TO: Texas Hazardous Waste Research Center

FROM: Shankar Chellam, University of Houston (currently at Texas A&M)

SUBJECT: Final Report

PROJECT NUMBER: 513UHH0033H

PROJECT TITLE: Enhanced removal of viruses and pharmaceuticals and personal care products by a hybrid electroflotation-microfiltration process

PROJECT PERIOD: September 1, 2013 – July 15, 2015

DATE: November 7, 2015

PROJECT DESCRIPTION

In this research, we evaluated methods to reduce microbiological contamination. One approach was to employ electrocoagulation, where aluminum coagulant was directly added to the feed water by *in situ* electrochemical dissolution of an elemental anode. The following are the aluminum electrocoagulation reactions:

Anode: Al^0 (s) = Al^{3+} + $3e^-$

Hydrolysis: Al^{3+} + $3H_2O$ = $Al(OH)_3$ (s) + $3H^+$

Cathode: $3H_2O$ + $3e^-$ = $3OH^-$ + $1.5H_2$

Overall: Al^0 (s) + $3H_2O$ = $Al(OH)_3$ (s) + $1.5H_2$

Importantly, cathodic production of OH^- ions neutralize H^+ generated by anodic hydrolysis of aluminum. Thus, electrolysis does not consume alkalinity (i.e. buffering capacity), which is a significant issue during conventional chemical coagulation due to the Brønsted acid behavior of alum. This is a significant process advantage of electrochemical treatment over alum addition.

Another aspect of the research performed was to investigate the role of functionalized bismuth nanoparticles on cells. Specifically, we looked at lipophilic bismuth dimercaptopropanol nanoparticles (BisBAL NPs) that have a very important antimicrobial activity. However, bismuth compounds are known to exhibit toxicity and reported side effects may include nephropathy, hepatitis, and encephalopathy. We hypothesized that complexing bismuth with dithiols would reduce their potential undesirable side-effects. This aspect of our research was performed in close collaboration with Prof. Claudio Cabral-Romero's group at the Facultad de Odontologia, Universidad Autonoma de Nuevo Leon (UANL), Monterrey, NL, Mexico. We comprehensively elucidated their effects on human blood cells including erythrocytes and leukocytes.

Objectives. The overall objectives are to (i) develop an innovative and multi-barrier aluminum electrochemical–microfiltration process for enhanced contaminant control and

(ii) determine whether BisBAL NPs trigger eryptosis (i.e. suicidal erythrocyte death) in a cell culture.

METHODOLOGY

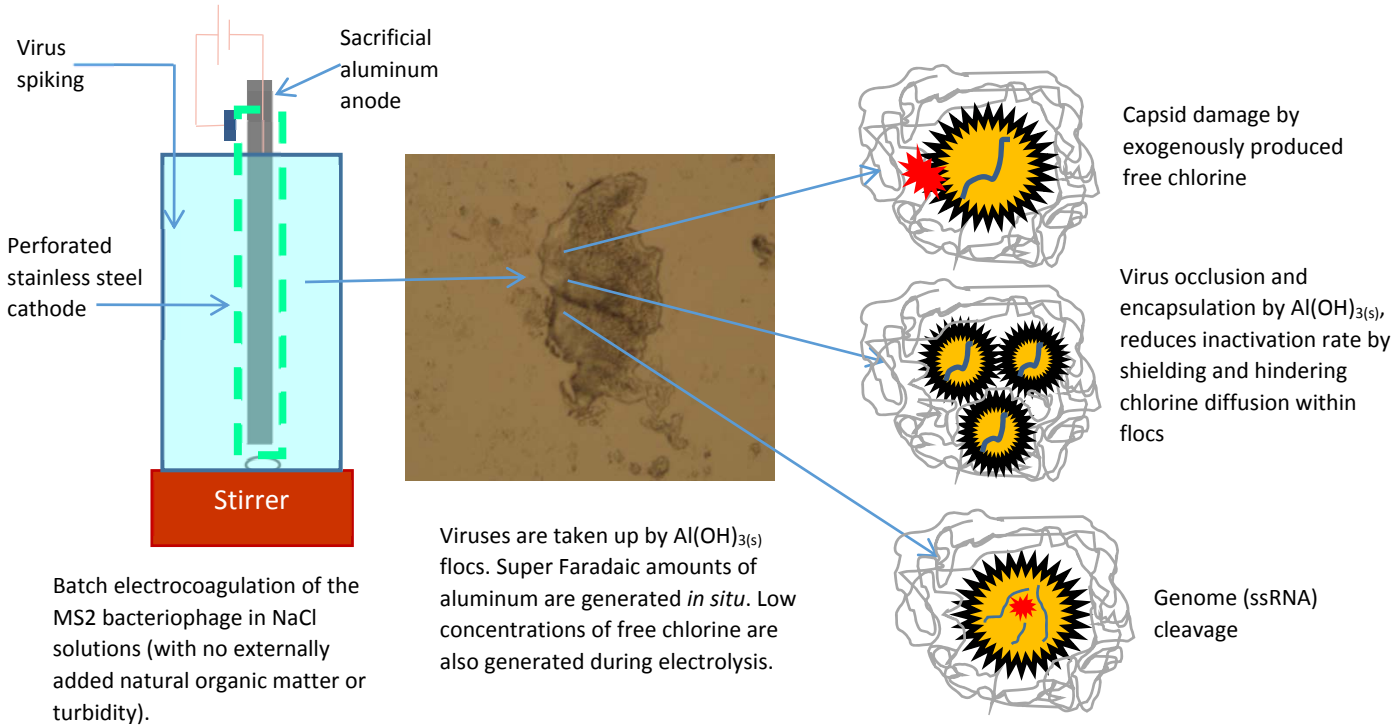

Figure 1. Schematic representation of virus coagulation by electrocoagulation.

