***Kevin H. Oshima*** *is an assistant professor of biology at New Mexico State University and specializes in environmental virology. Previously he was guest researcher with the Centers for Disease Control and Prevention in Atlanta, Georgia, and prior to that, a fisheries biologist with the Fish and Wildlife Service in Seattle. Kevin received an M.S. and Ph.D. in fisheries science from the University of Washington.*



# Concentration of Viral Particles from Water by Ultrafiltration

**Introduction**

A diverse number of human pathogens including viruses, bacteria, protozoan, helminthic and mycotic agents can have a waterborne mode of transmission. These organisms are typically excreted in the fecal and urinary wastes of humans and animals and may contaminate surface and groundwater.

More than 100 different enteric viruses are known to be excreted in human feces (APHA 1995; Farthing 1989; IAWPRC 1991). Viruses such as poliovirus, coxsackievirus, echovirus and other enteroviruses, adenoviruses, reoviruses, rotavirus, hepatitis A and Norwalk virus can be excreted in relatively large number in infected individuals (Table 1). They cause a number of illnesses that range in severity from gastroenteritis to paralysis.

Humans are exposed to enteric viruses through a variety of waterborne pathways including shellfish grown in contaminated ocean or estuary water, food crops, recreational water and drinking water (Metcalf et al. 1995). Surface water may be contaminated with virus from wastewater when on-site disposal systems fail or when treatment facilities do not have the capacity to treat large volumes of water during periods of heavy rainfall. In some cases there may be inadequate inactivation of viruses in drinking water.

Enteric viruses have been found in high concentrations in domestic sewage although levels can be highly variable (Metcalf et al. 1995). Enteric viruses in surface and groundwater are found at very low concentrations as a result of the dilution that takes place when treated or untreated sewage is mixed with natural waters. Hence, large volumes of water need to be concentrated before such viruses can be detected.

Although many viruses can have a waterborne component to their transmission, little is known about the nature and quantity of pathogenic viruses present in drinking, surface and groundwater and their relationship to disease. This has been due in part to the subclinical nature of many infections caused by waterborne enteric viruses, similarity of disease symptoms of enteric viruses to bacterial and parasitic diseases, and the lack of sensitive and reliable detection methodology (Sekia et al. 1980; Hughes et al. 1992). Determination of the incidence and concentration of viruses in water is therefore necessary for risk assessment and management for the purpose of protecting the public from disease outbreaks. To protect the public and to learn more about the risk of waterborne diseases to the public health, the U.S. Environmental Protection Agency (EPA) requires all utilities serving 100,000 or more to monitor their source water for a number of pathogenic agents including viruses.

Methods to detect viruses from water involve concentration steps to recover viruses in a much smaller volume. Various problems are associated with the concentration and detection of viruses from the aquatic environment including the small size of viral particles (20-100 nm in diameter), low virus

Table 1. Human enteric viruses that may be present in water[a].

| Virus Group | Genome | Serotypes |
|---|---|---|
| Enterovirus | +RNA | |
| Poliovirus | | 3 |
| Echovirus | | 34 |
| Coxsackievirus A | | 24 |
| Coxsackievirus B | | 6 |
| New Enterovirus types | | 4 |
| Hepatitis A | +RNA | 1 |
| Norwalk virus | +RNA | 4[b] |
| Rotavirus | ds segmented RNA | >20? |
| Reovirus | ds segmented RNA | 3 |
| Adenovirus | dsDNA | 39 |

[a] Farthing 1989
[b] Kapikian 1994

concentration, variability in amounts and types of virus present and presence of dissolved and suspended materials in water, and wastewater that interfere with virus concentration and detection. Methods to detect viruses also tend to be expensive and are highly specific, requiring many different assays to detect the various viruses found in water (Gurrman-Bass and Catalano-Sherman 1985; APHA 1995; Ijzerman et al. 1997; Rose et al. 1984; Sobsey and Glass 1984; Sobsey and Hickey 1985). Some such as Norwalk virus cannot be assayed or propagated in cell lines.

