Removal of Cryptosporidium using Coagulation
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Cooperative Research Centre for Water Quality and Treatment

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FOREWORD

Removal of Cryptosporidium Using Coagulation

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CRC for Water Quality and Treatment Project No. 3.1.2 – Polyelectrolytes in Water Treatment
EXECUTIVE SUMMARY

*Cryptosporidium parvum* is an enteric protozoan parasite which includes genotypes infectious to humans and most mammals. Surviving as excreted oocysts in the environment, they are small (4 to 6 μm), robust and resistant to chemical disinfection (particularly chlorination and chloramination). Cryptosporidium is now recognised as a major problem in the production of drinking water world-wide.

To combat the problem of *Cryptosporidium parvum* oocysts in drinking water, conventional water treatment technology has concentrated on two main areas: 1) removal by filtration and 2) disinfection (the inactivation of oocysts using a range of disinfectants). However, there is very little knowledge on how oocysts behave during the coagulation, flocculation and sedimentation process. The flocculation and sedimentation steps are both physical, and are dependent on the coagulation step for optimisation.

This research project addressed the process of coagulation and investigated the removal of oocysts using chemical processes. Within this framework the removal using aluminium sulphate (alum), \( \text{Al}_2(\text{SO}_4)_3 \), ferric chloride, \( \text{FeCl}_3 \) and poly-diallyl-dimethyl ammonium chloride (PolyDADMAC) for *Cryptosporidium parvum* oocysts was optimised. A number of factors were explored including:

- The effect of pH (across the range from 5 to 9).
- The effect of turbidity (both on the recovery of oocysts by centrifugation and fluorescent staining with monoclonal antibodies as well as the effect on floc formation and removal of oocysts during coagulation). Water used for turbidity experiments range from Hope Valley reservoir (5 NTU) up to River Murray water (> 60 NTU).
- How the floc and removal of oocysts is affected by the concentration of NOM (natural organic matter).

The important findings from this study include the following:

- The removal of oocysts is dependent on two different processes, direct interaction of oocysts with the coagulant and entrapment in flocculated material. In this respect, floc formation was found to be the most significant factor in the removal of oocysts by alum.
- Natural settling rates of oocysts during jar tests are significant and vary with water type.
- The activity (live or heat-inactivated) and age of the oocysts affect the surface charge of the oocysts, which can result in differing removal rates of oocysts during jar test experiments.
- The influence of NOM on the removal of oocysts by alum coagulation can be minimised by increasing the coagulant dose. This is important when using alum, but PolyDADMAC and ferric chloride appear unaffected by NOM concentration.
- Turbidity has a significant effect on floc formation and the subsequent removal of oocysts.
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ABBREVIATIONS

Alum  Aluminium Sulphate, $\text{Al}_2(\text{SO}_4)_3$
BSA  Bovine Serum Albumin
cm  Centimetres
DAFF  Dissolved Air Flotation and Filtration
DE  Diatomaceous Earth
DOC  Dissolved Organic Carbon

Ferric Chloride  Iron (III) Chloride, $\text{FeCl}_3$
FL  Fluorescein
g  Grams
HU  Hazen Units
Log  Logarithm, Base 10
mg  Milligrams
ml  Millilitres
MIEX®  Magnetic Ion Exchange Resin
NGS  Normal Goat Serum
NOM  Natural Organic Matter
NTU  Nephelometric Turbidity Units
PAC  PolyAluminium Chloride
PBS  Phosphate Buffer Saline
pH  $-\log([\text{H}^+])$

PolyDADMAC  Poly-DiAllyl-DiMethyl Ammonium Chloride
ppm  Parts Per Million
rpm  Revolutions Per Minute
RO  Reverse Osmosis
l  Microlitres
m  Micrometres
UV  Ultraviolet
UV$_\text{abs}$  Ultraviolet Absorbance
w/v  Weight per Volume
w/w  Weight per Weight
1 INTRODUCTION

1.1 Cryptosporidium

The genus Cryptosporidium comprises single-celled protozoan parasites which grow and reproduce within the digestive organs or respiratory tract of vertebrates. The organism was originally observed in the digestive trails of laboratory mice and formally described as the species Cryptosporidium muris (Tyzzer, 1907), with Cryptosporidium parvum discovered 4 years later. Several species of Cryptosporidium are recognised, infecting a wide range of hosts. Most mammals can be infected by Cryptosporidium parvum, although there is growing evidence that discrete, identifiable genotypes within this species show considerable host specificity. In particular, C. parvum from grazing animals (the ‘bovine’ genotype) is considerably less infectious for humans that the genotype which has been isolated from humans. Specific mammalian groups are host to other Cryptosporidium species; rodents harbour C. muris (which has also been detected in cattle and camels) while guinea pigs host C. wrairi. Birds can be infected by C. baileyi and C. meleagridas, reptiles by C. serpentis and fish by C. nasorum (Fayer et al., 1997, Graczyk et al., 1997, Morgan et al., 1999). Immune-deficient humans may have increased susceptibility to C. parvum genotypes that otherwise have low infectivity as well as other Cryptosporidium species (Pieniazek et al., 1999).

Cryptosporidium is monoxenous, that is, its life cycle is completed within one host. The parasite moves from host to host by means of the faecal-oral route and has a complex life-cycle as shown in Figure 1 (Fayer and Ungar, 1986). Mature oocysts are shed in the faeces of an infected host and then ingested by other hosts through direct contact or contamination of the food, water or environment. Cryptosporidium infection is called cryptosporidiosis. The life cycle of Cryptosporidium is complex (Figure 1) and involves both sexual and asexual reproduction within the epithelial cells. Each generation of Cryptosporidium parasites can develop to maturity within 12 to 14 hours. The oocysts can then sporulate in situ and either release sporozoites for autoinfection or else pass from the body in the host’s faeces.

1.2 Cryptosporidiosis

For the water industry the major source of Cryptosporidium parvum oocysts is through the faeces of calves, cows and sheep being washed into the rivers and reservoirs (Juranek, 1995), with one calf capable of releasing up to $10^{10}$ oocysts per day (Bukhari, 1999). Oocysts, which usually contain four sporozoites, are infectious as soon as they are released from the host into the environment (Peeters et al., 1989).

The first confirmed case of human cryptosporidiosis was recorded in 1976. The illness is acute but self-limiting in immunocompetent victims, generally abating in 8 to 20 days (Juranek, 1995). It is characterised by the onset of explosive, profuse, watery diarrhoea, usually 1 to 2 weeks after exposure, with less frequent symptoms including abdominal cramps, nausea, vomiting and fever.
There is currently no cure for cryptosporidiosis so people with compromised immune systems (including HIV-infected persons (AIDS)) are affected much more seriously by the illness, with long-term infections resulting in losses of up to 25 litres of water per day, often leading to death (Ryan et al., 1994).

1.3 Waterborne Outbreaks of Cryptosporidiosis

The first documented waterborne outbreak of cryptosporidiosis occurred in July 1984 in Texas (D’Antonio et al., 1985). Since then numerous waterborne outbreaks have been reported throughout the world, particularly from the UK, Canada and USA. The most publicised waterborne outbreak to date (in Milwaukee in 1993) affected more than 400,000 people and resulted in some deaths (Lisle and Rose, 1995). This outbreak demonstrates the potential danger of Cryptosporidium when exposed to a large population via a city’s drinking water supply.

One of the most recent reports of Cryptosporidium in water supplies occurred in Sydney, Australia, in 1998. Although no cases of illness were reported a large amount of negative publicity was generated within the water industry. This lead to a push for increased research in Australia into the detection and removal of Cryptosporidium parvum oocysts in water.

1.4 Removal of Cryptosporidium by Water Treatment

Previous research with respect to the problem of Cryptosporidium oocysts in drinking water has concentrated on two main areas:

1. **Removal by Filtration:** The removal of Cryptosporidium oocysts from drinking water using filtration including, dissolved air flotation and filtration (DAFF) (Plummer et al., 1995), microfiltration and ultrafiltration (membrane) (Jacangelo et al., 1995), slow-sand filtration (Fogel et al., 1993), filtration using...
diatomaceous earth (DE) (Ongerth and Hutton, 1997) and granular deep-bed filtration (Hatukai et al., 1997).

2. **Disinfection:** The inactivation of infectious Cryptosporidium oocysts using a range of disinfectants including, ozone (Tomiak et al., 1998), chlorine dioxide (Liyanage et al., 1997), ultraviolet irradiation (UV) (Clancy et al., 1998), ammonia (Jenkins et al., 1998) and mixed oxidants (Venczel et al., 1997).

*Cryptosporidium parvum* oocysts range from 4 to 6 μm in size, too small to be removed directly by rapid gravity filters. In addition, the oocysts are extremely resistant to disinfection by chlorine and chloramine, which are the most commonly used disinfectants for water treatment in Australia. Thus, in a conventional water treatment plant the removal of *Cryptosporidium* oocysts must be accomplished by optimisation of the coagulation, flocculation and sedimentation processes.
2 STUDY DESIGN

2.1 Introduction

The behaviour of Cryptosporidium parvum oocysts, during the coagulation, flocculation and sedimentation processes in conventional water treatment, is an area that is not well understood, with previous research being limited (Baudin et al., 1998). This study aimed to address some of the gaps in knowledge on how Cryptosporidium oocysts behave during these processes. The flocculation and sedimentation steps are both physical, and are dependent on the coagulation step for optimisation. Thus, the coagulation process was investigated to determine the optimal conditions for the removal of Cryptosporidium oocysts during this stage of water treatment. To achieve the maximum removal of oocysts, it is necessary to optimise the coagulation process, by:

1. Selection of the most efficient coagulant (including costs, hazards and availability).
2. Optimising the coagulant dose.
3. Optimising the pH of the water being treated.
4. Adjusting the coagulant dose for changes in raw water quality including natural organic matter (NOM) dissolved in the water and the turbidity of the water being treated.

These criteria are addressed individually in the following sections.

2.2 Type of Coagulant

The coagulants used during this study were alum, ferric chloride and PolyDADMAC. Alum or aluminium sulphate is supplied as a solution of approximately 50% w/w (500,000 ppm) Al₂(SO₄)₃·18H₂O, with between 8 and 16% w/w of Al₂O₃ depending on the supplier, and added to give the desired concentration. Alum is strongly acidic (about pH 3) and is the most commonly used coagulant in Australia. The aluminium ion, Al³⁺ (or Al(OH)³⁺) provides a strong positive charge in the water which attracts the negatively charged organic compounds and particles in water and facilitates the flocculation process. Ferric chloride is supplied as a solution of approximately 42% w/w (420,000 ppm) FeCl₃. Ferric chloride is strongly acidic (about pH 1) and is now the most commonly used coagulant in NSW. The ferric ion, Fe³⁺ (or Fe(OH)³⁺) provides a strong positive charge in the water, similar to Al³⁺ above. PolyDADMAC or Poli-diallyl-dimethyl ammonium chloride is a cationic polymer supplied as a solution of approximately 40% w/w (400,000 ppm) and is neutral or mildly acidic (pH 5 – 7). PolyDADMAC is used both alone and in conjunction with alum or ferric chloride as a coagulant aid.

2.3 Dose of Coagulant

Before choosing a dose of coagulant it is important to understand the nature of the water being treated. The water used in this study was collected from the Hope Valley reservoir (Figure 2). This water generally reflects the River Murray in character (fed from runoff from the small natural catchment and water pumped from the River Murray) but with lower turbidity and generally has the following characteristics:

- Turbidity between 4 and 5 NTU.
- pH between 8 and 8.3.
- TDS of approximately 350 mg/L.
- Alkalinity of approximately 70 mg/L CaCO₃.
- DOC (Dissolved Organic Carbon) of between 4 and 6 mg/L.
- True colour (456 nm) of between 11 and 14 HU.
- UV₉₅ (254 nm) of approximately 0.14 cm⁻¹.
Overall, Hope Valley is a reasonably clear water in appearance, with low turbidity and colour, and moderate salt content and alkalinity, and is typical of water used in the production of drinking water in South Australia.

Five different coagulant doses were chosen to give a range of coagulation and flocculation conditions including:

1. Low Dose, Poor Flocculation.
2. Low to Medium Dose, Mild Flocculation.
3. Optimal Dose (based on turbidity), Giving Good Flocculation.
5. Highest Dose, Used for Enhanced Coagulation (more effective for NOM removal).

The corresponding coagulant doses for the above conditions in Hope Valley water for alum, ferric chloride and PolyDADMAC are given in Table 1.

Table 1. A list of the coagulant doses used for this study

<table>
<thead>
<tr>
<th>Description</th>
<th>Floc</th>
<th>Alum (ppm)</th>
<th>Ferric Chloride (ppm)</th>
<th>PolyDADMAC (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Dose</td>
<td>Poor</td>
<td>15</td>
<td>16</td>
<td>1.0</td>
</tr>
<tr>
<td>Medium Dose</td>
<td>Medium</td>
<td>30</td>
<td>32</td>
<td>2.5</td>
</tr>
<tr>
<td>Average Dose</td>
<td>Good</td>
<td>45</td>
<td>47</td>
<td>4.0</td>
</tr>
<tr>
<td>High Dose</td>
<td>Good</td>
<td>60</td>
<td>63</td>
<td>6.0</td>
</tr>
<tr>
<td>Enhanced Coagulation</td>
<td>Very Good</td>
<td>75</td>
<td>79</td>
<td>10.0</td>
</tr>
</tbody>
</table>
Figure 2. Hope Valley Reservoir, including: a) city view, b) treatment plant overview, c) pumps, d) flocculation tanks, e) sedimentation tanks, f) filters
2.4 pH of the Water
The natural pH of the water (Hope Valley) was approximately 8.2. This pH was varied prior to each experiment (Section 3.3) in order to determine whether the removal of Cryptosporidium oocysts by alum coagulation is effected by pH. The pH values used for this study were 5, 7 and 9, in order to cover the range of pHs commonly found in natural waters. Additional experiments carried out to determine the effect of NOM concentration (Section 2.5) and Turbidity (Section 2.6) on the removal of Cryptosporidium oocysts by alum were carried out at pH 7.

