their ability to adsorb microorganisms in water. The maximum adsorption of microorganisms was achieved using particles coated with a combination of ferric hydroxide and aluminum hydroxide (see Table 3)

Table 3: Comparison of the removal of microorganisms by diatomaceous earth treated sequentially with two metal salts or a single metal salt

Single Treatment	PRD-1 Removal (Log ₁₀)	Sequential treatment Salt#1 → Salt#2	PRD-1 Removal (Log ₁₀)
AlCl ₃	2.54±0.04	0.25 M FeCl ₃ → 0.1M AlCl ₃	2.95±0.05
FeCl ₃	1.71±0.03	0.25 M FeCl ₃ → 0.5M AlCl ₃	3.56±0.05

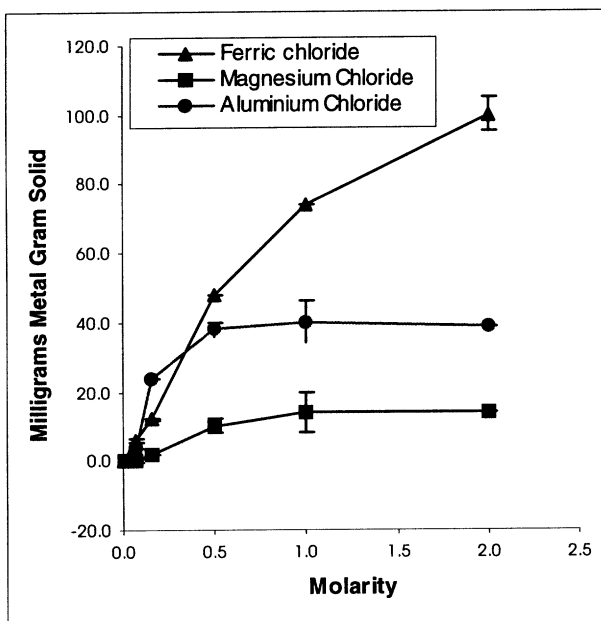


Figure 16 : Metal Composition of Modified Diatomaceous Earth

diatomaceous earth particles greatly increased the ability of the particles to adsorb bacteriophages in batch tests (Figure 18). Increasing the zeta potential to -10mV permitted a reduction of greater than $2.5\log_{10}$ in the numbers of the three phages tested.

Increasing the salt concentration increased the amount of metal deposited on the diatomaceous earth (Figure 16). The amount of aluminum and magnesium deposited did not increase much after the concentration of modifying solution was increased past 0.5 M while the concentration of iron deposited on diatomaceous continued to increase to 2 M. The addition of metallic hydroxides increased the zeta potential of the diatomaceous earth particles. The zeta potential continued to increase as the salt concentration of the modifying solution was increased (Figure 17). As previously mentioned, changing the zeta potential of

Data in Figure 18 were obtained using diatomaceous earth coated with aluminum hydroxides. Similar results were obtained with diatomaceous earth coated with ferric hydroxide or magnesium hydroxide (data not shown).

The adsorption of bacteria to diatomaceous earth coated with aluminum hydroxide was similar to adsorption of phages in that increasing the zeta potential increased the adsorption (see Figure 19). It is clear that the zeta potential of the modified solids is directly related to the adsorption of bacteria and bacteriophages. Efficient adsorption of these microorganisms occurs once the zeta potential is raised above approximately -30 mV (for phages) and -10 mV (for bacteria).

Removal of *Cryptosporidium* from water²⁹

In another study, (Shaw, Walker, Koopman)²⁹ the surface coating of hydrous iron aluminum oxide on Ottawa sand was investigated to improve the removal of *Cryptosporidium* oocysts from water by filtration. *Cryptosporidium* in water supplies is often a problem in United States and elsewhere²⁹. This protozoan causes severe

gastroenteritis that is potentially fatal to infants and immunocompromised individuals and that does not respond well to available therapies. *Cryptosporidium* carries a negative surface electrical charge in the pH range of natural water sources, as do natural filter media such as sand and diatomaceous earth. Thus, removal of the oocysts particles by filtration (without chemical conditioning) is difficult because of electrostatic repulsion between the oocysts and the filter media surface. Coatings of metallic hydroxides, oxides and peroxides on filter media improved the removal of bacteria and viruses from, and reduced turbidity in, water and waste water²⁷.

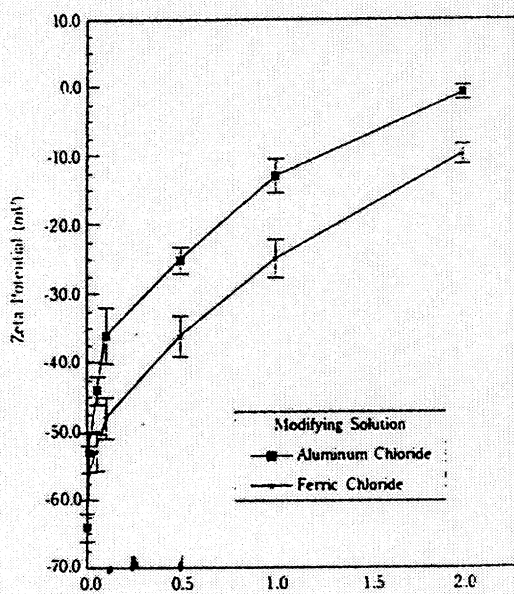


Figure 17. Zeta Potential (mV) of diatomaceous Earth vs Molarity of Modifying solution

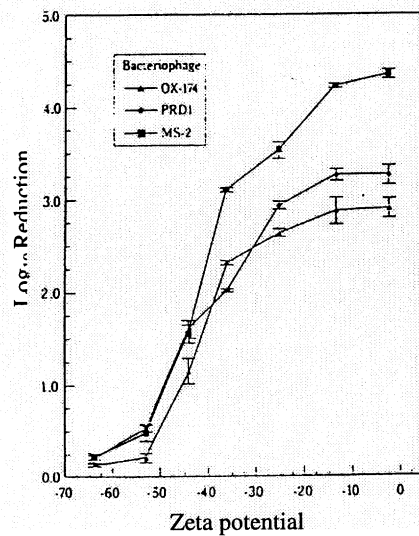


Figure 18 : Removal of Bacteriophage from water vs Zeta potential of modified Diatomaceous Earth.