Current methodology for concentrating viruses from water involve filtering virus-containing samples through electropositive or electronegative charged microporous filters (0.2-0.45 mm), eluting the adsorbed viral particles and concentrating the eluent to a final volume of <10 ml (APHA 1995). Environmental samples are usually collected in large volumes (100-1000 L) to increase the chance of detecting viruses that are in very low concentration (Ijzerman et al. 1997; Metcalf et al. 1995). Microporous filters are used to adsorb viruses to filters that have a pore size that is larger than the viral particles. Adsorption of viral particles to the filter is achieved by the electrostatic interactions between the viral particles and the filter (APHA 1995; Hou et al. 1980; Shields and Farrah 1983). Adsorbed viruses are then eluted from the surface of the filters by filtering a small volume of eluent, such as beef extract, aluminium hydroxide or glycine through the filter (Berman et al. 1980; Rose et al. 1984; APHA 1995). It is believed that eluents work by competing for binding sites or altering filter binding properties such that viruses are released back into solution (Shields and Farrah 1983).

Eluted viruses are still in volumes that are too large (1 L or more) to be conveniently and economically assayed directly in cell culture or by polymerase chain reaction (PCR). Thus they are further concentrated by methods such as aluminium hydroxide adsorption-prècipitation or polyethylene glycol hydroextraction-dialysis (PEG), or organic flocculation (APHA 1995; Metcalf et al. 1995). The objective is to efficiently concentrate viruses into < 1 ml.

The main advantage of the microfiltration is that filtration is achieved at a relatively fast rate such that large volumes of water can be filtered within 1-2 hours. This process however has several limitations.

These include variable viral recoveries as a result of different viruses binding with different affinities to the filters and variation in conditions such as pH, salt concentration organic matter and filter chemistry will alter the effectiveness of adsorption (APHA 1995; Shields and Farrah 1983; Bicknell et al. 1985; Goyal and Gerba 1982; Jasons and Bucens 1986; Melnick et al. 1984).

Adsorption of viral particles to filters are affected by water quality because organic matter in the water may compete with viruses for binding sites on the filter. When viruses were suspended in raw water containing high levels of soluble organics, viral adsorption with the electropositive filter much lower than when this same water was treated with activated carbon (Sobsey and Glass 1984).

The efficiency of elution may also be variable and eluents can have an adverse effect on downstream viral detection systems. Beef extract has been found to inhibit PCR (Ijzerman et al. 1997). Choice of eluents can be a significant factor in viral recovery (Toranzos and Gerba 1989).

Due to complex interactions resulting in variable recovery efficiency from microfiltration, other concentration methods for waterborne viruses should be considered. Ideally the concentration method should have the following attributes: increased detection sensitivity, consistent results even in different water qualities and among different viruses, rapid, simple and cost effective. In this study, the efficiency of viral recovery by ultrafiltration is being examined with these perspectives in mind.

Ultrafiltration concentrates viruses by size exclusion as opposed to viral adsorption to the filter and therefore its efficiency may be less affected by virus type or water quality (Belfort et al. 1975a and b; Berman et al. 1980; Bicknell et al. 1985; Garin et al. 1996; Jasons and Bucens 1986; Oshima et al. 1995). During the ultrafiltration process the water circulates in a cross-flow pattern across the membrane surface and this helps maintain suspended matter in solution thereby prolonging filter life. This flow pattern also serves to help maintain the viral particles in suspension in the retentate. Much of the research on characterizing and optimizing recovery has focused on the use of ultrafiltration on tap and distilled water (Belfort et al. 1975a and b; Berman et al. 1980; Garin et al. 1996). Less research has been done on virus concentration from surface and groundwater (Bicknell et al. 1985; Garin et al. 1996; Juliano and Sobsey 1997). Large-scale testing to characterize and optimize virus recovery from multiple water types and viruses has not been done. Nor has the impact of ultrafiltration been examined from the standpoint of downstream processes such as the polymerase chain reaction.

Although ultrafiltration units tend to be more expensive than those of microfiltration, the former can be cost effective as they are reusable after sanitation. The main disadvantage of ultrafilters is that their pore size has to be small enough to retain viruses and this reduces their flow rate. However, ultrafiltration may be a practical alternative to micro-filtration because larger modules can overcome the slower filtration rate and the potential for more consistent and efficiency recovery of viral agents.

Since ultrafiltration works by size exclusion, it has the potential to concentrate all microorganisms by a single method (Juliano and Sobsey 1997). Thus a single method could be used for viruses and parasitic organisms and even bacterial agents.

The aim of this study is to optimize the feasibility of two ultrafiltration systems (hollow fiber and tangential flow) to concentrate viruses from water. Initial tests were done with a laboratory scale system. Variables such as different viruses, water qualities and blocking/elution methods from 2 L of virus suspensions were tested. Results will be used as baseline data for future studies with field scale ultrafiltration systems.