2.5 Concentration of NOM in the Water
The concentration of NOM in Hope Valley water is generally between 4 and 6 ppm. Organic matter was isolated from Hope Valley water using the MIEX® process (Section 3.1) and then used to simulate an increase in concentration of NOM (+4 and +8 ppm NOM). This was accomplished by addition of the MIEX® extract at an appropriate concentration prior to treatment.

2.6 Turbidity of the Water
The method used to modify the turbidity of water samples during this project was filtration. Previous studies (Lucas, 1999) have shown that filtration with 0.2 m filters removes turbidity with no effect on the other characteristics of the water (such as concentration of NOM, pH and salt content). Thus, the water sample can be filtered and combined in different fractions with the raw water to give the desired turbidity. Hope Valley water with a range of 0 to 5 NTU was used for most turbidity experiments, with some additional turbidity experiments performed using River Murray water, with a much higher turbidity (greater than 60 NTU). The majority of the experiments in this study were performed using 0.2 m filtered Hope Valley water (approximately 0.15 NTU) so that any effect of turbidity could be ignored.

2.7 Electrophoresis
The surface charge of the Cryptosporidium oocysts is likely to have a direct impact on the coagulant dose needed to neutralise the oocysts and remove them from solution. Therefore, the surface charge properties were evaluated using Electrophoresis, a technique commonly used to measure the effective surface charge of particles in solution. The experimental technique and apparatus are discussed in detail in Section 3.2, below. For this study electrophoresis was performed on Cryptosporidium oocysts to determine how the surface charge of the oocysts varies upon:

- Heat-inactivating the oocysts.
- Changing the ionic strength or total dissolved solids (TDS) (10⁻² and 10⁻³ M).
- The effect of pH (from 2 to 12).
- The effect of turbidity (0 to 65 NTU).
- The effect of the concentration of Natural Organic Matter (NOM) (0 to 15 ppm of added NOM using a MIEX® extract).
3 EXPERIMENTAL

3.1 Solutions and Materials
Alum or aluminium sulphate (50% w/w Al₂(SO₄)₃·18H₂O, PIVOT Chemicals) was diluted to a stock solution of 20,000 ppm and used as required. Ferric chloride or Profloc-F (42% w/w FeCl₃, Orica) was diluted to a stock solution of 21,000 ppm and used as required. PolyDADMAC or Magnafloc LT35 (40% w/w, Ciba Specialty Chemicals) was diluted to a stock solution of 2,000 ppm and used as required. HCl and NaOH solutions were made up as 1 N stock solutions and then diluted to 1.0, 0.1 and 0.01 M and used as required. Phosphate Buffer Saline (PBS) was made by dissolving 17 g of potassium dihydrogen orthophosphate in Milli-Q water (475 ml), adjusting the pH to 7.2 using 10 M NaOH and making the volume up to 500 ml using Milli-Q water.

Suspensions of Cryptosporidium parvum oocysts (Moredun Animal Health) were diluted using PBS from stock of either 10⁸ oocysts in 2 ml for heat-inactivated oocysts or 10⁷ oocysts in 2 ml for live oocysts to give 10⁶ per ml.

Blocking buffer consisted of 1% bovine serum albumin (BSA), 10% normal goat serum (NGS), and 0.02% sodium azide (as a preservative) made up in PBS solution and used as required.

Labelling of Cryptosporidium parvum oocysts was accomplished using AquaGlo™ G/C Direct (Waterborne Inc.). This reagent consists of a monoclonal antibody directed against the oocyst wall, conjugated with fluorescein isothiocyanate (FITC), together with a similar antibody against Giardia lamblia cysts, in 1% bovine serum albumin (BSA) as an antibody stabiliser and 0.02% w/v sodium azide as a preservative.

A MIEX™ extract for NOM studies was made by extraction of NOM from Hope Valley water using prepared MIEX™ resin (Orica) and then concentrating using Reverse Osmosis (RO). This NOM extract consisted of only the dissolved portion of NOM from Hope Valley water and was very low in salt (0.4 %).

3.2 Electrophoresis
A diagram showing the Electrophoresis apparatus (Rank Brothers Particle Micro-Electrophoresis Apparatus Mark II) appears in Figure 3. The apparatus consists of a microscope with 100x magnification, a square 5 ml cell, and a lamp at the rear which shines through the cell to the microscope and illuminates the samples. The cell contains the sample solution and two platinum electrodes and is surrounded by a thermostatted water bath to keep the temperature constant at 25°C.

3.2.1 Calibration
The walls of the electrophoretic cell will generally be charged in the presence of solvent (usually negative in water). This leads to an effective positive charge in the centre of the cell and results in solvent streams once a potential is placed across the electrodes, with the water in the centre of the cell moving in the opposite direction to that on the sides (Poiseuille’s law – the sum of the pressures is proportional to the flow and resistance). There are only two points within the cell that the solvent itself is stationary and these need to be calibrated in order for accurate electrophoretic measurements to be carried out. The electrophoresis apparatus was calibrated using the following procedure:

- Water was placed in the cell and thermostatted water bath.
- The distance from the microscope to the inside of the two walls of the cell was measured, with the two measurements subtracted and the number assigned to \( d \).
- The height of the cell was measured and assigned to \( l \).
- Using the formula derived by Komataga (Equation 3.1) the value for the stationary levels (s) were calculated.
\[
\frac{s}{d} = 0.500 - \left[ 0.0833 + \frac{32 \ d}{\pi^3 \ l} \right]^{\frac{1}{2}}
\]

- The value for stationary levels was then added or subtracted from the original two measurements of the inside of the walls of the cell to give the optimum distance for electrophoretic measurements.
- The apparatus was then set to these values for the experiments.

Figure 3. Rank Brothers electrophoresis apparatus
3.2.2 Electrophoretic Measurements

Electrophoretic measurements were carried out using the following procedure:

- Solutions consisted of 100 ml of test water, which was pH adjusted and made up to the desired ionic strength by the addition of NaCl. If NOM was required then MIEX™ extract was added (as described in Section 3.1 above) prior to pH adjustment to give the desired concentration of NOM in solution.

- 5 ml samples were transferred by pipette to 10 ml tubes and Cryptosporidium oocysts added (using 50 l of a 10^6 oocysts per ml suspension) giving an overall concentration of 10^4 oocysts per ml.

- Samples were mixed thoroughly by shaking and transferred to the electrophoresis cell and the electrodes inserted. The suspension was allowed to equilibrate to 25°C for 5 minutes in a thermostatted water bath.

- The lamp on the Electrophoresis apparatus was switched on and electrophoretic measurements taken until sufficient points had been collected (generally between 20 and 80 time measurements of at least 3 different oocysts). Electrophoretic measurements consisted of applying a current and measuring the time the oocyst takes to move across a grid and then reversing the current and timing the oocyst to move back across the grid.

- Suspensions were then discarded and the cell washed through with Milli-Q water ready for the next sample.

The measured time for the oocyst to cross one square of the grid (65 m) was averaged for the total measurements made in both directions and the time (in seconds) was converted to the electrophoretic mobility, μ, using Equation 3.2:

\[ \mu = \frac{d}{t} \frac{R}{K} - \frac{l}{V} \text{ (m.cm.s.V)} \]

where

- \( l = RKA \) (cm)
- \( R \) = resistance across cell (Ω)
- \( K \) = specific conductivity (Ω^(-1).cm^(-1))
- \( A \) = cross sectional area (cm²)
- \( d \) = grid division = 65 m
- \( t \) = time (seconds)
- \( l \) = distance between electrodes (cm)
- \( V \) = applied voltage (V)

For the cell used, \( A = 0.10308 \) cm², \( RK = 66.56 \) cm⁻¹, so that \( l = 6.861 \) cm. Grid division is 65 m so that Equation 3.2 becomes:

\[ \mu = \frac{65}{t} - \frac{6.861}{V} \text{ (m.cm.s.V)} \]

For water at 25°C this can be converted to mV (Hunter, 1981) to give a Zeta potential, \( \zeta \):

\[ \zeta = 12.83 \]
3.3 Jar Tests

The jar test procedure is a method designed for the evaluation of coagulant and coagulant aids by simulation of a conventional water treatment process. The experimental setup is shown in Figure 4. It involves the use of a multi-position stirrer with variable speed control up to 200 rpm (used for flash mixing - see below) with the stirring paddles made of stainless steel to prevent corrosion. Illumination is also used to observe the floc formation with up to six Gator Jars per jar test apparatus. Gator jars used in this study were perspex and designed for use with 2-litre water samples.

The jar test involved filling each jar with 2-litres of the desired water (generally 0.2 m filtered Hope Valley water in this study). The water was pH adjusted when required by the addition of HCl or NaOH solutions. The amount of acid or base required was determined prior to the jar test by the pH titration of 500 ml of the water to be used with HCl or NaOH after coagulant addition until the desired pH was reached. This titre value was then multiplied by four to give the amount needed for pH correction of the two-litre volume for the full jar test. Any pH correction was performed while the water samples were being stirred at 200 rpm (flash-mixing speed) and the samples were further stirred for 5 minutes after addition to ensure solutions were thoroughly mixed.

For experiments involving NOM (Section 6.1) MIEX™ extract was added at this stage to give the desired concentration of NOM in each Gator jar.

The next stage involved the addition of Cryptosporidium oocysts when required, by the addition of 400 l of a 10⁶ / ml suspension with vigorous stirring (200 rpm) to give an overall concentration of 200 oocysts per ml (200,000 oocysts per litre and 400,000 oocysts per jar). After addition, the suspensions were stirred at 200 rpm for a further 5 minutes to ensure oocysts were uniformly distributed throughout the water samples.

The desired coagulant was added at a range of concentrations while samples were flash mixed (stirred at 200 rpm) for 1 minute. If a coagulant aid was used then the coagulants were combined and added at the start of flash mixing. Solutions were stirred at 20 rpm for a further 14 minutes after which the size / presence of any floc was recorded.

Solutions were allowed to settle (sedimentation step) for 15 minutes, during which the time for settling of 80% and 90% of the floc was recorded. The following samples were then taken from the Gator jars:

1. 50 ml of each suspension was taken from the tap and discarded to ensure no build-up of floc at the tap.
2. 50 ml of each suspension was taken from the tap to be used to measure turbidity.
3. 50 ml samples were taken (in duplicate) from the taps for each suspension containing Cryptosporidium oocysts for counting and collected in 50 ml centrifuge tubes.
4. 50 ml samples for any top samples required for suspensions containing Cryptosporidium oocysts were removed using 25 ml pipettes (in duplicate).

Processing and analysis of 50 ml samples for counting is described below in Section 3.4. If filtration was to be performed (to simulate the sand / anthracite filtration beds commonly used in water treatment) then the water is passed through a Whatman No 1 filter paper after the settling / sedimentation stage.

3.4 Recovery of Oocysts

The 50 ml samples were analysed for Cryptosporidium oocysts using the following procedure:

1. The 50 ml centrifuge tubes with the sample were centrifuged (using a Beckman GS-6 Centrifuge) for 20 minutes at 3800 rpm.
2. Samples were removed from centrifuge and the supernatant drawn off by vacuum pump to leave approximately 0.5 ml.
3. The remaining 0.5 ml in each sample was transferred to 1.5 ml pre-weighed Eppendorf mini-
centrifuge tubes. The 50 ml centrifuge tubes were washed with a further 1.0 ml of PBS (Phosphate
Buffer Saline) which was also added to the Eppendorf tubes.

4. The Eppendorf tubes were vortexed to thoroughly mix the contents and then centrifuged (MBC
Centrifuge, Hawksley, England) at 14000 rpm for 5 minutes.

5. Excess liquid was drawn off by pipette to leave a pellet plus approximately 200 l.

6. 500 l of blocking buffer was added to each Eppendorf tube, and the solutions vortexed to ensure
mixing. Samples with heavy floc were mixed by pipette to break up the floc and ensure oocysts
were uniformly distributed throughout the suspension. Solutions were then incubated at 38°C for
60 minutes to block active sites on ligands that might compete for the antibody stain.

7. The Eppendorf tubes were centrifuged at 14000 rpm for 5 minutes, with excess blocking buffer
removed as in step 5.

8. 40 l of a fluorescent probe solution (monoclonal antibody with a fluorescent ligand attached
preserved in blocking buffer solution) was added to the samples. Any floc was broken up using
micropipettes and the solutions vortexed to ensure they were thoroughly mixed.

9. Samples were incubated at 38°C for 30 minutes, vortexed, and incubated at 38°C for a further 15
minutes.

10. 1.0 ml of PBS was added to each Eppendorf tube, and the samples vortexed to ensure adequate
mixing prior to centrifugation. This process diluted the fluorescent stain remaining in solution so
that the oocysts could be more clearly viewed.