Electrolysis was performed in batch mode using a 450mL cylindrical cell with an annular electrode configuration; aluminum anode surrounded by a perforated 316-stainless steel cathode. The virus stock was fed and thoroughly mixed into the feed water. A sample was then collected to measure the initial concentration of viruses before electrolysis. Electrolysis was performed to dose the appropriate amount of aluminum (0-30 mgAl/L) at a constant current density. Coagulated and flocculated viruses were separated by centrifugation and dissolved in 6% beef extract at pH 9.5. Viruses in supernatant and flocs were assayed to calculate inactivation values. Free chlorine was measured using N,N-diethyl-*p*-phenylenediamine (DPD) colorimetric method using a Hach DR-4000 spectrophotometer. A schematic of our experimental apparatus and major findings are given in Figure 1.

For the synthesis of bismuth nanostructures, the following chemical reagents were used: bismuth pentahydrate ($Bi(NO_3)3•5H_2O$), 2,3-dimercapto-1-propanol (BAL), sodium borohydride (NaBH4), and propylene glycol were all analytical grade reagents purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water (Barnstead Nanopure Diamond) was used to prepare solutions and dilutions. A stock solution of 2:1 molar

ratio of Bi (Bis) to 2,3-dimercapto-1-propanol (BAL) served as a cationic precursor for the BisBAL nanoparticles and the choice of molar ratio was based on the previous work, which showed BisBAL was stable over a wide pH range (4-11) and effective against microbial biofilm formation. During the course of BisBAL reduction with $NaBH_4$, the pink color of soluble BisBAL instantly transformed to a black colored suspension composed of BisBAL nanoparticles. The stock suspensions of 25 mM of BisBAL nanoparticles in 10 mL batches were prepared and stored at 4ºC until use.

RESULTS AND DISCUSSION

Aluminum electrolysis. As seen in Figure 2 slightly more aluminum was reproducibly dissolved in replicate experiments than expected from purely electrochemical considerations.

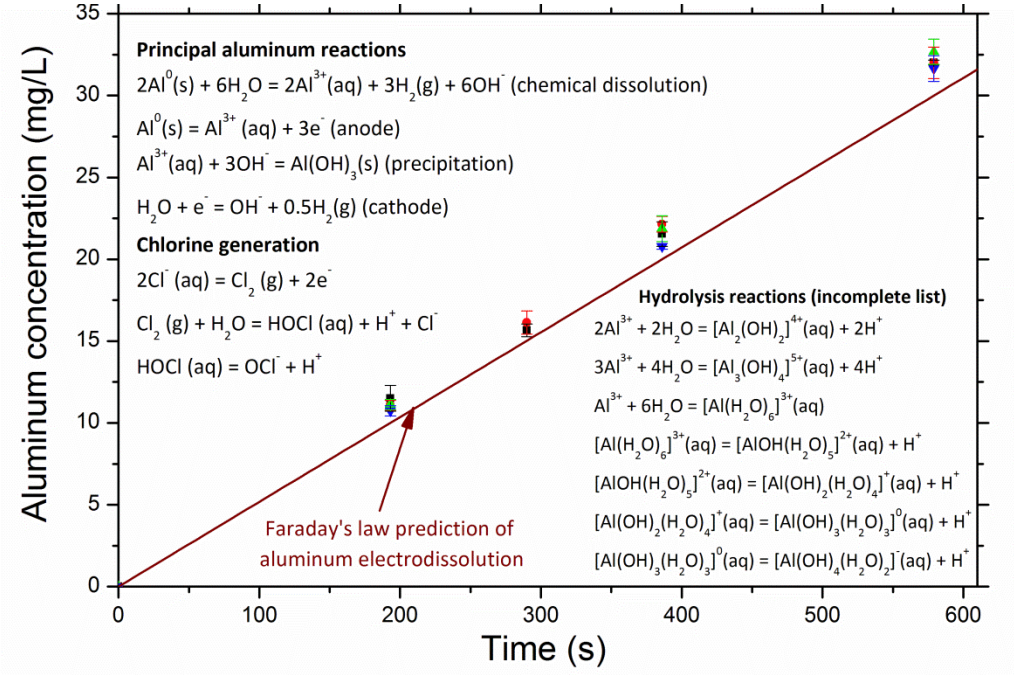

Figure 2. Aluminum electrochemical generation was augmented by chemical dissolution to release more coagulant than predicted by Faraday's law (solid brown line). Expected electrochemical and hydrolysis reactions are also shown. Note that free chlorine is predicted to be generated via oxidation of dissolved chloride ions.