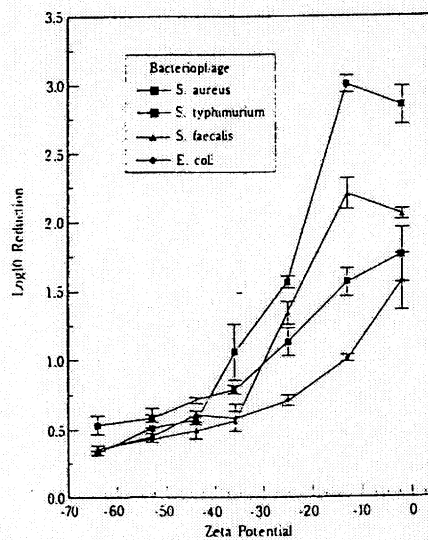


Figure 19 : Removal of Bacteria (*S. aureus*, *S. typhimurium*, *S. faecalis*, *E. Coli*) from Water vs Zeta Potential of Modified Diatomaceous Earth.

Coating Ottawa sand with the surface coating of hydrous iron aluminum oxide, increased the zeta potential from -40 to $+45$ mV at pH 7.0, increasing the potential for interaction with negatively charged (-25 mV at pH 7.0) *Cryptosporidium* oocysts. Water

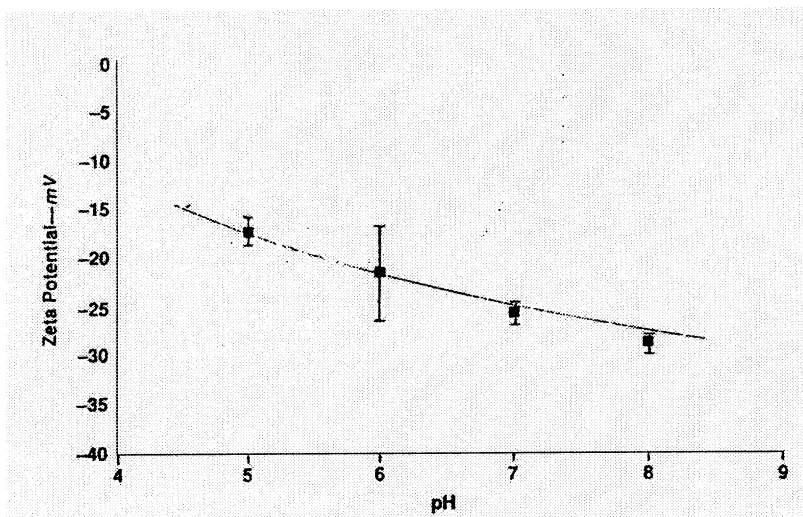


Figure 20 : Zeta Potential of Cryptosporidium oocysts versus pH.
(Error bars represent ± 1.0 standard deviations.)

seeded with Cryptosporidium oocysts was passed through parallel columns of uncoated and coated sand at superficial velocities of 200-800 m/d (3.15-14 gpm/sq. ft) and column lengths of 100-400 mm (4-16 in.). In every trial, coated sand removes significantly more oocysts than uncoated sand.

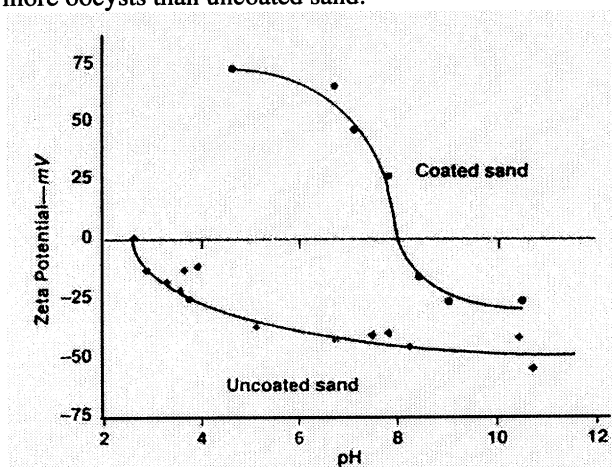


Figure 21 : Zeta potential of Uncoated sand and sand coated with iron and aluminium (hydr)oxide

The zeta potential of Cryptosporidium was negative and ranged from -17mV at pH5.0 to -29 mV at pH8 (Figure 20). The zeta potential of the uncoated and coated sand was determined by using streaming potential apparatus. The zeta potential of uncoated sand

ranged from 0 mV at pH 2.6 to –55 mV at pH 10.7 with isoelectric point at pH 2.5 (see Figure 21). Coating the sand with Fe Al (hydro)oxides made the zeta potential more electropositive. The isoelectric point of the coated sand was 8.0, which is between the range of 4.6-6.8 for Fe Oxides (Fe_2O_3 , hematite; FeOOH , goethite) and of 7.8-9.1 for Al oxides (Al_2O_3 , corundum; AlOOH , boehmite). The uncoated sand had an Fe content of 0.11 ± 0.026 mg/g sand and Al content of 0.014 ± 0.003 mg/g sand (Table 4)

Table 4. Fe and Al contents of coated and uncoated sand used in various studies.

Sand Size mm(in.)	Sand coated	Fe (mg/g sand)	Al (mg/g sand)
0.6-0.7(0.02-0.03)	No	0.11	0.01
0.6-0.7(0.02-0.03)	Yes	1.36	1.22

The Al to Fe molar ratio in the coating was 2:1 as compared with 1:1 in the coating solution. The increase in the Al content may be because the Al coating binds better to sand.

The change in the concentration of particles with respect to column length in a granular media filter can be modeled by the first order relation:

$$\frac{\partial c}{\partial z} = -\lambda c \quad (1)$$

in which c is the concentration at length z , and λ is the coefficient that characterizes the filter media. Under conditions in which the coverage of filter media by particles is low enough that it does not affect the rate of particle attachment, this expression may be integrated over the length of the filter column, yielding:

$$\frac{c_{eff}}{c_{in}} = e^{-\lambda L} \quad (2)$$

in which c_{in} is the influent concentration of particles, c_{eff} is the effluent concentration of particles. The fractional removal efficiency (η) can be expressed as:

$$\eta = \left[1 - \left(\frac{c_{eff}}{c_{in}} \right) \right] = 1 - e^{-\lambda L} \quad (3)$$

Equation (3) indicates that as the filter coefficient increases, the removal efficiency for a given column length also increases. The filter coefficient was calculate by least square nonlinear fit of equation (3) to the curve in Figure 22. Filter coefficients of coated sand were 2.9 times as high as those of uncoated sand, indicating that coating can significantly improve the removal of *Cryptosporidium* by granular media filters.