11. The Eppendorf tubes were centrifuged at 14000 rpm for 5 minutes, with the excess solution
removed as in step 5.

12. Samples were weighed and stored in a refrigerator until microscope counting.

3.5 Microscopy
The microscopes used to count the Cryptosporidium for this study were Olympus BX40, BX50 or BX60
fluorescence microscopes fitted with a 100 W mercury vapour lamp which was replaced after a
maximum use of 200 hours. Samples in Eppendorf tubes were mixed thoroughly by pipette (to ensure
any floc was broken up) and then approximately 50 l was transferred to a haemocytometer slide. The
haemocytometer slide has a 3 by 3 square with 9 sections of 0.1 l (each of which also contains a 3
by 3 square) (Figure 5) etched onto its surface. UV light was then passed through the sample and the
green fluorescence viewed using a microscope. The Cryptosporidium oocysts fluoresce green as
shown in Figure 6. The focus was varied through the depth of the slide to ensure all oocysts within the
area being counted were recorded.
1) Add H⁺ or OH⁻ if pH correction required.
2) Spike with Cryptosporidium parvum oocysts.
3) Add coagulant and flash mix for 1 minute @ 200 rpm.
4) Slow stir for 14 minutes @ 20 rpm.
5) Stop stirring and allow to settle for 15 minutes.
6) Discard first 50 ml from tap and then take samples for later analysis.
Figure 5. Typical grid pattern on a haemocytometer slide. Each double-bordered square holds a volume of 0.10 l. Total volume of grid is 0.90 l.

Figure 6. Cryptosporidium parvum oocysts showing green fluorescent stain under UV light (Plummer et al., 1995)
4 ELECTROPHORESIS

4.1 Introduction
Variation in the surface charge of particles will influence the coagulant dose required to neutralise those particles and remove them from suspension. Electrophoresis is a technique commonly used to measure the effective surface charge of particles in suspension. This study was performed to determine how the surface charge of Cryptosporidium parvum oocysts varies with a number of factors including:

- Heat-inactivating the oocysts to determine whether the surface charge is affected.
- Changing the ionic strength or total dissolved solids (TDS) \(10^{-2}\) and \(10^{-3}\) M).
- The effect of pH (from 2 to 12).
- The effect of turbidity (0 to 65 NTU).
- The effect of the concentration of NOM (0 to 15 ppm of added NOM using a MIEX™ extract).

These factors are discussed in more detail below. The maximum error associated with the electrophoretic potentials was approximately 20% (estimated using the standard deviation).

4.2 Effect of Ionic Strength and Heat-Inactivation Across a pH Range
Electrophoresis was performed on live and heat-inactivated Cryptosporidium oocysts at ionic strengths of \(10^{-3}\) and \(10^{-2}\). The pH ranges used were 3 to 12 for \(10^{-2}\) M ionic strength and 3 to 11 for \(10^{-3}\) M ionic strength, with the results shown in Figure 7 and given in Appendix I. Figure 7 shows the variation in zeta potential with pH for live and heat-inactivated oocysts at ionic strengths of \(10^{-2}\) and \(10^{-3}\) M. There is a decrease in zeta potential when the ionic strength is increased by a factor of 10 from \(10^{-3}\) to \(10^{-2}\) M. There is also a decrease in zeta potential when the oocysts are heat-inactivated. This suggests the surface charge of the oocysts decreases with inactivation.

A comparison of zeta potential of live and heat-inactivated oocysts, including literature values for live oocysts (Drozd et al., 1996 and Ongerth et al., 1996) performed at \(10^{-3}\) M is shown in Figure 8 and given in Appendix II. This shows a general trend as the zeta potential (effective surface charge) decreases from Ongerth (4-day old oocysts) to live oocysts from this work (2 to 4 weeks old oocysts) to heat-inactivated oocysts. (The line for Drozd is calculated from many points and estimates a cross-over at a pH of approximately 2.5).
Figure 7. Comparison of zeta potential of live and inactive oocysts at $10^{-2}$ and $10^{-3}$ M

Figure 8. Comparison of zeta potential of live and inactive oocysts at $10^{-3}$ M
4.3 Effect of Concentration of NOM

Electrophoresis was performed on heat-inactivated *Cryptosporidium* oocysts at $10^{-3}$ M and pH 6 and 8 with various concentrations of NOM. Results are shown in Figure 9 and given in Appendix III. The NOM isolate used was filtered to remove turbidity and had significantly higher levels of organics compared with inorganics (TDS) in order to assess the effect of NOM concentration without changing the ionic strength. At both pH 6 and 8 there was an increase in effective surface charge with increase in concentration of NOM. This may be a result of the oocysts interacting with the NOM, generating a complex with a more negative charge.

4.4 Effect of Turbidity

Electrophoresis was performed on heat-inactivated *Cryptosporidium* oocysts at $10^{-2}$ M and pH 7 with various turbidities. The concentration of NOM was the same for filtered and unfiltered samples, so any observed affect is a result of a change in turbidity only. Results are shown in Figure 10 and given in Appendix IV. In the range of 0 – 5 NTU (Hope Valley water) which encompasses clear and filtered waters, the variation is relatively small. However, in highly turbid waters (65 NTU, River Murray water), in comparison, the surface charge is considerably enhanced. Both waters show a similar trend, with the effective surface charge increasing with increasing turbidity.

4.5 Summary of Electrophoresis

The surface charge of the oocysts will affect the coagulant dose needed to neutralise the charge and remove the oocysts from solution. Thus, in theory a higher dose of coagulant would be needed to remove oocysts in cases when:

- The oocysts are live, with fresher oocysts having a higher surface charge and inactivated oocysts having a lower surface charge.
- The water has low salinity.
- The water has higher levels of NOM.
- The water has high turbidity.
- The pH is high.
Figure 9. Effect of NOM concentration on the zeta potential of inactive oocysts at pH 6 and 8 at $10^{-3}$ M

Figure 10. Effect of turbidity on the zeta potential of inactive oocysts at $10^{-3}$ M
5 PRELIMINARY WORK

5.1 Introduction

Before the major experimental work could be performed some preliminary experiments were required in order to ensure the results were valid for real conditions and to plan future jar tests. These initial experiments explored the effect of:

1. The state of *Cryptosporidium parvum* oocysts – The determination of whether heat-inactivated oocysts behave the same in water as live oocysts with respect to removal by coagulation during a jar test.

2. Sampling Points – Whether there is a significant difference in the removal rates between *Cryptosporidium* oocysts sampled from the top and bottom of the Gator jar (ie whether there is significant natural settling of oocysts).

3. Turbidity – The effect of turbidity and whether high turbidity has a negative impact on recovery rates of *Cryptosporidium* oocysts during analysis.

4. Whatman No. 1 Filter Papers – These filter papers are commonly used in jar tests as a surrogate for sand / anthracite filtration so their effect on the removal of oocysts was tested for possible parallel and future studies.

These experiments are explored in more detail below in Sections 5.2 to 5.5.

5.2 Live Versus Heat-Inactivated Oocysts

Two parallel jar tests were carried out to determine the effect of the oocysts being alive or heat-inactivated on the removal of *Cryptosporidium parvum* oocysts by alum coagulation at pH 7, with the results shown in Figure 11 and given in Appendix V. Figure 11 shows a significant difference between the removal of live (10%) and heat-inactivated (24%) *Cryptosporidium* oocysts at the lowest alum dose of 15 ppm at which there is a poor floc formed. This difference corresponds well to the difference in surface charge of the live and heat-inactivated *Cryptosporidium* oocysts being approximately 2:1 (Section 4.2). Hence, the higher surface charge of the live *Cryptosporidium* oocysts results in a greater requirement for coagulant in comparison with the heat-inactivated *Cryptosporidium* oocysts (in order to neutralise them). However, as the alum dose is increased from 15 ppm (poor floc) to 30 ppm and above (where a better floc is obtained) the difference between the removal of live and heat-inactivated *Cryptosporidium* oocysts is negligible (2 to 3% - within experimental error). The results from Figure 11 suggest that when a significant floc is formed in the coagulation / flocculation stage, live and heat-inactivated *Cryptosporidium* oocysts behave the same during the jar test.

Thus, for the remaining work in this study heat-inactivated *Cryptosporidium parvum* oocysts were used, as they are easier to work with and are less hazardous (infection from Cryptosporidiosis – Section 1.2).
5.3 Effect of Sampling Location

Two jar tests (one using alum and one using PolyDADMAC) were carried out to determine the effect of sampling for *Cryptosporidium parvum* oocysts at the top (by pipette - approximately 5 cm from the surface of the water (see Section 3.3)) and from the tap (approximately 5 cm above the bottom of the Gator jar) as demonstrated in Figure 12. The results of these jar tests are shown in Figure 13 and 14, for alum and PolyDADMAC, respectively, and given in Appendix VI. Figure 13 (alum coagulation) shows two lines with similar trends, with the difference between the apparent removal rates of oocysts from top and bottom sampling from the Gator jars attributed to the natural settling rates of the oocysts and floc during the 15 minute settling time. This leads to an apparent greater removal for the higher sampling point. The lower numbers of oocysts in the top Gator jar samples together with the higher uncertainty associated with pipette placement (taking the sample from the same point each time) also lead to a higher experimental error, so that future work within this project was carried out by taking samples from the tap of the Gator jar (bottom sampled). Figure 14 (PolyDADMAC coagulation) shows little difference between removal rates of top and bottom samples and suggests that particles are kept more in suspension and do not settle as rapidly. This is probably a result of the less stable floc (cloudy solution) formed when using PolyDADMAC, whereas alum forms a much more stable floc which settles more readily.

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**Figure 11.** Comparison of the removal of live and heat-inactivated *Cryptosporidium parvum* oocysts using alum

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**Figure 12.** Comparison of the removal of live and heat-inactivated *Cryptosporidium parvum* oocysts using alum
Figure 12. Gator jar showing sampling points for Section 3.3

Figure 13. Comparison of top and bottom sampling on the apparent removal of Cryptosporidium parvum oocysts using alum
5.4 Effect of Turbidity on Recovery Rates

The effect of turbidity on recovery rates was the final test to be determined in order to correctly attribute removal by the treatment process as distinct from differences in efficiency of detection of oocysts and was carried out using the following procedure:

- The water to be tested was separated into two equal lots, with one lot filtered through a 0.20 m filter to remove turbidity.
- The two fractions (filtered and unfiltered) were then combined in different percentages and a standard jar test carried out (without coagulant).
- Duplicate samples were taken from the taps of the Gator jars at the conclusion of the jar test, with recovery rates of the Cryptosporidium parvum oocysts compared with the turbidity of the solutions.

The experiment was carried out in two main parts:

1) Hope Valley water (Section 2.3), with three jars containing:
   i. Filtered (0.20 m)
   ii. 50:50 Filtered:Unfiltered
   iii. Unfiltered

to determine if the recovery of the oocysts varies across this limited range of turbidity (approximately 0 to 5 NTU).

2) River Murray water (Section 2.6), with five jars containing:
   i. Filtered (0.20 m)
   ii. 75:25 Filtered:Unfiltered
iii. 50:50 Filtered:Unfiltered
iv. 25:75 Filtered:Unfiltered
v. Unfiltered
to determine if the recovery of the oocysts varies across this broader range of turbidity (approximately 0 to 60 NTU).

The results of Part 1 with Hope Valley water are shown in Figure 15 and given in Appendix VII. Figure 15 shows a slight increase in recovery rate with increasing turbidity from 0.22 to 4.5 NTU. This slight increase may be attributed to the formation of a more solid pellet capturing the oocysts during the centrifugation (Section 3.4), leading to a smaller loss in recovery. However, the slight increase is probably within experimental error, indicating there are no complications associated with oocyst recovery for a range of turbidity between 0 and 5 NTU.

![Figure 15](image_url)

**Figure 15. Effect of turbidity on the recovery of Cryptosporidium parvum oocysts from Hope Valley water**

The results of Part 2 with River Murray water are shown in Figure 16 and given in Appendix VII. Figure 16 shows a number of trends and yielded the following results:

- A similar recovery to the Hope Valley results of around 20% was obtained at low turbidity (0.2 NTU). Low recoveries of oocysts in clean waters are attributed to a less firm pellet being formed upon centrifugation compared with the pellet in a more turbid sample.

- The recovery of the oocysts increased dramatically up to 70% at 16.5 NTU and further to 85% at 30 NTU. This is probably a result of the greater pellet being formed, trapping the oocysts more efficiently during centrifugation (Section 3.4).

- As the turbidity increased to 45 and 60 NTU there was a slight fall-off of recovery rates down to 80% and 70%, respectively. The significance of this decrease is unclear as higher errors (variation between duplicate samples) were associated with these latter two turbidities. It appears once the
turbidity of a sample increases past 30 NTU there is a decrease in recovery rates due to either in efficient staining of some of the oocysts or they are being hidden as a result of the large amount of debris in solution.

Thus, the following conclusions can be made on the effect of turbidity on the recovery rates of Cryptosporidium parvum oocysts during analysis:

- There is no decrease in recovery of Cryptosporidium oocysts in low turbidity waters such as Hope Valley water across the range of 0 to 5 NTU.
- As turbidity increases the recovery of oocysts also increases up to 30 NTU, probably as a result of the formation of a firmer pellet.
- As turbidity increases above 30 NTU there is a decrease in recovery, probably as a result of the amount of debris in solution or less efficient staining.

