MIMICKING FILTRATION OF *CRYPTOSPORIDIUM PARVUM* IN POROUS MEDIA USING BIOTIN AND GLYCOPROTEIN-COATED MICROSPHERES

L. Pang*[1], U. Nowostawska[2], L. Weaver[1], G. Hoffman[1], A. Karmacharya[1], A. Skinner[1], and N. Karki[1]

[1]Institute of Environmental Science & Research Ltd, Christchurch, New Zealand.

[2]Chemistry Department, University of Otago, Dunedin, New Zealand.

ABSTRACT

Cryptosporidium parvum is a highly infectious protozoan. Insufficient removal of *C. parvum* in drinking water has resulted in many cryptosporidiosis outbreaks worldwide. Due to its longevity and extreme resistance to chlorination, removal of *C. parvum* in porous media is predominantly by filtration. However, currently no reliable surrogates have been established for *C. parvum* removal by filtration.

Latex microspheres of similar size, density and shape as *C. parvum* oocysts were coated with biotin and glycoprotein, which had similar surface charges to that of *C. parvum*. Glycoprotein also mimicked the type of surface protein of the oocysts.

Filtration experiments with two different sand media demonstrated that, compared to the unmodified microspheres, the surrogates achieved a superior match to the retention of the *C. parvum* oocysts, showing the same log-reduction. In contrast, results from the unmodified microspheres differed one-order of magnitude. Detection of the fluorescently surrogates was rapid using a spectrofluorometer, contrasting to time-consuming microscopic examination of *C. parvum*. The surrogates remained stable in size and charge for at least 22 months.

When validation in pilot trials, the surrogates developed here could have potential uses in assessing *C. parvum* filtration in sand filters in water treatment and water recycling through land disposal.

KEYWORDS

***Cryptosporidium parvum*, surrogates, filtration, sand filter, water treatment, water quality.**

1 INTRODUCTION

Cryptosporidium parvum is a protozoan that can cause severe gastrointestinal disease. It is highly infectious and ingestion of fewer than 10 oocysts can lead to infection (WHO, 2004). *C. parvum* oocysts are commonly found in surface-waters, and have been detected in some drinking water supplies (LeChevallier et al., 1991, WHO, 2004). It can survive for many months in surface-water and years in groundwater, and is resistant to chlorination unless used at high levels above levels allowable for drinking water treatment. Failure of sand filters during water treatment and insufficient removal of *C. parvum* from groundwater has resulted in many waterborne outbreaks worldwide (USEPA 2005). For example in 1993, Milwaukee, USA, about 400,000 people were infected and more than 100 people died after contamination of drinking water by *C. parvum* (Mac Kenzie et al., 1994). Major outbreaks also occurred in other developed countries due to contamination of groundwater and drinking water by *C. parvum* (Willocks et al., 1998).

Being extremely infectious and highly resistant to chemical disinfection, *C. parvum* is often used in risk analysis of drinking-water supplies. However, because of the high analytical cost, *C. parvum* is not routinely monitored

in water treatment. Traditionally, water turbidity is used to monitor *C. parvum* removal in water supplies but turbidity would not quantify the concentration of *C. parvum* (Emelko et al., 2005). Some researchers have used unmodified polystyrene microspheres as a surrogate for studying *C. parvum* filtration, but the results are generally unsatisfactory (Harvey et al., 2010).

It is very difficult (often impossible) to study the retention and transport of actual pathogens in groundwater and drinking water treatment. This is because of the public health risk and high costs often involved. Our study was motivated by a need to minimise expensive and labour-intensive analysis of the actual pathogens and reduce the risk in dealing with pathogens. We aimed to develop surrogates that behave like *C. parvum* in its filtration in porous media and are environmentally friendly, cost-effective, and easy to detect. As *C. parvum* filtration in porous media is largely determined by its size, surface charge, surface macromolecular structure, shape and density, we hypothesized that a surrogate that mimics these properties may approximate *C. parvum* filtration.

2 METHODS

C. parvum oocysts are spherical or oval in shape, 3.9–5.9 μm in diameter. Live *C. parvum* was purchased from Waterborne™, Inc. (New Orleans, USA). To mimic the size, shape and density of the oocysts, fluorescent carboxylated polystyrene microspheres (4.87 μm in diameter) were purchased from Polysciences, Inc. (Warrington, USA). The buoyant density of polystyrene microspheres was 1.05 g/cm^3, which equals the geometric mean of the oocyst density (Medema et al., 1998). Fluorescent polystyrene microspheres are non-genotoxic and have been used in groundwater and surface water field studies previously (Behrens et al., 2001).