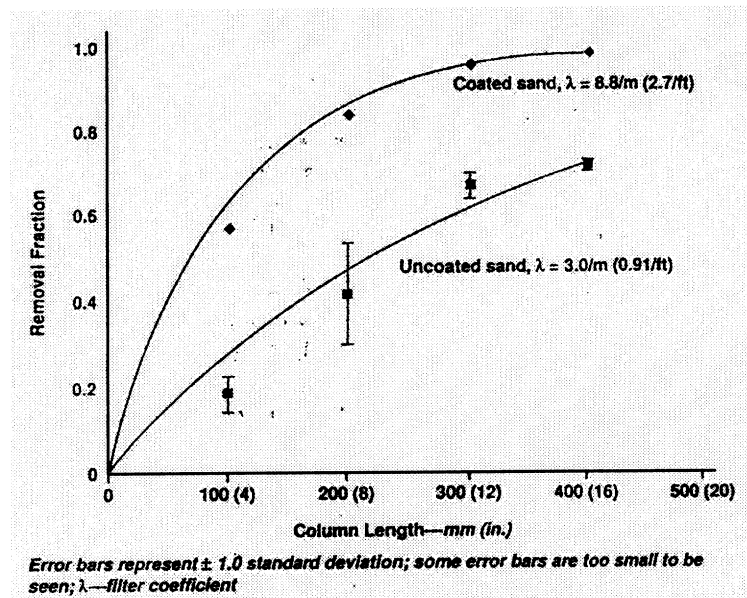


Figure 22 : Effect of column length on removal of *Cryptosporidium* oocyst in sand filters

Analysis of bacterial deposition on Metal (Hydr)oxide-coated Sand filter Media

Several recent papers have described enhanced removal efficiencies in filtration systems utilizing metal oxides and metal hydroxides^{30,6,31,28}. In spite of some success in this area, a fundamental understanding of why deposition is improved to a greater degree in some coatings and not in others often appears to be lacking. Improved understanding of the mechanisms influencing particle attachment could aid in the design of more efficient filter media in a wide range of industrial applications. Bacterial adhesion, however, is difficult to quantify at a fundamental level. Hydration forces³², hydrophobic interactions³³⁻³⁶, macromolecular bridging³⁷, surface roughness³⁸, and electrical double-layer and Van der Waals forces^{30,39-42} have all been proposed as possible influences on adhesion. Many of these factors, however, are difficult to experimentally measure for microbial attachment to granular media. An arguable exception to this observation is the measurement of zeta potential and its influence on particle interaction forces. This study attempts to exploit these electrical double-layer and Van der Waals forces with the use of metal hydroxide coatings in order to improve microbial adhesion.

The role of electrical double-layer and Van der Waals forces in particle deposition is often viewed in terms of DLVO theory^{43,44}. Figure 23 illustrates two regions of particle deposition predicted by DLVO theory for the interaction between two charged surfaces. The dashed line represents the interaction expected between a strong negatively charged filter such as Ottawa sand and a negative bacteria particle. This line falls in a region

where the rate of particle attachment to the surface is controlled by double-layer repulsive forces and the resulting interaction energy barrier. As the magnitude of these like charges on the interacting particles decreases the DLVO theory predicts the Van der Waals force to dominate at short separation distances. This eventually results in a finite energy barrier around 10 kT where the particle attachment event (controlled by the energy barrier) no longer determines particle capture (solid center line). For an oppositely charged filter and particle, the energy barrier disappears such that the rate of deposition is controlled by the rate of diffusion or convective transport of bacteria to the

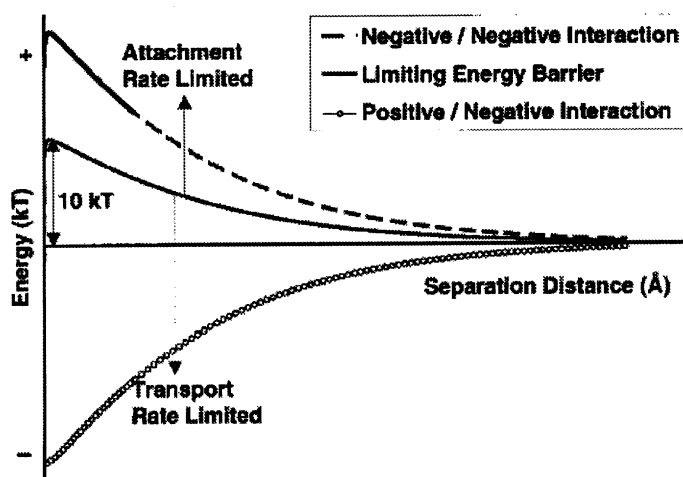


Figure 23. Set of hypothetical Helmholtz potential energy curves illustrating the expected interaction energies for oppositely charged particles (lower line), and similarly charged particles with increasingly larger charge magnitudes (upper dashed line). The solid center line represents the boundary between attachment rate limited deposition and transport limited particle deposition.

surface⁴⁵. Alteration of the surface potential of the granular filter medium to be positive, as observed for the metal-hydroxide-rich coatings used within this study, removes the repulsive double-layer interaction. Two oppositely charged surfaces should therefore experience stronger attraction over greater separation distances³⁰. This favorable interaction is shown in Figure 23 as the lower line and represents a case in which the kinetic rate constants of deposition should be controlled by particle transport to the filter surface. Most bacteria are negatively charged because of the predominance of the anionic groups present within the cell wall (carboxyl, amino, and phosphate groups); therefore, a positively charged filter surface should enhance particle deposition. Metal hydroxides were chosen for this study over metal oxides because of the experimental and theoretical observation that the hydroxides tended to provide a more positively charged surface^{46,47}.

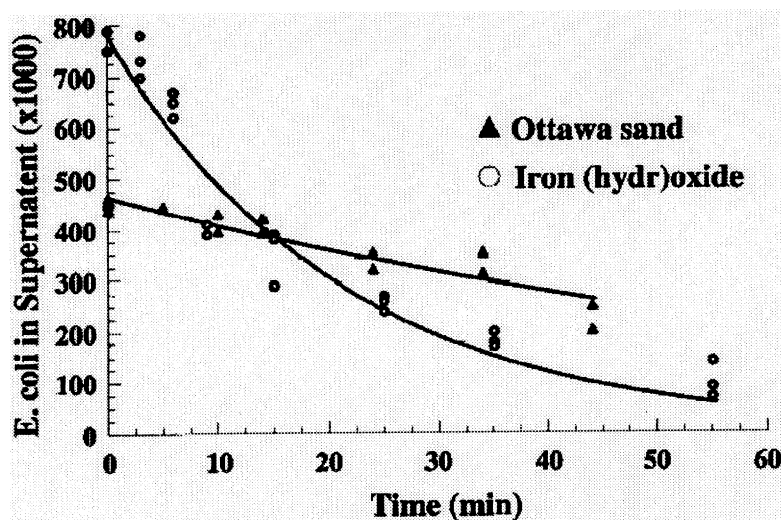


Figure 24. Sample set of data representing *E. coli*–Ottawa sand (solid triangles) and *E. coli*–iron (hydr)oxide (open circles). This graph is intended to show the differences between a filter with a higher kinetic rate constant, iron (hydr)oxide, and one with a low rate constant, Ottawa sand. All curves were fitted with an exponential curve using nonlinear least-squares regression.