5.5 Effect of Filtering with Whatman No. 1 Papers

In order to simulate a water treatment plant, a jar test must have a method for assessing the filtration step. Whatman No. 1 filter papers are commonly used for this purpose and an experiment was performed to test how those filter papers affect the removal of Cryptosporidium parvum oocysts to determine their applicability for this study (see Section 3.3). Results are shown in Figure 17 and given in Appendix IX. Figure 17 shows a plot of removal of oocysts versus alum dose with and without filtration at pH 7. Figure 18 is identical to Figure 17 except it uses log removal instead of percentage removal to enhance the upper section. Note that in the cases of > 99.99% and > 4 values in Table 10 no oocysts were detected and a lower limit is estimated.

Results from the Whatman No. 1 filter study are summarised below.

1. With no added coagulant (alum) Whatman No 1 filter papers remove 50% of the Cryptosporidium oocysts in solution, despite the average pore size of Whatman No. 1 filter papers being 12 m, compared to 5 m for Cryptosporidium parvum oocysts. This may be a result of the oocysts interacting with the paper fibres and becoming enmeshed.

2. Even for the lowest doses of alum (15 ppm) the removal of Cryptosporidium oocysts by the Whatman No 1 filter paper exceeds 98%.

3. For alum doses higher than 30 ppm no Cryptosporidium oocysts were observed.

These results suggest that regardless of the floc formed by alum, the Whatman No. 1 filter papers remove almost all Cryptosporidium oocysts in suspension. This may be a result of the floc layering the filter paper and forming a much more efficient barrier to oocysts than would be expected from an average pore size of 12 m or oocysts remaining aggregated within the floc. Figure 18 shows at least a 2 log removal of oocysts using a Whatman No. 1 filter paper, regardless of the alum dose used. Previous studies (Rachwal et al., 1996) have shown between 0.5 and 2 log removal of oocysts through filtration alone. This indicates that Whatman No.1 filter papers remove a higher proportion of oocysts than would be expected through a sand / anthracite filter, although further studies would be needed to fully assess their applicability as filter surrogates with respect to the removal of Cryptosporidium oocysts.

Thus, with Whatman No. 1 filter papers being a poor surrogate, the filtration step cannot be duplicated and so the remaining work can only be carried out with respect to coagulation / flocculation.
Figure 16. Effect of turbidity on the recovery of Cryptosporidium parvum oocysts in River Murray water

Figure 17. Effect of filtering using a Whatman no 1 filter paper on the removal of Cryptosporidium parvum oocysts after alum coagulation.
6 RESULTS AND DISCUSSION

6.1 Introduction

Initial validation (Section 5) demonstrated a number of trends for coagulation including:

- Differences between the removal rates of live and heat-inactivated Cryptosporidium parvum oocysts.
- The effect of sampling from the top or bottom sections of the Gator jar.
- The effect of turbidity on recovery of Cryptosporidium oocysts during analysis across a wide range of turbidities (0 to 60 NTU).
- The effect of filtration using Whatman No. 1 filter papers.

Unless stated otherwise, the experiments discussed below involve the use of 0.2 m filtered Hope Valley water (to remove turbidity) and heat-inactivated Cryptosporidium parvum oocysts with samples taken from the tap in the Gator jar (bottom). Filtration by Whatman No. 1 filter papers was not used for the reasons discussed in Section 5.5. A full description of the experimental procedure used is given in Section 3. The removal of Cryptosporidium oocysts using alum, PolyDADMAC and ferric chloride are investigated in Section 6.2, 6.3 and 6.4, respectively.

There are a number of variables that may influence the effect on the removal of Cryptosporidium oocysts using coagulation including:

1. The coagulant dose.
2. The pH of the water.
3. The concentration of NOM in the water.
4. The turbidity of the water.

Variation of Coagulant Dose
The coagulant dose was varied to give a good range of coagulation as shown in Table 1.

Variation of pH
The pH was 5, 7 and 9 to cover the pH range commonly associated with natural waters. The pH of the sample water was modified by the addition of acid or base prior to coagulant addition, as discussed in Section 3.3.

Variation of NOM Concentration
The concentration of NOM (the dissolved Natural Organic Matter in solution) was varied by addition of an extract isolated using MIEX® resin across the range of 0 to 13 ppm to determine the effect of NOM concentration on the removal of Cryptosporidium oocysts as discussed in Section 3.3. The NOM isolate used was filtered to remove turbidity and had significantly higher levels of organics compared with inorganics (TDS) in order to assess the effect of NOM concentration only.

Variation of Turbidity
The turbidity was varied across the range of 0 to 5 NTU (Hope Valley) and 0 to 65 NTU (River Murray) to determine the effect of turbidity on the removal of oocysts during coagulation (effect of turbidity on recovery of oocysts during analysis is covered in Section 5.4).

6.2 The Effect of Alum Coagulation on the Removal of Cryptosporidium parvum Oocysts

6.2.1 The Effect of Alum Dose and pH
The removal rates of Cryptosporidium oocysts using varying concentrations of alum at a pH of 5, 7 and 9 are shown in Figure 19 and given in Appendix X. Some significant trends were observed and summarised below:

1. As the alum dose increased in the range applied, the removal of Cryptosporidium oocysts also increased, regardless of pH.
2. At low alum doses where a poor floc was formed, the removal of Cryptosporidium oocysts was also poor (typically below 25%).
3. As soon as an alum dose was reached which results in the formation of a significant floc (30 ppm for pH 5 and 7 and 60 ppm for pH 9), the removal of Cryptosporidium oocysts increased to above 75%.
4. The highest removal of Cryptosporidium oocysts using alum occurred at pH 7 for the highest alum dose (75 ppm) and yielded 94% removal.
Figure 19. The removal of Cryptosporidium parvum oocysts using alum

Figure 20 is a three-dimensional colour contour plot which shows the optimal conditions for Cryptosporidium oocysts removal versus alum dose and pH. Figure 21 is similar to Figure 20 except it uses log removal instead of percentage removal. Both plots highlight the previous comments, with the red area yielding the highest removal of Cryptosporidium oocysts.

Figure 21 clearly shows the log removal of Cryptosporidium oocysts which can be obtained using alum and yields:

1. Approximately 0.1 log removal was obtained at low alum doses where a poor floc was observed.
2. Approximately 0.7 log removal was obtained once a significant floc was observed.
3. The best removal occurred at pH 7 for the highest alum dose (75 ppm) and yielded 1.2 log removal.
Figure 20. Three-dimensional plot showing the removal of *Cryptosporidium parvum* oocysts using alum

Figure 21. Three-dimensional plot showing the log removal of *Cryptosporidium parvum* oocysts using alum
6.2.2 The Effect of NOM Concentration on the Removal of Cryptosporidium parvum Oocysts

A jar test was carried out to determine the effect of NOM concentration on the removal of Cryptosporidium parvum oocysts using alum. A MIEX® extract (Section 3.1) was the source of NOM used to modify the NOM concentration. The NOM concentrations used were 4 ppm and 8 ppm of added NOM (Section 2.5). The jar test was carried out in two parts:

1. A jar test using the same alum dose for all jars, regardless of NOM concentration. The alum dose used was 45 ppm, the optimal dose for Hope Valley water (as determined by turbidity).

2. A jar test using optimal alum doses for each jar (as determined by turbidity), depending on NOM concentration. These alum doses were calculated using a preliminary jar test and were 45, 75 and 110 ppm for 0, 4 and 8 ppm of added NOM, respectively.

A plot of the results for the two experiments described above are shown in Figure 22. The jar test results are summarised in Appendix XI.

![Graph showing the effect of NOM concentration on the removal of Cryptosporidium parvum oocysts using alum before and after correction](image)

**Figure 22.** The effect of NOM concentration on the removal of Cryptosporidium parvum oocysts using alum before and after correction

For the uncorrected NOM experiment, Figure 22 shows a general decrease in the removal of oocysts, from 84% removal for Hope Valley water down to 70% removal for 4 ppm of added NOM and a further reduction to 50% removal for 8 ppm of added NOM. This trend suggests there is a decrease in the removal of oocysts with increasing concentration of NOM. However, the alum doses used in water treatment plants are generally optimised for the concentration of NOM, as this has been found to effect the formation of floc. Therefore, a less effective floc is expected to form as the concentration of NOM increases, unless the alum dose is also increased.

The corrected NOM experiment used alum doses which were optimised for the NOM concentration. Figure 22 shows a similar removal for Hope Valley water for no added NOM as expected (same alum dose, same concentration of NOM). A decrease in removal of oocysts is observed for both corrected
and uncorrected jar tests for 4 ppm added NOM. However, a difference was observed for the highest
NOM concentration, with the removal continuing to decrease for the uncorrected jar test and leveling
off for the corrected jar test.

The results of this NOM experiment are summarised below:

- When the concentration of NOM increased and the alum dose remained constant then a decrease
  in the removal of *Cryptosporidium* oocysts was observed.
- When the concentration of NOM increased and the alum dose also increased then the removal of
  *Cryptosporidium* oocysts showed either a slight decrease or remained constant. This suggests that
  if the concentration of NOM increases then the alum dose can be increased to prevent (or at least
  minimise) a decrease in the removal of *Cryptosporidium* oocysts.
- If the concentration of NOM decreased and the alum dose remained constant then the removal of
  *Cryptosporidium* oocysts would be expected to either increase slightly or remain the same.

### 6.2.3 The Effect of Turbidity on the Removal of *Cryptosporidium parvum* Oocysts

A jar test was performed to determine the effect of turbidity on the removal of *Cryptosporidium parvum*
oocysts using alum. Previous experiments involving alum dose, pH and NOM concentration were
 carried out using 0.20 m filtered Hope Valley water to remove turbidity (Section 2.6). Initial
experiments into turbidity (Section 5.4) showed no effect on the recovery of oocysts for Hope Valley
water (0 to 5 NTU) and that an increase in recovery could be expected for higher turbidity waters. This
experiment was therefore performed in two parts to explore:

1. The effect of using unfiltered Hope Valley water (4.5 NTU) on the removal of *Cryptosporidium*
oocysts and a comparison with the results from the corresponding jar test using 0.20 m
filtered water. This experiment was carried out at pH 7.
2. The effect on the removal of *Cryptosporidium* oocysts using alum (45 ppm) when one jar has a
much higher turbidity. This experiment was carried out at pH 7 using River Murray water with
one sample 0.20 m filtered and the other unfiltered (65 NTU).

The two parts of the turbidity experiment are discussed in Sections 6.2.3.1 and 6.2.3.2.

#### 6.2.3.1 Effect of Turbidity in Hope Valley Water

The results of these jar tests are shown in Figure 23 and given in Table 2. Figure 23 shows two major
trends:

1. At low alum doses (15 and 30 ppm) the removal of *Cryptosporidium* oocysts increases
   approximately 10% for unfiltered Hope Valley water compared with filtered Hope Valley water. This
   suggests that at low alum doses (less than optimum dose) the higher turbidity of the water slightly
   aids the flocculation process, resulting in the formation of a better floc.
2. At higher alum doses (45 ppm or more) turbidity has little effect on the removal of *Cryptosporidium*
oocysts.

These results indicate that over the turbidity range of Hope Valley water (0 to 5 NTU), turbidity has little
effect on the removal of *Cryptosporidium* oocysts using alum.

From Table 2, the turbidity of the settled water decreases as the removal of oocysts increases, and is
significant at low alum doses (15 to 30 ppm). However, no significant trend is observed for settled
water turbidity with higher alum doses (above 30 ppm).
The effect of turbidity of Hope Valley water on the removal of Cryptosporidium parvum oocysts using alum

Table 2. Effect of turbidity on the removal of Cryptosporidium parvum oocysts using alum in Hope Valley water

<table>
<thead>
<tr>
<th>Alum Dose (ppm)</th>
<th>0.20 μm Filtered (0.2 NTU)</th>
<th>Settled Turbidity (NTU)</th>
<th>Unfiltered (4.5 NTU)</th>
<th>Settled Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% / Log Removal</td>
<td>% / Log Removal</td>
<td>% / Log Removal</td>
<td>% / Log Removal</td>
</tr>
<tr>
<td>15</td>
<td>18% / 0.09</td>
<td>0.55</td>
<td>28% / 0.14</td>
<td>10.5</td>
</tr>
<tr>
<td>30</td>
<td>74% / 0.59</td>
<td>0.38</td>
<td>87% / 0.89</td>
<td>3.9</td>
</tr>
<tr>
<td>45</td>
<td>86% / 0.85</td>
<td>0.36</td>
<td>86% / 0.85</td>
<td>3.7</td>
</tr>
<tr>
<td>60</td>
<td>93% / 1.15</td>
<td>0.40</td>
<td>88% / 0.92</td>
<td>3.6</td>
</tr>
<tr>
<td>75</td>
<td>94% / 1.22</td>
<td>0.36</td>
<td>92% / 1.10</td>
<td>3.6</td>
</tr>
</tbody>
</table>

6.2.3.2 Effect of High Turbidity in River Murray Water
The results of these jar tests are given in Table 3 along with the log removal. Table 3 shows a significant increase in the removal of Cryptosporidium oocysts using alum for the highly turbid River Murray water (65 NTU). Previous work (Section 3.4) demonstrated that the recovery of Cryptosporidium oocysts was not adversely affected by higher turbidity waters, suggesting that this increase (from 75% to 95% or a log increase of 0.6 to 1.4) is a result of the larger particles (floc
capturing the oocysts in suspension and removing them more efficiently. This result indicates that water of high turbidity increases the removal of *Cryptosporidium* oocysts using alum.