To mimic the surface charge of the oocysts, biotin and glycoprotein, with isoelectric point values similar to that of the *C. parvum* oocysts, were purchased from Sigma-Aldrich (Auckland, New Zealand). Biotin can be found in a wide range of foods (e.g., yeast, liver, kidney, egg yolk, soybeans, nuts, and cereals). Glycoprotein can be found in plants, animals and humans, and is the predominant type of protein that *C. parvum* oocysts produce on its cell surfaces. Both biomolecules were harmless and non-carcinogenic. A cross-linker, EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) was used for coupling the selected biomolecules to the microspheres, following the established procedures.

The surface charges of the *C. parvum* oocysts, biomolecules, modified and unmodified microspheres were determined using laser Doppler microelectrophoresis (Malvern Instruments Zetasizer Nano ZS). A newly developed qNano Analyser (IZON Science, Christchurch) was used for characterizing particle concentrations and size distribution.

Filtration experiments were carried out using glass columns (22 cm long and 5 cm in diameter) packed with coarse sand (0.78 mm grain size) and very coarse sand (1.37 mm grain size), respectively. In each experiment a pulse of tracer solution containing one type of test particles and bromide (Br) were injected. A constant flow rate (0.8–1.0 m/day) was applied using a peristaltic MilliGAT Pump. The same background electrolyte of 1 mM NaCl at pH≈7 was used in all the experiments. The condition of ionic strength 1 mM and pH≈7 was similar to that of the tap-water (sourced from groundwater) in Christchurch city. The Br samples were analysed using a bromide ion selective electrode. Microscopic examinations were performed using an epifluorescence microscope (Leica instruments) for the oocysts. Oocysts were stained with a specific monoclonal antibody stain (Waterborne Inc.). The antibody stain is coupled to a fluorescent marker which allows for specific detection under fluorescence microscopy. The microsphere samples were analysed by spectrofluorometry. The experimental data were analysed for concentration reduction, mass recovery and filtration efficient coefficient.

3 RESULTS AND DISCUSSION

The zeta potentials of biotin, glycoprotein and *C. parvum* oocysts were very similar with isoelectric point pH≈2. In contrast, the unmodified microspheres exhibited significantly greater negative charges than the oocysts. The zeta potentials of the microspheres changed significantly after being coated with biotin and glycoprotein, becoming more similar to those of the oocysts. The surrogates remained stable in size and charge for at least 22 months.

In all the filtration experiments, the modified microspheres achieved a superior match to the oocysts than the unmodified microspheres (Figure 1), in concentration reduction, mass recovery, and filtration efficient coefficient. They showed the same log reduction in concentration as oocysts, whereas results from unmodified microspheres deviated by one order of magnitude.

Compared to biotin-coated microspheres, glycoprotein-coated microspheres better resembled oocyst concentration, despite having charge similar to biotin-coated microspheres. This suggests that surface protein also played an important role in particle attachment on solid surfaces.

Although the unmodified microspheres had less retention than the oocysts in the sand media investigated in this study because they were more negatively charged, unmodified microspheres will not necessarily be conservative in all types of porous media. The possibility of greater removal exists for unmodified microspheres than for the oocysts in the presence of positively charged media. For example, metal (hydr)oxide-coated sand filters have been developed for effective removal of microorganisms and other contaminants in water and wastewater treatment. Therefore using unmodified microsphere in such a treatment plant may over predict the ability of the system to remove oocysts.

If the filtration media used is net-negatively charged, the use of a surrogate that will more accurately predict oocysts concentrations could save the unnecessary treatment costs than if predicted using the overly conservative unmodified microspheres. Although their concentration difference was about one order of magnitude for the sand media investigated here, this difference may vary in other porous media. For field scale operations treatment costs may increase significantly if treatment level required is 1-log higher. Therefore the use of a surrogate such as glycoprotein-coated microspheres, which could more accurately predict oocyst removal than unmodified microspheres, is more appropriate.

4 CONCLUSIONS

Our study has shown that glycoprotein- and biotin-coated beads are better surrogates than unmodified beads to study the filtration of *C. parvum* oocysts in porous media, and can provide more accurate prediction of *C. parvum* concentration reduction.

With further pilot studies, the surrogates developed in this study could be a new tool to assess the performance of filters in water and wastewater treatment and to study oocysts retention and transport in soils and aquifers after land disposal of effluent and biosolids.

By using a cost-effective new tool, significantly costs could be saved for drinking water and wastewater utilities. With a tool that enables the quantitative prediction of contamination risk, we can better protect public health.

In this study we have proved that protein-coated microspheres can be used as effective surrogates to study retention and transport of a pathogen in porous media. This new approach could potentially revolutionise the way we study the movement and removal of waterborne pathogens in the environment. Adapting the same principle, we are currently developing surrogates for rotavirus and adenovirus under a Marsden Fund grant from the Royal Society of New Zealand.