In this study, bacterial deposition kinetics were measured for precipitated coatings rich in metal hydroxides to investigate the role of DLVO forces (electrical double-layer and Van der Waals forces) in filtration. The surfaces examined were Ottawa sand, aluminum (hydr)oxide coated Ottawa sand, iron (hydr)oxide coated Ottawa sand, and mixed iron–aluminum (hydr)oxide coated Ottawa sand. Rates of microbial deposition were compared for these different granular media and bacterial strains *Streptococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Escherichia coli*. Through the analysis of deposition results, the influence of surface potential of the metal (hydr)oxide coatings on bacterial deposition were determined within systems of granular media.

Deposition results

The bacterial deposition data was modeled as a first-order process (Eq. $C(t) = C_0 e^{-kt}$) and characterized with corresponding first-order kinetic rate constants. The model was observed to adequately fit the deposition data as a function of time, and a few sample sets of data have been included as Figure 24. The resulting kinetic rate constants, shown in Figure 25, indicated a statistically significant increase in the removal of microbes for the metal (hydr)oxide surface coatings. In addition, the average kinetic rate constants observed for the aluminum (hydr)oxide and the mixed (hydr)oxide were statistically similar as $k = 0.0604 \pm 0.006 \text{ min}^{-1}$ and $k = 0.0650 \pm 0.006 \text{ min}^{-1}$, respectively, suggesting bacterial deposition to be transport limited in the case of these two coatings. These two electropositive coatings had an average kinetic rate constant 1.5 times larger than what

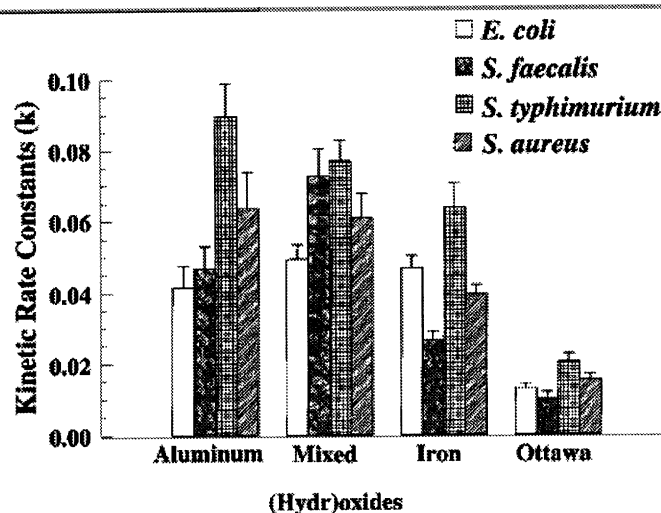


Figure 25. Bar chart showing the kinetic rate constants of deposition of the four microbial species on the four filter surfaces. Values were determined from fitting an exponential model to the deposition data. The data has been presented as a function of the different metal (hydr)oxide coatings (labeled along the bottom axis). The reported uncertainties for the parameters are the standard errors from the regression analysis.

was observed for the iron (hydr)oxide coating and 5 times larger than the rate constant values observed for the untreated Ottawa sand. Even the iron-coated medium with a zeta potential of -50 mV (40 mV more positive than the uncoated Ottawa sand) showed rate constants on the average 3.5 times greater than the constants seen for the Ottawa sand media.

DETOXIFICATION OF BLOOD OF DRUG OVERDOSED PATIENTS

Interfacial and colloidal strategies discussed above are not only relevant to the filtration of water, but they can be extended further to solve a major medical problem of saving patients suffering from drug overdose. Drug toxicity in humans as a result of accidental ingestion, therapeutic miscalculation, illicit drug usage, or suicide attempt is a major health care problem globally (2.4 million cases per year), not only in terms of cost but also in the context of increased patient morbidity and mortality. For example, almost 300,000 people in the U.S. are admitted annually to the hospital through emergency rooms because of drug toxicity, costing the country more than ten billion dollars per year in health care expenses and lost employee productivity. Unfortunately, the vast majority of life threatening drug intoxications do not have specific pharmacological antidotes to reverse their adverse effects.

Precedents for human internal use of bioparticles include Intralipid (propofol) and cationic liposome (delivery of gene therapy products), which are used as drug vehicles for highly lipophilic drugs, and hydroxyethylstarch (hetastarch), a blood expander. However, use of engineered biocompatible nanoparticles to reverse overdosed drug intoxication is a completely novel idea and approach.

The nanoparticles proposed for drug toxicity reversal may be of several types including “soft” particles (microemulsions, gels), “hard” particles (porous silica or metal oxide

particles or nanotubules of silica or carbon), and soft/soft or soft/hard particles (particles with oil core and gel, silica, siloxane, or metal oxide shells).

Two drugs were selected for testing; amiodarone, a very effective antiarrhythmic agent, and amitriptyline, the most widely prescribed tricyclic antidepressant in the U.S. Overdose of these drugs is a major cause of hospital admissions due to cardiac toxicity and is a major vehicle for committing suicide. Both of these drugs are highly lipophilic (hydrophobic). A flow sheet showing the types of nanoparticles in relation to the drug removal mechanisms is shown in the Figure 26 below.

Different types of novel nanoparticles are developed that can effectively reduce the free blood concentration of toxic drugs. We are trying to develop a fundamental understanding of how particle design (e.g. material type, porosity, templating, functionality) affects drug removal by alternate mechanisms (absorption, adsorption, enzymatic modification) in simple and complex media, and test the efficacy of the nanoparticles for reversing drug toxicity *in vitro* and *in vivo*.