Table 3. Effect of turbidity on the removal of *Cryptosporidium parvum* oocysts using alum in River Murray water

<table>
<thead>
<tr>
<th>Turbidity (NTU)</th>
<th>% Removal</th>
<th>Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56</td>
<td>76%</td>
<td>0.62</td>
</tr>
<tr>
<td>65</td>
<td>96%</td>
<td>1.40</td>
</tr>
</tbody>
</table>

6.3 The Effect of PolyDADMAC Coagulation on the Removal of *Cryptosporidium parvum* Oocysts

6.3.1 The Effect of PolyDADMAC Dose and pH

The removal rates of *Cryptosporidium parvum* oocysts using varied concentrations of PolyDADMAC at a pH of 5, 7 and 9 are shown in Figure 24 and given in Appendix XII. Some significant trends are observed for Figure 24 and summarised below:

1. For the range of PolyDADMAC doses used in these experiments as the PolyDADMAC dose increased the removal of *Cryptosporidium* oocysts also increased, regardless of pH, except for the highest PolyDADMAC dose (10 ppm) at pH 5. This is probably a result of “particle restabilisation” (Edzwald, 1981), with the polymer dose being in excess for the amount of NOM in solution.

2. At low PolyDADMAC doses (1.0 ppm) where a slightly cloudy solution was formed the removal of *Cryptosporidium* oocysts was also poor at pH 7 and 9 (typically below 40%). However, for the lowest dose at pH 5, a greater than 70% removal was observed. This suggests that a cationic polymer is very effective at *Cryptosporidium* removal at acidic pH.

3. As soon as a PolyDADMAC dose was reached which resulted in the formation of a significant floc (1.0 ppm for pH 5 and 2.5 ppm for pH 7 and 9), the removal of *Cryptosporidium* oocysts increased significantly to above 65%.

4. The optimal removal of *Cryptosporidium* oocysts using PolyDADMAC occurred at either pH 5 for the second highest PolyDADMAC dose (6 ppm) or at pH 7 for the highest PolyDADMAC dose (10 ppm) and yielded 95% removal.

Figure 25 is a three-dimensional colour contour plot which shows the optimal conditions for *Cryptosporidium* oocysts removal versus PolyDADMAC dose and pH. Figure 26 is similar to Figure 25 except log removal replaces percentage removal. Both plots highlight the previous comments, with the red area yielding the highest removal of *Cryptosporidium* oocysts.

Figure 26 clearly shows the log removal of *Cryptosporidium* oocysts which can be obtained using PolyDADMAC and yields:

1. Approximately 0.2 log removal is obtained at low PolyDADMAC doses where a poor floc is obtained (1.0 ppm at pH 7 and 9).

2. Approximately 0.6 log removal can be obtained once a significant floc is obtained (1.0 ppm at pH 5 and 2.5 ppm at pH 7 and 9).
3. The best removal occurs either at pH 5 for the second highest PolyDADMAC dose (6 ppm) or at pH 7 for the highest PolyDADMAC dose (10 ppm) and yields 1.3 log removal.

Figure 24. The removal of Cryptosporidium parvum oocysts using PolyDADMAC

Figure 25. Three-dimensional plot showing the removal of Cryptosporidium parvum oocysts using PolyDADMAC
6.3.2 The Effect of NOM Concentration on the Removal of Cryptosporidium parvum Oocysts

A jar test was carried out to determine the effect of NOM concentration on the removal of Cryptosporidium parvum oocysts using PolyDADMAC. A MIEX® extract (Section 3.1) was the source of NOM used to modify the NOM concentration. The NOM concentrations used were 4 ppm and 8 ppm of added NOM (Section 2.5). The jar test was carried out using the same PolyDADMAC dose for all jars, regardless of NOM concentration. The PolyDADMAC dose used was 4.0 ppm, the optimal dose for Hope Valley water.

A plot of the results for this NOM experiment are shown in Figure 27. The jar test results are summarised in Appendix XIII.

Figure 27 shows no decrease in the removal of oocysts upon adding NOM and suggests that the coagulation of water using PolyDADMAC is independent of NOM concentration.
6.3.3 The Effect of Turbidity on the Removal of Cryptosporidium parvum Oocysts

A jar test was performed to determine the effect of turbidity on the removal of Cryptosporidium parvum oocysts using PolyDADMAC. As stated above, previous experiments involving PolyDADMAC dose, pH and NOM concentration were carried out using 0.20 m filtered Hope Valley water to remove turbidity (Section 2.6). Initial experiments into turbidity (Section 5.4) showed no effect on the recovery of oocysts from Hope Valley water (0 to 5 NTU) and that an increase in recovery could be expected for higher turbidity waters. This experiment was therefore performed in two parts to explore:

1. The effect of using unfiltered Hope Valley water (4.5 NTU) on the removal of Cryptosporidium oocysts by PolyDADMAC and a comparison with the results from the corresponding jar test using 0.20 m filtered water. This experiment was carried out at pH 7.
2. The effect on the removal of Cryptosporidium oocysts using PolyDADMAC (4 ppm) when one jar has a much higher turbidity. This experiment was carried out at pH 7 using River Murray water with one jar 0.20 m filtered and the other unfiltered (65 NTU).

The two parts of the turbidity experiment are discussed in Sections 6.3.3.1 and 6.3.3.2 below.

6.3.3.1 Effect of Turbidity in Hope Valley Water

The results of these jar tests are shown in Figure 28 and given in Table 4. Figure 28 shows two major trends:

1. There was an increase in removal of oocysts for all PolyDADMAC doses used for raw Hope Valley water compared with filtered Hope Valley water. This suggests that the higher turbidity of the water significantly aids the flocculation process, when using PolyDADMAC for the removal of Cryptosporidium oocysts. This may result from the formation of a better floc which captures the oocysts, removing them from solution.
2. The increase in removal of oocysts due to higher turbidity water was most significant for the optimal doses of PolyDADMAC used (between 2.5 and 6 ppm). A less significant increase was observed for the lowest and highest dose of PolyDADMAC (1.0 and 10 ppm, respectively).

These results indicate that over the turbidity range of Hope Valley water (0 to 5 NTU), turbidity has a substantial effect on the removal of Cryptosporidium oocysts using PolyDADMAC.

From Table 4, the turbidity of the settled water decreases as the removal of oocysts increases, except for the lowest dose of PolyDADMAC (1.0 ppm). This trend appears significant for the filtered water (0.20 m) and less significant for the raw water.

**Table 4. Effect of added turbidity on the removal of Cryptosporidium parvum oocysts using PolyDADMAC in Hope Valley water**

<table>
<thead>
<tr>
<th>Poly-DADMAC (ppm)</th>
<th>0.20 m Filtered Turbidity (0.2 NTU)</th>
<th>Settled Turbidity (NTU)</th>
<th>Unfiltered Turbidity (4.5 NTU)</th>
<th>Settled Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>37% / 0.20</td>
<td>3.1</td>
<td>73% / 0.56</td>
<td>13.8</td>
</tr>
<tr>
<td>2.5</td>
<td>68% / 0.50</td>
<td>6.3</td>
<td>92% / 1.12</td>
<td>20.9</td>
</tr>
<tr>
<td>4.0</td>
<td>78% / 0.66</td>
<td>4.2</td>
<td>96% / 1.35</td>
<td>21.1</td>
</tr>
<tr>
<td>6.0</td>
<td>88% / 0.92</td>
<td>3.4</td>
<td>97% / 1.55</td>
<td>15.2</td>
</tr>
<tr>
<td>10.0</td>
<td>95% / 1.30</td>
<td>0.59</td>
<td>97% / 1.53</td>
<td>8.7</td>
</tr>
</tbody>
</table>

**Figure 28. The effect of turbidity of Hope Valley water on the removal of Cryptosporidium parvum oocysts using PolyDADMAC**
6.3.3.2 Effect of High Turbidity in River Murray Water

The results of these jar tests are given in Table 5 along with the log removal. Table 5 shows a significant increase in the removal of Cryptosporidium oocysts using PolyDADMAC for the highly turbid River Murray water (68 NTU). Previous work (Section 5.4) demonstrated that the recovery of Cryptosporidium oocysts was not adversely affected by higher turbidity waters suggesting that this increase (from 91% to 99% or a log increase of 1.0 log) is a result of the larger particles (floc) capturing the oocysts in solution and removing them more efficiently. This result suggests that water of high turbidity increases the removal of Cryptosporidium oocysts using PolyDADMAC.

Table 5. Effect of turbidity on the removal of Cryptosporidium parvum oocysts using PolyDADMAC in River Murray water

<table>
<thead>
<tr>
<th>Turbidity (NTU)</th>
<th>% Removal</th>
<th>Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>91%</td>
<td>1.05</td>
</tr>
<tr>
<td>68</td>
<td>99.2%</td>
<td>2.10</td>
</tr>
</tbody>
</table>

6.4 The Effect of Ferric Chloride Coagulation on the Removal of Cryptosporidium parvum Oocysts

6.4.1 The Effect of Ferric Chloride Dose and pH

The removal rates of Cryptosporidium parvum oocysts using varied concentrations of ferric chloride at pH 5, 7 and 9 are shown in Figure 29 and given in Appendix XIV. Some significant trends are observed for Figure 29 and these are summarised below:

1. For the range of ferric chloride doses used as the ferric chloride dose increased the removal of Cryptosporidium oocysts also increased, regardless of pH, although a minor drop in removal was observed for the highest ferric chloride doses at pH 5 and 7 (within experimental error).
2. Even at the lowest ferric chloride doses, the removal of Cryptosporidium oocysts was good (greater than 80%).
3. Once the optimal dose of ferric chloride (47 ppm) was reached (based on turbidity), the removal of Cryptosporidium oocysts increased to above 95%.
4. For pH 5 and 7, there was no apparent improvement in the removal of oocysts for ferric chloride doses above 47 ppm.
5. The optimal removal of Cryptosporidium oocysts using ferric chloride occurs at pH 5 and 7 for ferric chloride doses 47 ppm or higher and yields 98% removal.
Figure 29. The removal of *Cryptosporidium parvum* oocysts using ferric chloride

Figure 30 is a three-dimensional colour contour plot which shows the optimal conditions for *Cryptosporidium* oocysts removal versus ferric chloride dose and pH. Figure 31 is similar to Figure 30 except it shows log removal instead of percentage removal. Both plots highlight the previous comments, with the red area yielding the highest removal of *Cryptosporidium* oocysts.

Figure 31 clearly indicates the log removal of *Cryptosporidium* oocysts which can be obtained using ferric chloride and yields:

1. Approximately 0.8 log removal was obtained at the lowest ferric chloride dose (16 ppm).
2. Greater than 1.3 log removal was obtained when the optimal dose of ferric chloride (47 ppm) was used.
3. The best removal occurred at pH 5 and 7 for ferric chloride doses of 47 ppm and higher and yielded 1.7 log removal.
Figure 30. Three-dimensional plot showing the removal of Cryptosporidium parvum oocysts using ferric chloride

Figure 31. Three-dimensional plot showing the log removal of Cryptosporidium parvum oocysts using ferric chloride
6.4.2 The Effect of NOM Concentration on the Removal of *Cryptosporidium parvum* Oocysts

A jar test was carried out to determine the effect of NOM concentration on the removal of *Cryptosporidium parvum* oocysts using ferric chloride. A MIEX<sup>®</sup> extract (Section 3.1) was the source of NOM used to modify the NOM concentration. The NOM concentrations used were 4 ppm and 8 ppm of added NOM (Section 2.5). The jar test was carried out in two parts:

1. A jar test using the same ferric chloride dose for all jars, regardless of NOM concentration. The ferric chloride dose used was 47 ppm, the optimal dose for Hope Valley water.

2. A jar test using optimal ferric chloride doses for each jar, depending on NOM concentration. These ferric chloride doses were calculated using a preliminary jar test and were 47, 79 and 115 ppm for 0, 4 and 8 ppm of added NOM, respectively.

A plot of the results for both the corrected and uncorrected NOM experiments are shown in Figure 32. The jar test results are summarised in Appendix XV.

For the uncorrected NOM experiment Figure 32 shows a very slight decrease in the removal of oocysts with increasing NOM concentration, from 98% removal for Hope Valley water down to 96% removal for 8 ppm of added NOM. This trend indicates there may be a minor decrease in the removal of oocysts with increasing concentration of NOM although the effect is probably not significant.

For the corrected NOM experiment (involving the optimisation of the ferric chloride dose required in each jar for the new NOM concentration) Figure 32 shows a lesser falloff in removal rates of oocysts when correcting for NOM concentration, although the effect is minor and the values are probably within experimental error.

Thus, NOM concentration appears to have only a minor effect, if any, on the removal of oocysts using ferric chloride.
6.4.3 The Effect of Turbidity on the Removal of Cryptosporidium parvum Oocysts

A jar test was performed to determine the effect of turbidity on the removal of Cryptosporidium parvum oocysts using ferric chloride. As stated above previous experiments involving ferric chloride dose, pH and NOM concentration were carried out using 0.20 m filtered Hope Valley water to remove turbidity (Section 2.6). Initial experiments into turbidity (Section 5.4) showed no effect on the recovery of oocysts for Hope Valley water (0 to 5 NTU) and that an increase in recovery could be expected for higher turbidity waters. This experiment was therefore performed in two parts to explore:

1. The effect of using unfiltered Hope Valley water (4.5 NTU) on the removal of Cryptosporidium oocysts and a comparison with the results from the corresponding jar test using 0.20 m filtered water. This experiment was carried out at pH 7.
2. The effect on the removal of Cryptosporidium oocysts using ferric chloride (47 ppm) when one jar has a much higher turbidity. This experiment was carried out at pH 7 using River Murray water with one jar 0.20 m filtered and the other unfiltered (100 NTU).