The objective is to develop methods to concentrate viruses and *Crptosporidium* from a variety of different types of water. Optimization of recovery using the same method for all types of water as well as methods that are tailored for each type of water will be developed.

**Description of Filtration Systems**

Filters

Polyacrylonitrile (PAN) 50,000 MWCO (AHP-0013) hollow fiber ultrafilter (Microza; Pall Corp. Glen Cove NY) and a polyethersulfone 10,000 MWCO tangential flow ultrafiltration system (Filtron, Ann Arbor MI) were used to concentrate viruses from water. The hollow fiber had 0.017 $m^2$ of membrane material. Tangential flow system had 0.312 $m^2$ surface area.

Filtration Systems

Hollow Fiber - The hollow fiber ultrafilter module was fitted into a filtration system (PS24001;Asahi Chemical Industry Co., Tokyo, Japan) containing a gear type pump and valves to control transmembrane pressure and flow rate (Figure 1). Before and after each experiment, the ultrafilter module was sanitized by circulating sodium hypochlorite through the system. The free sodium hypochlorite concentration

was determined at the end of sanitation by measuring the absorbance at 530 nm (PR/200; Hach, Loveland, CO). The ultrafilter module was flushed with ultrapure water (UPW) until the residual concentration of free sodium hypochlorite was < 0.04 mg/L.

Each experiment was designed such that the retentate was recirculated back to a central reservoir (Figure 1). Filtration was terminated when only the hold-up volume (volume of fluid contained in the filter apparatus) remained. For each challenge, virus concentration was determined for the initial virus suspension, retentate and overall bulk permeate. The recovery of virus in the retentate was determined by the following equation:

$$\frac{\text{Initial total PFU of virus in the 2 L virus suspension}}{\text{X 100\% recovery}} = \text{Total PFU of virus in the retentate}$$