Advantages of Proposed Nanoparticulate Systems over Alternative Therapies for

<u>Functionalized Particulate Systems</u>	<u>Features</u>
Soft Particles (microemulsions)	<ul style="list-style-type: none"> • Nanoscale oil core • Fluid surface film
Soft/Hard Particles (Core-Shell/Porous/ Gels/Nanotubes)	<ul style="list-style-type: none"> • Hydrophobic core • Porosity allows drug penetration
Templated Particles (Porous/Gels/Nanotubes)	<ul style="list-style-type: none"> • Hard surfaces activated for specific adsorption of toxin
P450 Enhanced Nanoparticulates	<ul style="list-style-type: none"> • Enzyme in oil core or bound to hard surfaces degrades toxin

Figure 26 : Types of Nanoparticles and their features

Drug Overdoses

Alternative approaches to drug toxicity reversal include: (1) infusion of water-soluble plasma enzymes, (2) purified drug-specific antibodies, (3) hemodialysis, and (4)

specific pharmacological antidotes. In each case, the proposed nanoparticles have significant advantages over the alternative, as described below.

One of the major objectives of this research project is to use nanoparticles, containing an optimized hydrophobic internal environment with regards to P450 (an oxidase enzyme that degrades many biological substrates in humans) efficiency, as “enzymatic platforms” in the blood. Since P450 enzymes degrade a wide variety of xenobiotics, including drugs, particles containing these enzymes could thus augment the drug detoxification function of the liver and other organs. Prompt and efficient biodegradation of a wide variety of drugs can be achieved by a complementary strategy of designing a nanoparticle with soft and hard characteristics and an embedded enzyme. Partitioning of a toxic drug from the blood stream into the lipid environment of a nanoparticle containing P450 enzyme genetically engineered for high efficiency should not only effectively and promptly reduce the free blood concentration of drugs in the blood, but also concentrate the toxic drug in an area adjacent to the active enzyme. This in turn would promote extremely efficient catalysis and degradation of the toxic molecules. This approach is advantageous relative to direct infusion of enzymes into the bloodstream, because local concentrations of enzymes in the hydrophobic particle interior will be higher than those in the blood, and the P450 enzyme system to be used in the present research metabolizes a far wider range of drugs than water-soluble plasma enzymes such as butyrylcholinesterase.

Hemodialysis is not appropriate for the vast majority of life threatening drug overdoses for several reasons. First, many drugs that cause toxicity in humans are not susceptible to removal from the body by hemodialysis, either due to their physicochemical properties or their huge volumes of distribution. Second, the rapidity of removal of drug from the blood must be taken into account. Hemodialysis is slow (e.g., hours) to remove drugs at toxic levels from the blood stream. The intoxications of amiodarone and amitriptyline are frequently life threatening because of their severe effects on cardiac function, and treatment under these conditions must be initiated immediately or the patient may die. Furthermore, arterial and venous lines must be placed in the patient, delaying the initiation of treatment.

Finally, pharmacological antidotes that can be used to quickly and selectively reverse the deleterious physiological effects of drug overdoses are rare. This is not surprising, because pharmacological antidotes are most effective in cases where the drug in question mediates a single type of receptor. However, many, if not most, therapeutic drugs (e.g., amiodarone, amitriptyline) have toxic tissue effects that are not mediated by one specific type of receptor. More commonly, these drugs modulate the function of a number of different cellular targets (e.g., receptor, ion channels, and/or second messenger pathways).

A. Drug Removal by Mechanism A: Absorption of Drug into Particles

1. Soft Particle Synthesis - Microemulsions

Microemulsions are established media for absorption, transport, and release of biological materials.⁴⁸⁻⁵¹ The interfacial energy of oil-in-water microemulsions is extremely low and, therefore, provides a potential method for the extraction of lipophilic molecules like amiodarone and amitriptyline into the oil core. A

number of in vitro studies have been performed for the solubilization of lipophilic drugs and local anesthetics into oil-in-water microemulsions using anionic and ionic surfactants.⁵²⁻⁵⁴ The same media have been evaluated for parental delivery of fluripufen⁵⁵. No attempt has previously been made to employ microemulsions for toxicity reversal of overdosed drugs in blood. To evaluate which and to what degree the experimental variables may impact successful overdose drug removal, oil-in-water microemulsions have been formed and characterized using biocompatible Pluronic emulsifiers and pharmaceutically accepted oils.

2. Soft-Hard Particle Synthesis

a) Polymerizable microemulsions.

This area of investigation follows directly from that described above. Use of a polymerizable surfactant can result in encapsulation of the oil core, thus providing enhanced physical integrity to the drug-absorbing particle. The thus-formed polymer membrane will have different diffusivity than the monomeric surfactant layer around the core.

b) Microgels

Polymerization of acrylamide monomers in reverse microemulsions yields highly porous polymeric sponges referred to as microgels.^{56,57} The vast amount of interstitial space of these particles accommodates absorption or filtration of dissolved or solid materials. They are used, for example, in the filtration of beer prior to bottling. Both the size of microgel particles and their pore volumes can be controlled by emulsion chemistry. "Smart" gels can be envisioned and prepared which may have selective sorption characteristics^{58,59,60,61} allowing uptake of only desired targets, i.e. amiodarone and amitriptyline.

c) Micellar cores with hard shells

Micelles, like microemulsion droplets, are capable of absorbing overdosed drugs into their oil cores. Single micelles or a dispersion of micelles can be encapsulated inside a porous shell composed of metal oxide or carbonate. The shell material may or may not contain pores templated to allow selective molecular filtering and facilitate drug removal by the nanoparticles. Silica and other metal oxide films and particles have previously been templated for selective transport of or binding to a number of organic molecules including dyes and biomolecules⁶²⁻⁶⁴. The application of this technology to the formation of a molecularly selective capsule around a microemulsion or micellar core has not taken place even though the formation of untemplated metal oxide shells around many other cores has been achieved^{65,66}. Experiments have been carried out to obtain fundamental information on how a micellar core effects or controls the ability to template pores of a shell metal oxide or carbonate for molecular filtering of amiodarone and amitriptyline. Another dimension of this effort has been to hydrophobize by silation the pore surfaces in the metal oxide shell as a possible way to improve the removal of drug from a water-based medium. A hydrophobic drug should be transported to the particle core more efficiently if pore surfaces are made hydrophobic.

d) Polymer Cores with Hard Shells

1. Hydroxyethylstarch Cores

Hydroxyethylstarch, otherwise referred to as hetastarch, is commonly used in solution as a biocompatible injectable in medicine. Its polar, polyhydroxylated structure is not conducive to dissolving or binding a hydrophobic drug. Therefore, hetastarch will be acylated to convert some hydroxyl groups into the corresponding esters, making it a better reservoir for amiodarone and amitriptyline. An example of an acylating agent is 3,5-dinitrobenzoyl chloride. Either the solid hetastarch or its solutions is encapsulated inside a templated metal oxide shell, using techniques similar to those developed above in section A.2.c. The templated pores are small enough to prevent diffusion of the core macromolecules out of the particle, and also to prevent diffusion of esterases, which could liberate irritant 3,5-dinitrobenzoic acid, into the core. The aromatic rings to be attached to hetastarch by acylation are pi-electron deficient and form complexes with drug analogues having pi-electron rich aromatic rings in their structures. This phenomenon may occur at least with amiodarone and, if so, will extend the technique beyond complexing of biologically significant molecules by trinitrobenzene⁶⁷. There is no precedent for this technology applied to drug removal.