The two parts of the turbidity experiment are discussed in Sections 6.4.3.1 and 6.4.3.2 below.

6.4.3.1 Effect of Turbidity in Hope Valley Water

The results of these jar tests are shown in Figure 33 and given in Table 6. Figure 36 shows two major trends:

1. At low ferric chloride doses (16 and 32 ppm) the removal of Cryptosporidium oocysts increased significantly for unfiltered Hope Valley water compared with filtered Hope Valley water. This suggests that at low ferric chloride doses (less than optimum dose) the higher turbidity of the water significantly aids the flocculation process, resulting in the formation of a better floc.
2. For all ferric chloride doses used there was an improvement in the removal of oocysts for the unfiltered Hope Valley water (5 NTU) compared with the filtered Hope Valley water (0.2 NTU).

These results suggest that over the turbidity range of Hope Valley water (0 to 5 NTU), turbidity has a substantial effect in aiding the removal of Cryptosporidium oocysts using ferric chloride.

From Table 6, the turbidity of the settled water decreases as the removal of oocysts increases, except for the highest dose of Ferric Chloride (79 ppm). This trend appears significant for the filtered water (0.20 m) and less significant for the raw water.

Table 6. Effect of added turbidity on the removal of Cryptosporidium parvum oocysts using ferric chloride in Hope Valley water

<table>
<thead>
<tr>
<th>Ferric Chloride Dose (ppm)</th>
<th>0.20 m Filtered (0.2 NTU)</th>
<th>Settled Turbidity (NTU)</th>
<th>Unfiltered (4.5 NTU)</th>
<th>Settled Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>83.1% / 0.77</td>
<td>1.61</td>
<td>97.7% / 1.64</td>
<td>1.54</td>
</tr>
<tr>
<td>32</td>
<td>90.9% / 1.04</td>
<td>1.46</td>
<td>99.7% / 2.47</td>
<td>1.28</td>
</tr>
<tr>
<td>47</td>
<td>97.9% / 1.69</td>
<td>1.33</td>
<td>99.9% / 3.17</td>
<td>0.83</td>
</tr>
<tr>
<td>63</td>
<td>98.1% / 1.72</td>
<td>0.85</td>
<td>99.7% / 2.60</td>
<td>0.68</td>
</tr>
<tr>
<td>79</td>
<td>97.4% / 1.59</td>
<td>0.93</td>
<td>99.7% / 2.52</td>
<td>1.11</td>
</tr>
</tbody>
</table>
6.4.3.2 Effect of High Turbidity in River Murray Water

The results of these jar tests are given in Table 7 along with the log removal. Table 7 shows a significant increase in the removal of Cryptosporidium oocysts using ferric chloride for the highly turbid River Murray water (100 NTU). Previous work (Section 3.4) demonstrated that the recovery of Cryptosporidium oocysts was not adversely affected by higher turbidity waters suggesting that this increase (from 98% to 99.99% or a log increase of 1.8 to 4.0) is a result of the larger particles (floc) capturing the oocysts in solution and removing them more efficiently. This result suggests that water of high turbidity increases the removal of Cryptosporidium oocysts using ferric chloride.

![Figure 33. The effect of adding turbidity to Hope Valley water on the removal of Cryptosporidium parvum oocysts using ferric chloride](image)

**Table 7. Effect of turbidity on the removal of Cryptosporidium parvum oocysts using ferric chloride in River Murray water**

<table>
<thead>
<tr>
<th>Turbidity (NTU)</th>
<th>% Removal</th>
<th>Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>98.5%</td>
<td>1.84</td>
</tr>
<tr>
<td>100</td>
<td>≥ 99.99%</td>
<td>*4.00</td>
</tr>
</tbody>
</table>

* This is a lower limit only, as no oocysts were detected in this sample.
7. COMPARISON OF COAGULANTS AND COAGULANT AIDS

7.1 Introduction

Section 6 has explored the removal rates of Cryptosporidium parvum oocysts using alum, ferric chloride and PolyDADMAC coagulation. A comparison of the effectiveness of the three coagulants is explored in this section, including pH and coagulant dose (Section 7.2.1), NOM concentration (Section 7.2.2) and turbidity (Section 7.2.3) as well as the effectiveness of PolyDADMAC as a coagulant aid with alum and ferric chloride (Section 7.3).

7.2 Comparison of Alum, PolyDADMAC and Ferric Chloride Coagulation on the Removal of Cryptosporidium parvum Oocysts

7.2.1 Comparison of the Effect of pH and Coagulant Dose

A comparison of the removal of oocysts between the coagulants, alum, PolyDADMAC and ferric chloride at pH 5, 7 and 9 is given in Table 8 and shown in Figure 34, 35 and 36, respectively. To make a direct comparison, the ferric chloride results should be compared with aluminium on a molar basis. As alum concentrations are expressed using \( \text{Al}_2(\text{SO}_4)_3.18\text{H}_2\text{O} \), the number of moles of ferric ions is approximately twice that of the aluminium ions. The % removals for ferric chloride doses are expressed as aluminium equivalents and shown in Table 8 and Figures 34, 35 and 36.

![Figure 34. Comparison of the removal of oocysts at pH 5 for alum, PolyDADMAC and ferric chloride coagulation](image-url)
Table 8. Summary of the removal of oocysts using alum, PolyDADMAC and ferric chloride

<table>
<thead>
<tr>
<th>pH</th>
<th>Coagulant Dose (ppm)</th>
<th>% Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alum</td>
<td>Poly-</td>
</tr>
<tr>
<td>5</td>
<td>15 1.0</td>
<td>16 4.0</td>
</tr>
<tr>
<td></td>
<td>30 2.5</td>
<td>24 4.0</td>
</tr>
<tr>
<td></td>
<td>45 4.0</td>
<td>40 6.0</td>
</tr>
<tr>
<td>7</td>
<td>15 1.0</td>
<td>16 4.0</td>
</tr>
<tr>
<td></td>
<td>30 2.5</td>
<td>24 4.0</td>
</tr>
<tr>
<td></td>
<td>45 4.0</td>
<td>40 6.0</td>
</tr>
<tr>
<td>9</td>
<td>15 1.0</td>
<td>16 4.0</td>
</tr>
<tr>
<td></td>
<td>30 2.5</td>
<td>24 4.0</td>
</tr>
<tr>
<td></td>
<td>45 4.0</td>
<td>40 6.0</td>
</tr>
<tr>
<td></td>
<td>60 6.0</td>
<td>32 6.0</td>
</tr>
<tr>
<td></td>
<td>75 10.0</td>
<td>40 10.0</td>
</tr>
</tbody>
</table>

* Doses expressed as molar equivalents of Al and Fe (Al₂(SO₄)₃·18H₂O and FeCl₃)

Figure 35. Comparison of the removal of oocysts at pH 7 for alum, PolyDADMAC and ferric chloride coagulation
At pH 5, Figure 34 shows the following:

- At low levels of coagulation, alum performed poorly compared with PolyDADMAC and ferric chloride. This is probably a result of flocculation by alum being dependent on the concentration of NOM in solution, whereas PolyDADMAC is less dependent (Section 7.2.2).
- PolyDADMAC and alum showed a similar removal of oocysts at medium, optimal and enhanced coagulation, with PolyDADMAC having a higher removal at high levels of coagulant.
- Ferric chloride showed the highest removal of oocysts at pH 5 except for the high dose of PolyDADMAC.
- PolyDADMAC (a cationic polymer) and ferric chloride showed the highest removals at acidic pH.

At pH 7, Figure 35 shows the following:

- Ferric chloride showed the highest removal at pH 7 at low coagulant doses.
- Alum and ferric chloride behaved similarly at pH 7 at higher coagulant doses.
- When using enhanced coagulation all three coagulants showed similar removal rates of oocysts.
- PolyDADMAC showed the lowest removal of oocysts at pH 7 for all coagulant doses below the highest (enhanced).

At pH 9, Figure 36 shows the following:

- Alum showed the lowest removal of oocysts at pH 9, followed by PolyDADMAC
- Ferric chloride showed the highest removal of oocysts at pH 9.
- All three coagulants showed increasing removal of oocysts with increasing coagulant dose.
The difference in the removal of oocysts between high and enhanced coagulant levels was very similar for all three coagulants.

In summary, the following conclusions can be made about the removal rates of oocysts between the three coagulants used, alum, PolyDADMAC and ferric chloride:

- All three coagulants showed a general increase in the removal of oocysts with increasing coagulant dose, except for PolyDADMAC at pH 5 which decreased from high to enhanced coagulant dose due to particle restabilisation (Edzwald, 1981).
- Ferric chloride showed the best removal of oocysts for the whole range of pH used (5, 7 and 9).
- Ferric chloride showed the least difference between removal rates at different pH (5, 7 and 9).
- For low coagulant doses, alum showed the lowest removal of oocysts at all pH (5, 7 and 9).

### 7.2.2 Comparison of the Effect of NOM Concentration on the Removal of Cryptosporidium parvum Oocysts Using Alum, PolyDADMAC and Ferric Chloride Coagulation

A comparison of the effect of NOM concentration on the removal of oocysts using alum, PolyDADMAC and ferric chloride is given in Table 9 and shown in Figure 37.

<table>
<thead>
<tr>
<th>Added NOM (ppm)</th>
<th>% Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alum</td>
</tr>
<tr>
<td></td>
<td>Corrected / Uncorrected</td>
</tr>
<tr>
<td>0</td>
<td>84% / 87%</td>
</tr>
<tr>
<td>4</td>
<td>71% / 71%</td>
</tr>
<tr>
<td>8</td>
<td>52% / 73%</td>
</tr>
</tbody>
</table>
Figure 37. Removal of oocysts with added NOM concentration for alum, PolyDADMAC and ferric chloride coagulation

Figure 37 shows several trends as summarised below:

- The removal of oocysts using PolyDADMAC is independent of the concentration of NOM in solution.
- The removal of oocysts using alum is very much dependent on the concentration of NOM in solution, although this reduction in removal can be compensated to a degree by the addition of higher alum doses.
- The removal of oocysts using ferric chloride appears only marginally effected by increasing NOM concentration, however, when comparing molar equivalents to the alum dose a much higher concentration of ferric chloride was used (approximately twice the concentration of ferric compared with aluminium) (Section 7.2.1).

7.2.3 Comparison of the Effect of Turbidity on the Removal of Cryptosporidium parvum Oocysts Using Alum, PolyDADMAC and Ferric Chloride Coagulation

A comparison of the effect of turbidity on the removal of oocysts using alum, PolyDADMAC and ferric chloride is given in Table 10 and shown in Figure 38. Table 10 also includes the settled water turbidity for comparison. Figure 39 is identical to Figure 38 except that it shows log removal instead of percent removal. Figure 38 and 39 show a number of trends:

- All three coagulants showed a higher removal of oocysts when the turbidity was increased.
- Alum and PolyDADMAC showed a similar increase in removal (1 log) upon increasing the turbidity by 60 NTU.
- Ferric chloride yielded no oocysts at 100 NTU, so a 2 log increase was estimated, however, at 60 NTU ferric chloride may be expected to have at least a 1 log increase in the removal of oocysts.
- If molar equivalents are used for alum and ferric chloride then the ferric dose was approximately twice that of the aluminium dose.
From Table 10, the removal of oocysts increases as the initial turbidity increases, despite the increase in settled water turbidity which may be expected to yield higher recovery rates (Section 5.4).

Table 10. Removal of oocysts with turbidity for alum, PolyDADMAC and ferric chloride coagulation

<table>
<thead>
<tr>
<th>Initial Turbidity (NTU)</th>
<th>Settled Turbidity (NTU)</th>
<th>% / Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alum</td>
</tr>
<tr>
<td>0.20 / 3.9 / 1.56</td>
<td>76% / 0.62</td>
<td>91%  / 1.05</td>
</tr>
<tr>
<td>65 / 58 / 100</td>
<td>96% / 1.40</td>
<td>99.2% / 2.1</td>
</tr>
</tbody>
</table>

Figure 38. Removal of oocysts with added turbidity for alum, PolyDADMAC and ferric chloride coagulation
7.3 The Use of PolyDADMAC as a Coagulant Aid with Alum and Ferric Chloride and its Effect on the Removal of Cryptosporidium parvum Oocysts

Polymeric cationic compounds such as PolyDADMAC have been effectively used as coagulant aids in the past to aid coagulation and reduce the amount of coagulant (eg alum) needed to treat the water.

Two jar tests were performed to determine the effectiveness of PolyDADMAC as a coagulant aid for the removal of Cryptosporidium with both alum (Section 7.3.1) and ferric chloride (Section 7.3.2).

7.3.1 Alum with PolyDADMAC

A jar test was carried out using two alum doses (30 and 45 ppm) and three PolyDADMAC doses (0.5, 1.0 and 2.0 ppm) to determine the effectiveness of PolyDADMAC for oocyst removal. The results are shown in Figure 40 and given in Table 11.
Figure 40 shows a significant increase in removal upon the addition of low levels of PolyDADMAC for alum, probably as a result of the formation of a better floc which captures the oocysts and removes them from suspension. As the PolyDADMAC dose increases the removal of oocysts also increases for both alum doses (30 and 45 ppm).