More information can be found in:

Pang L., Nowostawska U., Weaver L., Hoffman G., Karmacharya A., Skinner A., and Karki A. (2012) Biotin- and Glycoprotein-Coated Microspheres: Potential Surrogates for Studying Filtration of *Cryptosporidium parvum* in Porous Media. Environ. Sci. Technol. 46, 11779-11787.

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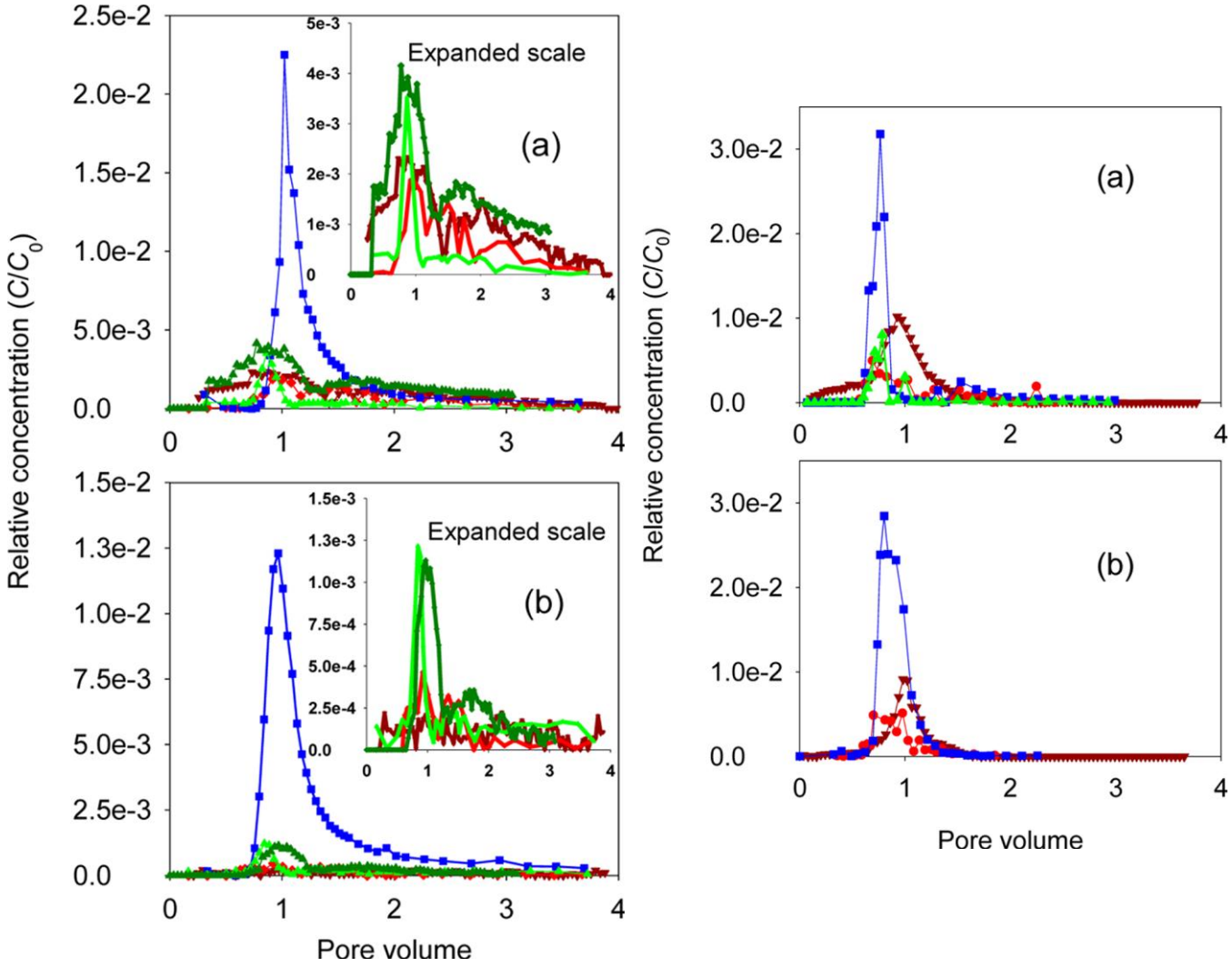

Figure 1: Concentration recoveries of Cryptosporidium parvum (red), glycoprotein-coated microspheres (brown), biotin-coated microspheres (green) and unmodified microspheres (blue) after passing through 0.78 mm sand columns (left) and 1.37 mm sand columns (right). (a) columns repacked with clean sand, and (b) columns flushed after clean-sand experiments without repacking. Inset graphs with the expanded scale show all data except for unmodified microspheres.

ACKNOWLEDGEMENTS

This study was internally funded by ESR under a Capability Fund.

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