2. Poly-N-vinylpyrrolidone cores

Poly-N-vinylpyrrolidone can be classified as a "miracle molecule" for medical application. It is soluble in water and is known to strongly bind several common drugs and eliminate them through the kidney⁶⁸. These discoveries were reported prior to 1978, after which time little new data on use of the polymer for medical purposes has appeared. In the present study, the solid polymer or an aqueous solution is encapsulated inside a hard shell composed of a material capable of allowing diffusion of amiodarone and amitriptyline into the core and come into contact with the polymer, but not allowing the latter macromolecule to diffuse out. As with research discussed in section A.2.d.(1), the pores in the shell material may be templated to allow selective molecular filtering of only the target toxic drugs to enter the core.

e) Oil-filled Nanotubules

It is well known that there are now available several types of nanotubules having different compositions, diameters and lengths. Very little is known about their pharmacological properties, but speculation is that they will be found to be generally safe for internal medicine and will be employed in drug delivery formulations. No one yet has evaluated them as components for removal of toxic drugs. Pioneering research at the University of Florida has demonstrated that nanotubules having variable compositions can be prepared using a low temperature template synthesis method.⁶⁹⁻⁷¹ Chemistry is carried out on the walls of the straight pores in a microporous aluminum membrane to deposit a tubular film of new material. After dissolution of the membrane template, the nanotubules can be isolated for further scientific evaluation. It is proposed that such tubules, as well as ones obtained by other high-energy processing routes, be filled with pharmaceutically acceptable oils like those to be used in section A.1. for making microemulsions.

B. Drug Removal by Mechanism B: Adsorption of Drug to Hard Particle Surfaces

1. Hydrophobized Nanoparticle Synthesis

a) Nanoparticle exteriors

Partition coefficients for distribution of many lipophilic drugs between aqueous and organic liquid phases are known either experimentally or can be accurately calculated using the Hansch quantitative structure-activity relation (QSAR) approach⁷². The "pi" constants from QSAR are widely used to predict pharmacological activity of analogues within a class of drugs before synthetic efforts begin. Attempts are being made to correlate QSAR constants with the ability of a fatty chain, attached to various types of metal oxide nanoparticles via silation, to absorb amiodarone and amitriptyline from aqueous solution. This concept has been successfully investigated in the development of chromatography columns, but not for removal of toxic drugs. A series of metal oxide nanoparticles having different porosities were synthesized by treatment with silating agents using traditional methods, or by uniform coating with a controlled thickness of various organic polymers using *in situ* syntheses to induce selective partitioning of drug molecules into hydrophobic core.^{65,73-76}

b) Nanotubule interiors

Mentioned above in section A.2.e. was an approach to drug removal employing nanotubules prepared by the University of Florida template synthesis method. The same group of investigators has treated the inside of the silica nanotubules with C₁₈ fatty hydrocarbon units and shown that enhanced absorption/binding of a model substrate occurs. This type of surface modification inside nanotubules has been extended and refined to include attachment of proteins for sensor applications.^{77,69} It is planned to carry out selectively both the internal and internal plus external silation of silica and titania nanotubules by the same methods but using reagents having different length fatty hydrocarbon chains.

2. Cross-linked poly-N-vinylpyrrolidone synthesis

a. Chemically cross-linked polymer

Several commercial samples of polymers formed using various ratios of N-vinylpyrrolidone and, e.g., divinylbenzene comonomers, are available. Their water solubilities decrease with increasing amount of cross-linker. Samples of water insoluble latexes have been evaluated for ability to adsorb and remove either of the two toxic drugs listed in this proposal. The data will expand on that known for drug absorption by poly-N-vinylpyrrolidone solutions⁶⁸ (cf. A.2.d.(2)).

b. Thermally cross-linked polymer

Recently, it has been discovered that when poly-N-vinylpyrrolidone is heated, it undergoes interchain cross-linking to become water insoluble but otherwise retains its amide chemical features. This new material is available in various particle sizes and may be able to strongly adsorb molecules like amiodarone and amitriptyline.

3. Molecularly templated metal oxide synthesis

Above in section A.2.c., the section on soft-hard particle synthesis, a plan was described for forming hard shells of various compositions and porosities around liquid or polymer cores. It was also noted that the shells could be templated for possible selective molecular filtering of absorbates as they pass through the shell and enter the core. Using the same quoted literature on successful templating of films^{62,63,64} as precedent, research is being carried out to template pores for selective adsorption of amiodarone and amitriptyline in several types of metal oxide nanoparticles. Specifically, sol-gel chemistry is being carried out with amiodarone or amitriptyline, or applicable derivatives, present to prepare porous molecularly templated silica, titania⁷⁸, and zirconia⁷⁹ nanoparticles, as well as ones composed of mixed metal oxides, which have previously been shown to undergo controlled surface area modification by thermal or plasma⁸⁰ treatment. Indeed, titania particles have been templated with pores the size and shape of borneol, a compound structurally related to camphor and cocaine. Fundamental information is lacking on exactly how much pore volume in such porous particles will be accessible to the target molecules, how selective the templated pores will be for only the target molecule when competitor adsorbates are present, and how irreversible the binding of the target drugs will be in the particles. Successful synthesis of the desired particles, physicochemical analysis of their ability to remove the target drugs from solution, and in vitro and in vivo evaluation of their efficiency to reverse toxicity, will greatly enhance understanding of new, unprecedented ways to employ colloidal material in medicine.

C. Drug Removal by Mechanism C: Enzymatic Degradation of Drug in Particle

1. Soft Particle Synthesis • Microemulsions

The research described for microemulsions in section A.1. can be expanded to include in the formulations an appropriate enzyme like cytochrome P450, an oxidase enzyme that degrades many biological substrates in humans. Cytochrome P450 derivatives are composed mostly of protein and therefore are compatible for use in microemulsion chemistry. With an oxidase enzyme present in the oil core, any overdose drug absorbed into it will theoretically be degraded and therefore be detoxified (assuming that the oxidation products are less toxic).