**Table 11. Removal of oocysts using alum coagulation with and without PolyDADMAC as a coagulant aid**

<table>
<thead>
<tr>
<th>Alum (ppm)</th>
<th>PolyDADMAC (ppm)</th>
<th>% Removal</th>
<th>Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>none</td>
<td>58%</td>
<td>0.38</td>
</tr>
<tr>
<td>30</td>
<td>1.0</td>
<td>79%</td>
<td>0.68</td>
</tr>
<tr>
<td>30</td>
<td>2.0</td>
<td>84%</td>
<td>0.80</td>
</tr>
<tr>
<td>45</td>
<td>none</td>
<td>62%</td>
<td>0.42</td>
</tr>
<tr>
<td>45</td>
<td>0.5</td>
<td>65%</td>
<td>0.46</td>
</tr>
<tr>
<td>45</td>
<td>1.0</td>
<td>74%</td>
<td>0.59</td>
</tr>
<tr>
<td>45</td>
<td>2.0</td>
<td>82%</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Thus, the addition of PolyDADMAC as a coagulant aid is effective in increasing the removal of *Cryptosporidium* oocysts using alum coagulation. In addition, higher doses of PolyDADMAC would be expected to yield a higher removal of oocysts.
7.3.2 Ferric Chloride with PolyDADMAC

A jar test was carried out using two ferric chloride doses (32 and 47 ppm) and three PolyDADMAC doses (0.5, 1.0 and 2.0 ppm) to determine the effectiveness of PolyDADMAC for oocyst removal. The results are shown in Figure 41 and given in Table 12.

Figure 41 shows a significant increase in removal upon the addition of low levels of PolyDADMAC for ferric chloride, probably as a result of the formation of a better floc which captures the oocysts and removes them from suspension. As the PolyDADMAC dose increases the removal of oocysts increases only marginally for both ferric chloride doses (32 and 47 ppm). This is in contrast with the further increase in removal which is observed for alum with higher levels of PolyDADMAC. However, this may be attributed to the higher dose of ferric used (if using molar equivalents) of approximately double the aluminium dose. Thus, the addition of low levels of PolyDADMAC as a coagulant aid is effective in increasing the removal of Cryptosporidium oocysts using ferric chloride coagulation, however, higher levels of PolyDADMAC yield no additional improvement (for high ferric doses).

![Figure 41. Removal of Cryptosporidium using ferric chloride coagulation with and without PolyDADMAC as a coagulant aid](image)

<table>
<thead>
<tr>
<th>Ferric Chloride (ppm)</th>
<th>PolyDADMAC (ppm)</th>
<th>% Removal</th>
<th>Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>none</td>
<td>83%</td>
<td>0.77</td>
</tr>
<tr>
<td>32</td>
<td>1.0</td>
<td>97%</td>
<td>1.54</td>
</tr>
<tr>
<td>32</td>
<td>2.0</td>
<td>98%</td>
<td>1.76</td>
</tr>
<tr>
<td>47</td>
<td>none</td>
<td>91%</td>
<td>1.04</td>
</tr>
<tr>
<td>47</td>
<td>0.5</td>
<td>98%</td>
<td>1.75</td>
</tr>
<tr>
<td>47</td>
<td>1.0</td>
<td>98%</td>
<td>1.80</td>
</tr>
<tr>
<td>47</td>
<td>2.0</td>
<td>99%</td>
<td>2.01</td>
</tr>
</tbody>
</table>
8. SUMMARY AND CONCLUSIONS

This study has explored the use of alum, ferric chloride and PolyDADMAC (both alone and as a coagulant aid) for the removal of Cryptosporidium parvum oocysts. A summary of the results of this study is given below.

Preliminary

- The surface charge of the oocysts varies in response to variation in pH, ionic strength, concentration of NOM and turbidity. These effects were explored using electrophoresis as a predictive tool for jar tests. In general, results correlated well at low coagulant doses, but the charge of the oocysts was only a minor factor at higher coagulant doses. The pH effect predicted by the surface charge of the oocysts was also not observed in the jar test results.
- Heat-inactivated Cryptosporidium parvum oocysts behave in a similar manner to live oocysts with respect to removal by alum coagulation during a jar test, provided a significant floc is obtained. In cases where a poor floc is formed, a higher proportion of heat-inactivated oocysts will be removed by alum coagulation compared with live oocysts (in an approximate ratio of 2 : 1).
- There is no significant difference in removal trends between sampling from the top (pipette) or the bottom (tap) of the Gator jar. The higher number of Cryptosporidium oocysts recorded when tap sampled (compared with pipette sampled) is a result of natural settling of the oocysts and floc during the settling / sedimentation stage.
- Whatman No. 1 filter papers are not a suitable surrogate for sand / anthracite filters using alum coagulation for removal rates of Cryptosporidium oocysts, although pilot plant studies would be necessary to confirm this.

Turbidity – Recovery Effects

- No difference in recovery rates of Cryptosporidium oocysts during analysis is observed when the turbidity is varied between 0 and 5 NTU.
- Recovery rates of Cryptosporidium oocysts increase during analysis as the turbidity is increased to 30 NTU due to the formation of a firmer pellet which traps the oocysts during the centrifugation step (Section 2.4).
- There appears to be a decrease in recovery rates of Cryptosporidium oocysts when the turbidity is increased above 30 NTU, possibly as a result of the amount of debris in solution masking or interfering with the fluorescent staining of oocysts.

Turbidity – Removal Effects

- Over the turbidity range of Hope Valley water (0 to 5 NTU), turbidity has little effect on the removal of Cryptosporidium oocysts using alum.
- Over this range turbidity has a significant effect on the removal of Cryptosporidium oocysts using both PolyDADMAC and ferric chloride, with removal increasing by 0.5 and 1 log, respectively, when the turbidity is increased from 0 to 5 NTU.
- Water of high turbidity (60 NTU or greater) increases the removal of Cryptosporidium oocysts using alum, PolyDADMAC and ferric chloride coagulation by at least 1 log.

pH Effects

- For the range of alum doses studied (15 to 75 ppm) as the alum dose increases the removal of Cryptosporidium oocysts also increases, irrespective of the pH of the water being treated.
- For the range of PolyDADMAC doses used (1.0 to 10.0 ppm) as the PolyDADMAC dose increases, the removal of Cryptosporidium oocysts also increases, irrespective of the pH of the water being treated.
water being treated, with one exception (10.0 ppm at pH 5) presumably as a result of particle restabilisation (Edzwald, 1981).

- As the ferric chloride dose increases (16 to 47 ppm), the removal of Cryptosporidium oocysts also increases, irrespective of pH. The removal of oocysts then levels off beyond 47 ppm, also irrespective of pH.
- At low alum doses where a poor floc is formed (Section 5.2.1), the removal of Cryptosporidium oocysts is also poor (typically below 25%) at all pH values.
- When an alum dose is reached which results in the formation of a significant floc, the removal of Cryptosporidium oocysts increases significantly to greater than 75%.

NOM
- When the concentration of NOM increases and the alum dose remains constant a decrease in the removal of Cryptosporidium oocysts is expected.
- When the concentration of NOM increases and the alum dose also increases to compensate then the removal of Cryptosporidium oocysts should be able to be maintained at a constant level.
- For PolyDADMAC and ferric chloride the removal of Cryptosporidium oocysts was independent of NOM concentration. However, for ferric chloride this may be a result of the higher dose (in molar equivalents) compared with the alum dose (Section 7.2.1).

Optimum Conditions
- The highest removal of Cryptosporidium oocysts using alum is 94% and occurs at a pH 7 with the highest alum dose (enhanced coagulation using 75 ppm).
- The highest removal of Cryptosporidium oocysts using PolyDADMAC is 95% and occurs at both pH 5 (6.0 ppm) and pH 7 (10.0 ppm).
- The highest removal of Cryptosporidium oocysts using ferric chloride is 98% and occurs at both pH 5 (47 ppm and higher) and pH 7 (47 ppm and higher).

Coagulant Aids
- PolyDADMAC is an effective coagulant aid and yields an increase in the removal of Cryptosporidium oocysts for both alum and ferric chloride.
- When PolyDADMAC is used as a coagulant aid with alum, as the concentration of PolyDADMAC increases the removal of Cryptosporidium oocysts also increases.
- When PolyDADMAC was used as a coagulant aid with ferric chloride, as the concentration of PolyDADMAC increased the removal of Cryptosporidium remained the same. This may be a result of the higher ferric dose (in molar equivalents) compared with the alum dose (Section 7.2.1).
9. RECOMMENDATIONS FOR THE WATER TREATMENT ENGINEER

The applications for the water treatment plant generated from this research are summarised below. The following recommendations are based on the use of alum, ferric chloride and PolyDADMAC as the coagulants for the removal of Cryptosporidium parvum oocysts. In addition, the recommendations are based on limited jar tests and have not been verified with pilot plant studies. Both of these points are explored further in Future Studies (Section 9).

9.1 General Recommendations

General recommendations for the water treatment engineer include:

- Optimising the coagulation process is an important step towards removing Cryptosporidium oocysts in conventional water treatment.

- The health of the oocysts (whether they are alive or inactive) is likely to influence the coagulant dose required, especially at lower coagulant doses.

- Ferric chloride is likely to be the most effective coagulant for the removal of Cryptosporidium.

- Ferric chloride is likely to be the least dependent on pH correction.

- Higher turbidity is likely to aid the removal of Cryptosporidium.

- PolyDADMAC is likely to be very effective as a coagulant aid when used with both alum and ferric chloride, and result in higher removals of oocysts.

9.2 Alum Coagulation

- Higher alum doses are likely to yield higher removal rates of Cryptosporidium oocysts, regardless of the pH, so that the use of enhanced coagulation is beneficial.

- The optimum removal of Cryptosporidium oocysts is achieved at a pH of between 6 and 7 (see Figure 19 and 20).

- The addition of higher concentrations of alum can minimise the effect of NOM on the removal of Cryptosporidium oocysts.

9.3 PolyDADMAC Coagulation

- Higher PolyDADMAC doses are likely to yield higher removal rates of Cryptosporidium except when the dose results in particle restabilisation (Edzwald, 1981).

- The optimal removal of oocysts is achieved at a pH of between 5 and 7 (see Figure 28 and 29).

- The concentration of NOM in the water is not important when using PolyDADMAC as the coagulant.

9.4 Ferric Chloride Coagulation

- Higher ferric chloride doses are likely to yield higher removal rates of Cryptosporidium oocysts until the optimum dose is achieved, after which no significant improvement is gained.

- The optimal removal of Cryptosporidium oocysts is achieved at a pH of between 5 and 7.

- The concentration of NOM in the water is not likely to be important when using ferric chloride coagulation.
10. FUTURE WORK

This study explored the effectiveness of coagulation for the removal of Cryptosporidium parvum oocysts, and highlighted several areas that warrant future investigation.

The principal need required is for pilot plant studies to determine how directly applicable the jar test results from this study will be to water treatment plants. Full-scale treatment plants use a continuous flow of water so that oocysts would experience continuous currents in the water column throughout the treatment process. This means that the high settling rate of oocysts observed in laboratory experiments may not be observed in the water treatment plant.

Furthermore, a pilot plant study using sand / anthracite filters could be included to determine their effectiveness as a barrier to Cryptosporidium oocysts and as a comparison with Whatman No 1 filter papers.

Other areas which warrant further investigation include:

1. The use of lower ferric chloride doses or higher alum doses for some experiments so that a direct comparison can be made (using molar equivalents) on the effectiveness of the two coagulants for removing oocysts.

2. The use of surrogates such as Clostridium perfringens or Bacillus subtilis spores to assess the efficiency of removal of Cryptosporidium by water treatment plants.

3. Exploring different coagulants such as polyaluminium chloride (PAC) and ferric sulphate, \( \text{Fe}_2(\text{SO}_4)_3 \) to determine whether they differ significantly with respect to oocyst removal.

4. The use of other coagulant aids such as cationic Polyacrylamides, to determine whether they can be used to enhance the removal of Cryptosporidium oocysts when combined with other coagulants.

5. A more intense study into the effects of turbidity on both the recovery and removal of oocysts. In addition, fine particulate matter could be added to the water treatment train in order to assess whether the removal of oocysts can be enhanced using this technique.

6. Additional studies exploring the properties of different waters and their effects on the removal of oocysts.
11. ACKNOWLEDGMENTS

I would like to thank the CRC for Water Quality and Treatment for funding this research and the Australian Water Quality Centre where this research was carried out.

