Experimental study of Human Adenoviruses interactions with TiO_2 Nanoparticles

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Introduction and Purpose

Waterborne viral infection is one of the most important causes of human morbidity, and relates diseases continue to have public health and socioeconomic implications worldwide. Human Adenoviruses (HAdVs) is commonly found in environmental waters and is very resistant to water disinfection an environmental stressors. Titanium dioxide Nanoparticles (TiO_2 NPs) are one group of the most widely used nanomaterials in consumer products including sunscreens, cosmetics, paints, and solar cell energy. Recently, they were increasingly applied for the photocatalytic degradation of pollutants in water, air, and soil matrices.

With the rapid growth of the production, consumption and disposal of nanomaterials, they will inevitably and ultimately enter the environment. Accidental and/or deliberate introduction of TiO_2 NPs into subsurface environments may lead to contamination of drinking water. **The objective of this study was to investigate the adsorption of HAdV in TiO_2 NPs systems. The Nanoparticles used as a model were Titanium dioxide (TiO_2). The survival of HAdVs onto these nanoparticles was characterized at 25 ^oC under the effect of visible light (VL) and the effect of dark (D).**

Materials and Methods

❑ TiO2 NPs Suspension Preparation

Titanium dioxide power (TiO_2, anatase, <25nm in diameter, purity greater than 99.9%

- TiO_2 NPs stock suspension (1000mg/L) in dH_2O
- Sonication for 30min and determination of TiO_2 size distribution in dH_2O after settling for 7 days: 180±31 nm (ZetaSizer Nano-ZS90).

❑ Virus Stock Preparation

- Human Adenovirus serotype 35 (HAdV35) as model virus
- Cultivation in human lung carcinoma cell line A549
- The initial concentration of adenoviral stock: 10^8-10^9 genome copies/ml
- Quantification by Real-Time Polymerase Chain Reaction (qPCR).

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Materials and Methods (cont.)

❑ Batch experiments

Kinetic Adsorption Experiments

- Effect of visible light (VL) and dark (D)
- DNase I reaction buffer solution at pH 7.6
- Static experiments at room temperature (25 ^{o}C)
- Control tubes (virus in the absence of NPs)
- Reactor tubes (virus in the presence of NPs at a concentration of 1 gr per liter)
- Virus concentration: 10^9, 10^5, 10^4 copies/ml
- Dark batch experiments: reactor and control tubes covered with aluminum foil, throughout the experiment
- Samples were collected every 24 h for a period of 7 days and centrifuged at 2000g (6000 rpm) for 30 min

❑ Virus Extraction and virus detection by Real – Time PCR (qPCR)

- Treatment with DNase I (free – RNase) before DNA extraction to degrade DNA released from damaged viral capsids
- Extraction of viral nucleid acid was performed using a commercial viral RNA kit (QIAamp viral RNA mini kit – Qiagen)
- Hexon gene was used as the target area
- All samples were tested in duplicate (two neat and two diluted)
- Use of TaqMan Universal PCR Master Mix (Applied Biosystems) and a carry-over contamination prevention system, uracil N-glycosylase

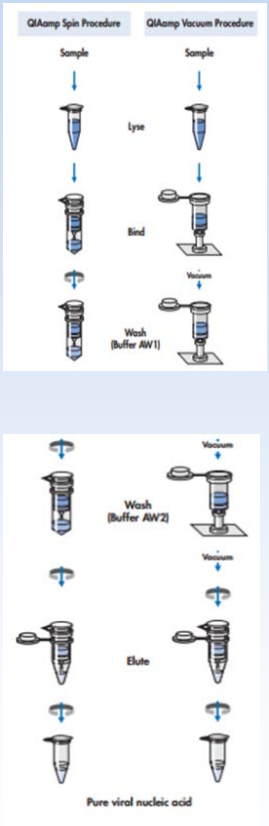

Figure 1: QIAamp Viral RNA Mini Kits represent a well established general-purpose technology for viral RNA preparation. The kit combines the selective binding properties of a silicagel-based membrane with the speed of microspin or vacuum technology and is ideally suited for simultaneous processing of multiple samples.

Materials and Methods (cont.)

The effect of NPs on Human Adenoviruses adsorption NPs were determinate by calculating the

$\log_{10}$ of C/Co

Where

- $C = C_{o(t)} - C_{(t)}$,
- $C_{o(t)}$, virus concentration at time t in the controls tubes (copies/mL)
- $C_{(t)}$, virus concentration at time t in the reactors tubes (copies/mL)
- C, concentration of viruses sorbed onto NPs

Results

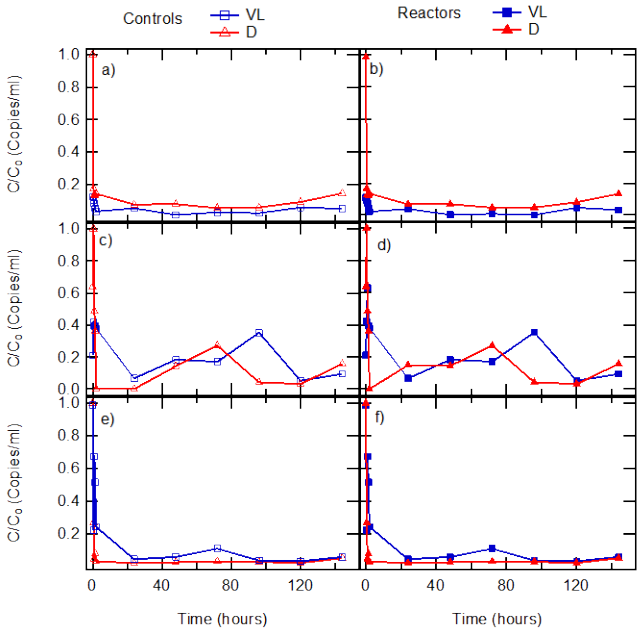

Figure 2: Effect of NPs on Human Adenoviruses adsorption under Visible Light (VL) and Dark (D) static batch conditions at 25 ^{O}C. The first row of graphs (a and b) corresponds to virus initial concentration C_o=3,31 x 10^9 copies/ml, the second rows (c and d) to C_o=6,37 x 10^5 copies/ml and the third row (e and f) corresponds to C_o=6,53 x 10^4 copies/ml. The open and the solids symbols represent experiments in Controls and in Reactors respectively.

Conclusions

- ✓ Exposure time intervals in the range of seven days resulted in a load reduction of 0.25 to 2.50 logs for VL experiments and a reduction of 0.15 to 2.85 for D experiments
- ✓ Virus adsorption onto TiO_2 was systematically more persistent at effect of VL
- ✓ The adsorption rates of the Human Adenoviruses under controlled conditions, they increase with increasing time
- ✓ The increased reduction of waterborne viruses by their contact with TiO_2 NPs systems could play an important role in the prevention of viral waterborne diseases.

Conclusions

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