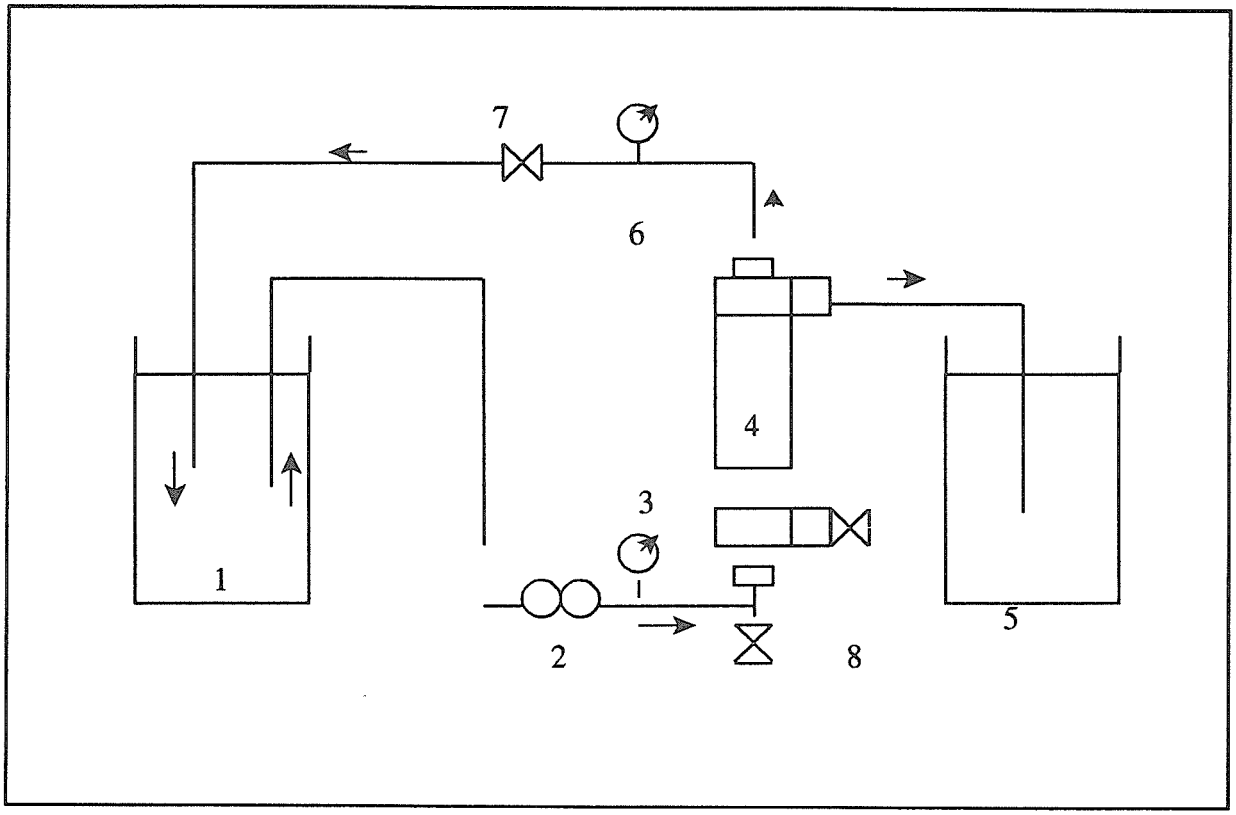


Figure 1. Diagram of filtration scheme for the hollow fiber filtration system. 1) retentate reservoir; 2) circulation pump; 3) pressure gauge; 4) pencil module; 5) permeate reservoir; 6) pressure gauge at module outlet; 7) valve to control module outlet pressure; 8) drain valve.

Tangential Flow Ultrafiltration System

This system contains two sheets of filter membrane. A cross-flow pattern is maintained during filtration using a peristaltic pump (Figure 2). Filtration is again terminated when only the hold-up ~ 80 ml remains in the retentate. Like the hollow fiber system, this system is sanitizable. Because of stainless steel fittings NaOH was used as the sanitant.

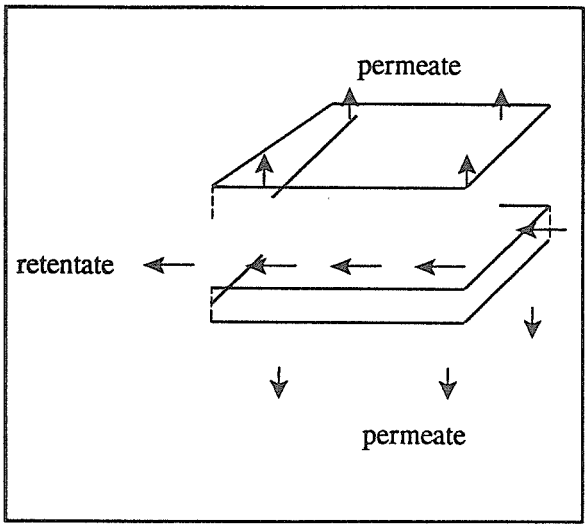


Figure 2. Diagram filtration scheme for tangential flow filtration. The overall filtration scheme is very similar to the hollow fiber system.

**Viruses**

Escherichia coli (ATCC 11303) was used as the host strain for growth and assay of phage T1 (ATCC 11303-B1). Pseudomonas aeruginosa (ATCC 15692-B2) was used for growth and assay of phage PP7 (ATCC 15692-B2). The plaque assay was conducted as described previously (Oshima et al.1995).

The Sabin 2 vaccine strain was used as the challenge virus for experiments with poliovirus. The virus was grown in HeLa cells and the plaque assay conducted as described previously (Oshima et al. 1995) (Table 2).

**Characterization and Optimization of Recovery**

A number of variables for obtaining efficient and predictable virus recovery has been evaluated. Filter pretreatments and/or elution of viruses from the filter after filtration have been tested from reagent grade water, tap, surface and groundwater.

**Results and Discussion**

Results are being readied for publication thus specific details cannot be reported here. However, methods have been identified in small-volume testing (2 L) for each type of water as well as a single method for all water types have been identified. Recovery goals are to concentrate > 60% of particularly PP7 and poliovirus from all types of water and to develop a single filtration process for all types of water. These methods will then be applied to a prototype field scale system that is currently being readied for testing. This system should be able to filter 100-1000 L of water in less than 2 hours. The volume will depend on the source of water (100 L for surface water, 1000 L for tap). Future studies will involve spiking virus to different types of water under field-scale conditions and also testing the system with *Cryptosporidium*.

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Table 2. Physical characteristics and host of model viruses to be used in this study.

| Virus | Size | Host | Envelope | Nucleic Acid |
|---|---|---|---|---|
| Phage T1 | 50 nm head<br>150 nm tail | *E.coli* | No | dsDNA |
| Phage PP | 725 nm | *P. aeruginosa* | No | ssRNA |
| Poliovirus (Sabin 2 strain) | 25 nm | HeLa | No | ss RNA |

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