I would also like to thank Jim Morran (Water Treatment, AWQC) for his leadership, advice and friendship, Bret Robinson (Protozoology, AWQC) for his excellent protozoology background, expertise and ideas, Phil Dobson (Protozoology, AWQC) for his technical expertise and helpfulness and Mary Drikas (Water Treatment, AWQC) for her management of the project funding.
12. REFERENCES


APPENDIX I

Zeta potential of inactive and live oocysts at $10^{-2}$ and $10^{-3}$ M, pH 3-12 (see Figure 7)

<table>
<thead>
<tr>
<th>pH</th>
<th>Zeta Potential, (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inactive Oocysts $10^{-3}$ M</td>
</tr>
<tr>
<td>3</td>
<td>-2.09</td>
</tr>
<tr>
<td>4</td>
<td>-5.26</td>
</tr>
<tr>
<td>5</td>
<td>-8.92</td>
</tr>
<tr>
<td>6</td>
<td>-14.32</td>
</tr>
<tr>
<td>7</td>
<td>-15.93</td>
</tr>
<tr>
<td>8</td>
<td>-16.71</td>
</tr>
<tr>
<td>9</td>
<td>-17.98</td>
</tr>
<tr>
<td>10</td>
<td>-19.06</td>
</tr>
<tr>
<td>11</td>
<td>-23.22</td>
</tr>
<tr>
<td>12</td>
<td>-32.20</td>
</tr>
</tbody>
</table>
## APPENDIX II

Zeta potential of inactive and live oocysts at $10^{-3}$ M, pH 3-12, including data from Ongerth and Drozd (see Figure 8)

<table>
<thead>
<tr>
<th>pH</th>
<th>Zeta Potential, $(mV)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Live Oocysts $10^{-3}$ M</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-3.52</td>
</tr>
<tr>
<td>4</td>
<td>-7.00</td>
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<tr>
<td>5</td>
<td>-13.05</td>
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<tr>
<td>6</td>
<td>-18.25</td>
</tr>
<tr>
<td>7</td>
<td>-24.09</td>
</tr>
<tr>
<td>8</td>
<td>-25.32</td>
</tr>
<tr>
<td>9</td>
<td>-32.62</td>
</tr>
<tr>
<td>10</td>
<td>-33.69</td>
</tr>
<tr>
<td>11</td>
<td>-53.21</td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

* Actual pH shown in brackets afterwards.
APPENDIX III

Effect of NOM concentration on inactive oocysts at $10^{-3}$ M and pH 6 and 8 (see Figure 9)

<table>
<thead>
<tr>
<th>NOM Concentration (ppm)</th>
<th>Zeta Potential, (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 6</td>
</tr>
<tr>
<td>0</td>
<td>-20.65</td>
</tr>
<tr>
<td>2</td>
<td>-21.75</td>
</tr>
<tr>
<td>4</td>
<td>-24.45</td>
</tr>
<tr>
<td>6</td>
<td>-25.61</td>
</tr>
<tr>
<td>8</td>
<td>-28.81</td>
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<tr>
<td>10</td>
<td>-30.68</td>
</tr>
<tr>
<td>12</td>
<td>-34.26</td>
</tr>
<tr>
<td>15</td>
<td>-36.07</td>
</tr>
</tbody>
</table>
### APPENDIX IV

Effect of turbidity on inactive oocysts at $10^{-3}$ M (see Figure 10)

<table>
<thead>
<tr>
<th>Water Source</th>
<th>Turbidity</th>
<th>Zeta Potential, $(mV)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hope Valley</td>
<td>0.42</td>
<td>-16.32</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>-16.87</td>
</tr>
<tr>
<td></td>
<td>5.3</td>
<td>-17.99</td>
</tr>
<tr>
<td>River Murray</td>
<td>0.37</td>
<td>-15.20</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>-17.64</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>-32.82</td>
</tr>
</tbody>
</table>
### APPENDIX V

Removal rates of live and heat-inactivated *Cryptosporidium parvum* oocysts (see Figure 11)

<table>
<thead>
<tr>
<th>Alum Dose (ppm)</th>
<th>Heat-Inactivated Oocysts % Removal</th>
<th>Live Oocysts % Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10%</td>
<td>24%</td>
</tr>
<tr>
<td>30</td>
<td>74%</td>
<td>76%</td>
</tr>
<tr>
<td>45</td>
<td>79%</td>
<td>82%</td>
</tr>
<tr>
<td>60</td>
<td>81%</td>
<td>85%</td>
</tr>
<tr>
<td>75</td>
<td>84%</td>
<td>87%</td>
</tr>
</tbody>
</table>
APPENDIX VI

Comparison of removal rates of *Cryptosporidium parvum* oocysts on top (pipette) and bottom (tap) sampling for alum and PolyDADMAC (see Figure 13 and 14)

<table>
<thead>
<tr>
<th>Alum Dose (ppm)</th>
<th>Bottom-Sampled Oocysts (Tap) % Removal</th>
<th>Top-Sampled Oocysts (Pipette) % Removal</th>
<th>Poly-DADMAC (ppm)</th>
<th>Bottom-Sampled Oocysts (Tap) % Removal</th>
<th>Top-Sampled Oocysts (Pipette) % Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>24%</td>
<td>46%</td>
<td>1.0</td>
<td>37%</td>
<td>47%</td>
</tr>
<tr>
<td>30</td>
<td>76%</td>
<td>90%</td>
<td>2.5</td>
<td>68%</td>
<td>64%</td>
</tr>
<tr>
<td>45</td>
<td>82%</td>
<td>97%</td>
<td>4.0</td>
<td>78%</td>
<td>75%</td>
</tr>
<tr>
<td>60</td>
<td>85%</td>
<td>91%</td>
<td>6.0</td>
<td>88%</td>
<td>86%</td>
</tr>
<tr>
<td>75</td>
<td>87%</td>
<td>93%</td>
<td>10.0</td>
<td>95%</td>
<td>96%</td>
</tr>
</tbody>
</table>
### APPENDIX VII

**Turbidity experiment using Hope Valley reservoir water (see Figure 15)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Turbidity (NTU)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered (0.20 m)</td>
<td>0.22</td>
<td>17%</td>
</tr>
<tr>
<td>50:50 Filtered:Unfiltered</td>
<td>2.2</td>
<td>18%</td>
</tr>
<tr>
<td>Unfiltered</td>
<td>4.5</td>
<td>19%</td>
</tr>
</tbody>
</table>
APPENDIX VIII

Turbidity experiment using River Murray water (see Figure 16)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Turbidity (NTU)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered (0.20 m)</td>
<td>0.20</td>
<td>16%</td>
</tr>
<tr>
<td>75:25 Filtered:Unfiltered</td>
<td>16.5</td>
<td>71%</td>
</tr>
<tr>
<td>50:50 Filtered:Unfiltered</td>
<td>31.0</td>
<td>87%</td>
</tr>
<tr>
<td>25:75 Filtered:Unfiltered</td>
<td>45.0</td>
<td>81%</td>
</tr>
<tr>
<td>Unfiltered</td>
<td>60.0</td>
<td>71%</td>
</tr>
</tbody>
</table>
APPENDIX IX

Removal rates of *Cryptosporidium parvum* oocysts before and after filtration (Whatman no. 1 filter paper) (see Figure 17 and 18)

<table>
<thead>
<tr>
<th>Alum Dose (ppm)</th>
<th>Pre-Filtered % Removal</th>
<th>Whatman Filtered % Removal</th>
<th>Pre-Filtered Log Removal</th>
<th>Whatman Filtered Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 %</td>
<td>50%</td>
<td>0.0</td>
<td>0.30</td>
</tr>
<tr>
<td>15</td>
<td>18%</td>
<td>98.84%</td>
<td>0.09</td>
<td>1.94</td>
</tr>
<tr>
<td>30</td>
<td>74%</td>
<td>99.98%</td>
<td>0.59</td>
<td>3.64</td>
</tr>
<tr>
<td>45</td>
<td>86%</td>
<td>&gt; 99.99%</td>
<td>0.85</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>60</td>
<td>93%</td>
<td>&gt; 99.99%</td>
<td>1.15</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>75</td>
<td>94%</td>
<td>&gt; 99.99%</td>
<td>1.22</td>
<td>&gt; 4</td>
</tr>
</tbody>
</table>
APPENDIX X

Removal of *Cryptosporidium parvum* oocysts after alum treatment at a pH of 5, 7 and 9 (see Figure 19, 20 and 21)

<table>
<thead>
<tr>
<th>Alum Dose (ppm)</th>
<th>Removal % pH 5</th>
<th>Removal % pH 7</th>
<th>Removal % pH 9</th>
<th>Log Removal pH 5</th>
<th>Log Removal pH 7</th>
<th>Log Removal pH 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>18%</td>
<td>18%</td>
<td>24%</td>
<td>0.09</td>
<td>0.09</td>
<td>0.12</td>
</tr>
<tr>
<td>30</td>
<td>80%</td>
<td>74%</td>
<td>47%</td>
<td>0.70</td>
<td>0.59</td>
<td>0.28</td>
</tr>
<tr>
<td>45</td>
<td>80%</td>
<td>86%</td>
<td>51%</td>
<td>0.70</td>
<td>0.85</td>
<td>0.31</td>
</tr>
<tr>
<td>60</td>
<td>82%</td>
<td>93%</td>
<td>75%</td>
<td>0.75</td>
<td>1.16</td>
<td>0.60</td>
</tr>
<tr>
<td>75</td>
<td>86%</td>
<td>94%</td>
<td>76%</td>
<td>0.85</td>
<td>1.22</td>
<td>0.62</td>
</tr>
</tbody>
</table>
APPENDIX XI

Effect of added NOM on the removal of Cryptosporidium parvum oocysts using same alum dose (45 ppm) and corrected alum dose in Hope Valley water (see Figure 22)

<table>
<thead>
<tr>
<th>Added NOM (ppm)</th>
<th>Total NOM (ppm)</th>
<th>Uncorrected % Removal</th>
<th>Corrected % Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>84%</td>
<td>87%</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>71%</td>
<td>71%</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>52%</td>
<td>73%</td>
</tr>
</tbody>
</table>
# APPENDIX XII

Removal of *Cryptosporidium parvum* oocysts after PolyDADMAC treatment at a pH of 5, 7 and 9 (see Figure 24, 25 and 26)

<table>
<thead>
<tr>
<th>PolyDADMAC (ppm)</th>
<th>pH 5</th>
<th>pH 7</th>
<th>pH 9</th>
<th>Log Removal pH 5</th>
<th>Log Removal pH 7</th>
<th>Log Removal pH 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>74%</td>
<td>37%</td>
<td>38%</td>
<td>0.59</td>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td>2.5</td>
<td>82%</td>
<td>68%</td>
<td>71%</td>
<td>0.74</td>
<td>0.50</td>
<td>0.54</td>
</tr>
<tr>
<td>4.0</td>
<td>84%</td>
<td>78%</td>
<td>71%</td>
<td>0.80</td>
<td>0.65</td>
<td>0.54</td>
</tr>
<tr>
<td>6.0</td>
<td>95%</td>
<td>88%</td>
<td>83%</td>
<td>1.30</td>
<td>0.91</td>
<td>0.77</td>
</tr>
<tr>
<td>10.0</td>
<td>84%</td>
<td>95%</td>
<td>83%</td>
<td>0.80</td>
<td>1.31</td>
<td>0.77</td>
</tr>
</tbody>
</table>
APPENDIX XIII

Effect of added NOM on the removal of *Cryptosporidium parvum* oocysts using same PolyDADMAC dose in Hope Valley water (see Figure 27)

<table>
<thead>
<tr>
<th>Added NOM (ppm)</th>
<th>Total NOM (ppm)</th>
<th>% Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>86%</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>84%</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>85%</td>
</tr>
</tbody>
</table>
## APPENDIX XIV

Removal of *Cryptosporidium parvum* oocysts after ferric chloride treatment at a pH of 5, 7 and 9 (see Figure 29, 30 and 31)

<table>
<thead>
<tr>
<th>Ferric Chloride (ppm)</th>
<th>pH 5 Removal %</th>
<th>pH 5 Log Removal</th>
<th>pH 7 Removal %</th>
<th>pH 7 Log Removal</th>
<th>pH 9 Removal %</th>
<th>pH 9 Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>84%</td>
<td>0.80</td>
<td>83%</td>
<td>0.77</td>
<td>85%</td>
<td>0.81</td>
</tr>
<tr>
<td>32</td>
<td>90%</td>
<td>1.02</td>
<td>91%</td>
<td>1.04</td>
<td>93%</td>
<td>1.14</td>
</tr>
<tr>
<td>47</td>
<td>98%</td>
<td>1.74</td>
<td>98%</td>
<td>1.69</td>
<td>96%</td>
<td>1.37</td>
</tr>
<tr>
<td>63</td>
<td>97%</td>
<td>1.55</td>
<td>98%</td>
<td>1.72</td>
<td>97%</td>
<td>1.54</td>
</tr>
<tr>
<td>79</td>
<td>97%</td>
<td>1.48</td>
<td>97%</td>
<td>1.59</td>
<td>97%</td>
<td>1.57</td>
</tr>
</tbody>
</table>
## APPENDIX XV

Effect of added NOM on the removal of *Cryptosporidium parvum* oocysts using same ferric chloride dose and corrected ferric chloride dose in Hope Valley water (see Figure 32)

<table>
<thead>
<tr>
<th>Added NOM (ppm)</th>
<th>Total NOM (ppm)</th>
<th>Uncorrected % Removal</th>
<th>Corrected % Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>99%</td>
<td>98%</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>99%</td>
<td>98%</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>98%</td>
<td>96%</td>
</tr>
</tbody>
</table>
The Cooperative Research Centre for Water Quality and Treatment is an unincorporated joint venture between:

- ACTEW Corporation
- Australian Water Quality Centre
- Australian Water Services Pty Ltd
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- City West Water Limited
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- Griffith University
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- Monash University
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- Power and Water Corporation
- Queensland Health Pathology & Scientific Services
- RMIT University
- South Australian Water Corporation
- South East Water Ltd
- Sydney Catchment Authority
- Sydney Water Corporation
- The University of Adelaide
- The University of New South Wales
- The University of Queensland
- United Water International Pty Ltd
- University of South Australia
- University of Technology, Sydney
- Water Corporation
- Water Services Association of Australia
- Yarra Valley Water Ltd