Measured concentrations followed a linear trend with a statistically significant (95% confidence) higher slope ($0.0558\pm5.81\times10^{-4}$ mg/L-s) than Faraday's law (0.0518 mg/L-s). Hence, chemical dissolution of the electrode augmented aluminum dosing in the presence of NaCl, which is in contrast to low salinity surface water where coagulant dosing quantitatively follows electrochemical considerations alone. Theoretical predictions were made using Faraday's law.

Characterization of BisBAL NPs. Bismuth nanoparticles obtained were spherical in shape with the number-weighted average hydrodynamic diameter of 53 nm (Figure 3). The nanoparticles are composed of rhombohedral crystallites (≈ 18nm) with di-thiols as

lipophilic surface chemical groups and the lattice spacing of individual crystallite was 0.325, which is consistent with nanoscale bismuth nanoparticles. UV-Vis absorbance measurements revealed that the nanoparticles had greater (≈70%) affinity towards 1-octanol rather than water, which further suggests that lipophilic property of the nanoparticles, arises from the di-thiols bounds to nanoparticle surface.

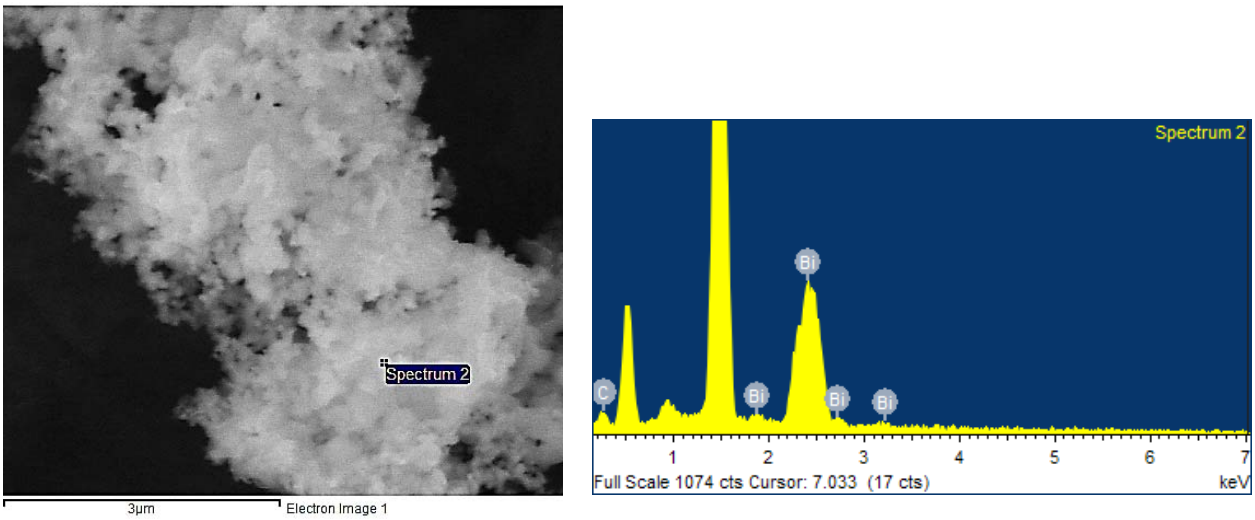

Figure 3. Lipophilic bismuth nanoparticles visualized by scanning electron microscopy. The dominant population of spherical shaped nanoparticles (<100 nm), showed the nanoparticle clusters interspersed among the lesser electron dense material is shown in the TEM images (left). The spectrum of elements in the sample observed by SEM is shown on the right hand side.

Genotoxic assays revealed no damage to genomic DNA of blood cells after 24 h of exposition to BisBAL NPs. 100‑1000 μM of bismuth nanoparticles promotes apoptosis between blood cells after 24 h of incubation. In other words, BisBAL NPs at concentrations lower than 100 μM do not cause damage on blood cells; they could potentially be used by humans without affecting erythrocytes and leukocytes. The action mechanism of how bismuth or its compounds damage cells is still unknown. Early reports using doses lower than 20mg kg^{-1} of bismuth nanoparticles PLGA encapsulated were internalized into cells and remain into the cytoplasm without side effects. Based on our experiments with Calcein AM and fluorescence microscopy, we can argue that in high doses (500–1000 μM) BisBAL nanoparticles enter the cell and stock into cytoplasm of host cells. The nanoparticles entry will alter the plasmatic membrane permeability of host cells,modifying their homeostasis and metabolism and finally leading to apoptosis.

PEER REVIEWED PUBLICATIONS

One paper was published in a scientific journal after peer-review jointly with Prof. Cabral Romero's group. More information on our findings can be found in:

Hernandez-Delgadillo, R., A.R. Badireddy, V. Zaragoza-Magaña, R.I. Sánchez-Nájera, S. Chellam, and C. Cabral Romero (2015). Effect of lipophilic bismuth nanoparticles (BisBAL NPs) on Erythrocytes. *Journal of Nanomaterials.* Article ID 264024.