2. Soft-Hard Particle Synthesis

a. Polymerizable microemulsions

The scientific concepts, approaches and goals of this part of the research were presented in section A.2.a. They are being expanded to include cytochrome P450 enzyme in the microemulsions stabilized with polymerizable surfactant, and the formed polymerized shell-liquid core particles are being evaluated for biochemical application to overdose drug removal and degradation.

b. Micellar cores containing enzyme and hard shell

Similar to encapsulation of enzymes in microemulsions with polymerizable surfactants, the research discussed in section A.2.c. above is being extended to forming core micellar media containing cytochrome P450 derivatives inside a shell composed of either semipermeable

polymer or inorganic oxide. The oxide shell may have molecular selectivity for transport of amiodarone and amitriptyline into the core.

3. Hard Particle Synthesis

a. Encapsulation of crystalline enzyme

Immobilization of enzyme substrates on the outside of particles is mature technology employed in several types of chemical manufacturing processes. The reverse situation, the inclusion and/or chemical attachment of enzymes inside a shell of porous metal oxide, has not been reported, but would be a natural extension of methods reported for coating organic latex particles^{65,66}. It is proposed to determine experimental conditions to encapsulate crystalline cytochrome P450 derivatives inside several types of porous metal oxide shells.

b. Enzymes inside nanotubules

Discussed above in section B.1.b. were issues and experiments on using uniquely prepared nanotubules with functionalized interior surfaces to remove overdosed drug. The study plan is to prepare samples of nanotubules from either inorganic or organic precursors with P450 enzyme attached to the interior. If initial characterization is promising, the outer surface of the nanotubules can also be passified before in vivo application to prevent protein adsorption. Fundamental information is being obtained on the diffusivity of the target drug molecules into the nanotubules, the ability of the immobilized enzyme on the interior to detoxify the drug, and the turnover number of drug molecules destroyed per unit of enzyme or nanotubules in the solution.

Below is a succinct description of the results of studies that are relevant to issues of particle design, the cardiac effects of the study drugs, and the attenuation of these

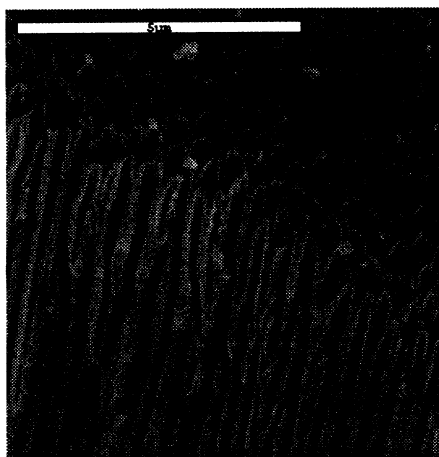


Figure 27. Scanning electron micrograph of the surface of the microporous alumina template membrane.

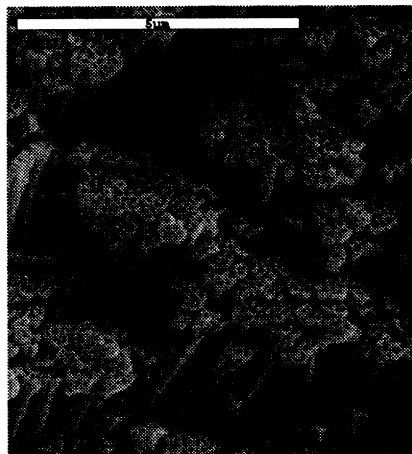


Figure 28. Scanning electron micrograph of the silica nanotubules prepared within the pores of the template membrane shown in Figure 27.

effects by lipids and enzymes. Taken together, the data provided below demonstrate the potential of nanoparticles in mitigating the adverse electrophysiological effects of these types of drugs, and demonstrate preliminary proof of concept as they relate to the aims of the current research proposal.

Preliminary data that suggest that it is possible to synthesize nanoparticles to remove drugs or drug analogs from solution has been successfully obtained. Partch and coworkers have shown that when a cocaine-like biomolecule is incorporated into the reaction mixture during sol-gel synthesis, followed by its removal, the particles have pores which, by inference from Makote and Collinson⁶⁴, should preferentially reabsorb the same molecule over others from solution. Equally exciting is the observation that pi-acceptor aromatic rings that are to be covalently tethered to silica nanoparticles interact efficiently with pi-donor aromatic rings common to local anesthetics used in medicinal treatment. Furthermore, the aromatic rings are toxic if overdosed. This experimental result extends the bioapplicability of work on pi complex formation between trinitrobenzene and dopamine derivatives⁶⁷.

Martin and coworkers used the microporous alumina template membranes shown in Figure 27 to prepare silica nanotubules. The silica nanotubules were prepared within the pores of this template membrane using a sol-gel method. A scanning electron micrograph of the template-prepared silica nanotubules is shown in Figure 28. A commercially available long-chain alkyl thiol (C_{18}) was attached to the inside surfaces of the silica nanotubules. These hydrophobic-interior nanotubules were then used to extract the hydrophobic target molecule (7,8-benzoquinoline) from dilute aqueous solution. The investigators have since dissolved the template membrane and collected the hydrophobic tubules by filtration. These tubules were dispersed into a solution of the same hydrophobic target molecule. The tubules were then removed by filtration. Absorption data again showed that the tubules extracted the target molecule.

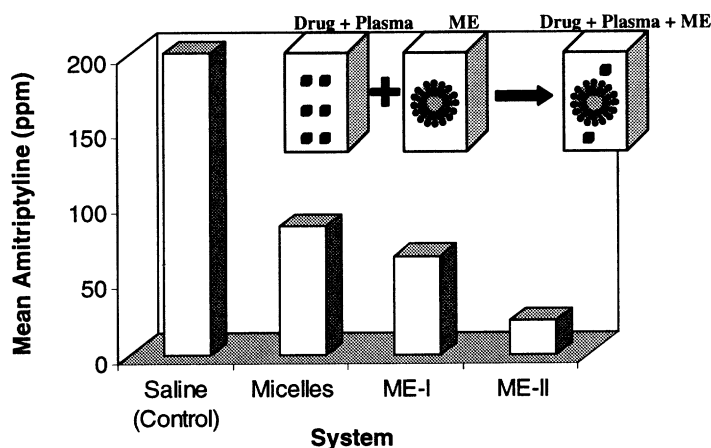


Figure 29 : Effect of Pluronic L-44 Micelles and Microemulsions in Reducing the concentration of Amitriptyline in Plasma

Attenuation of the *In Vitro* and *In Vivo* Electrophysiological Effects of Bupivacaine by Intralipid

Dennis, Morey, Shah, and coworkers carried out *in vitro* and *in vivo* pilot studies with bupivacaine, a widely used local anesthetic known for its cardiotoxic effects, to demonstrate the feasibility of using partitioning into macroemulsion-based soft bioparticles as a means to attenuate the tissue effects of a potent, highly lipid soluble drug. Figure 29 schematically illustrates how addition of microemulsion to plasma containing drug reduces the concentration of free drug molecules in the plasma. This is due to partitioning of the drug molecules in the oil core as well as in the interface of the microemulsion droplet. Furthermore, in Figure 29 we studied the effect of emulsifier micelles (Pluronic L-44 and fatty acid soap) as well as two microemulsions (ME-I and ME-II) having increasing volume fraction of oil. The concentration of free drug molecules decreases in 10-15 minutes of equilibration between microemulsion and saline. Figure 30 shows the importance of the size of emulsion droplet in reversing the toxicity caused by bupivacaine on isolated guinea pig heart. Here, the ME-3, having a

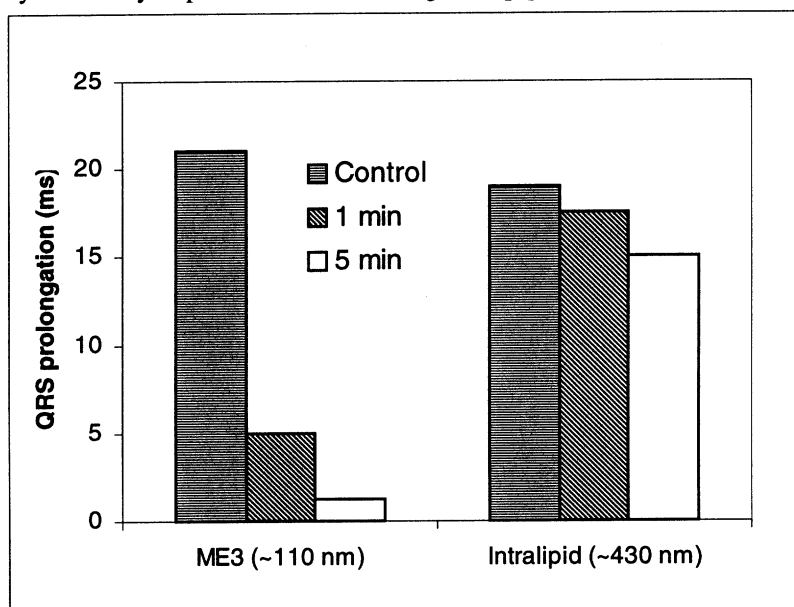


Figure 30 : Removal toxic effect of Bupivacaine on Isolated guinea-pig heart by particles of different size

droplet diameter of 110 nm, was more effective in decreasing the QRS interval than the Intralip droplets, having 434 nm diameter. The magnitude of QRS signal is directly related to toxicity induced by bupivacaine molecules. Bupivacaine (1 μ M) caused a time-dependent lengthening of the SA, QRS, QT and A-H interval in isoflurane-anesthetized rats (Figure 31).

For the sake of brevity, only the QRS interval, a Na channel dependent conduction time that is a major target for these types of drugs, is illustrated for both types of experiments. In both studies, Intralipid was found to attenuate the toxic effects of bupivacaine. Taken together, these pilot data provide preliminary evidence that even a crude macroemulsion such as Intralipid (10%), which has a relatively low lipid partition coefficient and binding capacity for lipid drugs compared to the new microemulsions, can effectively attenuate the *in vitro* and *in vivo* effects of the very potent, highly lipophilic local anesthetic bupivacaine.

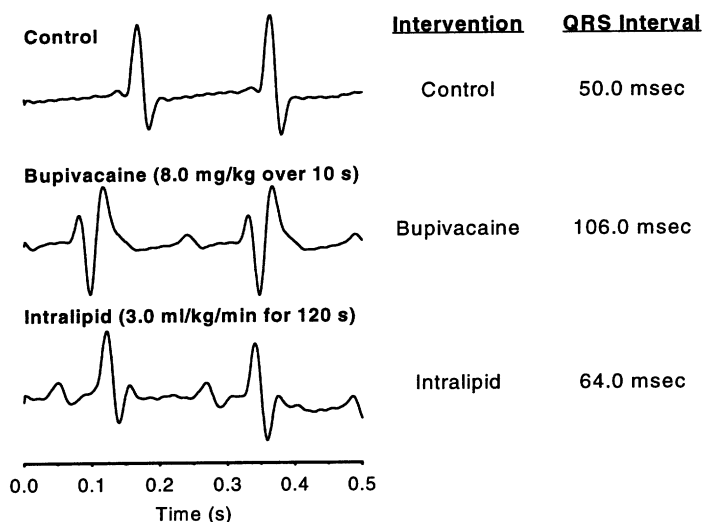


Figure 31: Attenuation of the *in vivo* cardiotoxic effects of bupivacaine in isoflurane anesthetized rats. Shown is the effect of an IV bolus of bupivacaine (8mg/kg over 10 sec). Compared to time matched controls, Intralipid (3 ml/kg/min over 2 min) more rapidly attenuated bupivacaine-induced prolongation of the QRS interval. Two additional experiments were carried out, and gave similar results.

Path Forward

Using a multidisciplinary team approach, tremendous opportunities now exist for synergistic cooperation between nanoparticle scientists, engineers, physicians, medicinal chemists, and enzymologists, not only to improve the quality of healthcare in the world, but also to develop new technologies that have great economic value. The long term goal of this research project is to integrate cutting edge technologies of nanoparticle science and technology into those of pharmacogenomics (genetic

polymorphisms) and molecular biology (optimizing the efficiency and stability of human cloned P450 enzyme fractions) to effectively treat the large majority of drug poisonings in the world in a broad and highly effective manner, and to extend these technologies to new areas of medicine (e.g., treatment of life threatening cases of fungal and gram-negative sepsis using antibiotics incorporated into a lipid environment within the nanoparticle). The full basic and therapeutic potential of nanoparticles will depend on the further development of systems with optimal particle design, drug partitioning, and enzymatic activity characteristics.

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