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REMOVAL OF CRYPTOSPORIDIUM OOCYSTS BY WATER TREATMENT METHODS AT LABORATORY SCALE

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INTRODUCTION

The aim of this work was to investigate the removal of *Cryptosporidium parvum* oocysts from water by conventional treatment processes. Certain physical properties of oocysts, relevant to their removal from water were also considered, as were properties related to their detection in water.

Physical properties such as size, electrophoretic mobility, and light scattering have been determined. Removal of oocysts by coagulation and sedimentation, rapid filtration through granular media and slow sand filtration have been measured.

Results of all of these investigations will be given in this report, together with interpretation and discussion. In those cases where the results have been previously given in quarterly progress reports, some consolidation and rearrangement of the information has been made to make a more logical sequence and to avoid duplication. New information on rapid sand filtration was obtained in the last phase of the project and will be reported in considerable detail.

SOURCE OF OOCYSTS

During the whole of this work, oocyst samples have been obtained from Dr V McDonald, Parasitology Department, St Pancras Hospital for Tropical Diseases. In the early stages samples were from human patients, but these were not available subsequently. Later samples were from infected calves. In most cases, the purified oocysts were supplied in dichromate medium, and one sample was supplied in phosphate-buffered saline (PBS) solution. The latter sample became heavily contaminated by bacteria.

The question of the origin, separation procedure and storage medium for the samples may be relevant to some of the properties reported below, but such matters have not been studied systematically. It remains an open question whether, for instance, the surface properties of oocysts isolated from human or bovine hosts and subjected to a range of physical and chemical manipulations, are representative of those for oocysts found in surface waters.

Concentrations of stock oocyst suspensions were usually determined by particle counting and the appropriate dilutions made for the different tests.

PHYSICAL PROPERTIES

Size:

Particle size of oocysts, diluted into 1% NaCl solution, were initially determined by a Coulter Counter, model TA, with a 50 μm orifice. This gave a mean diameter of 4.7 μm, with a very narrow size distribution. Later measurements with a newly-acquired particle counter (Elzone 280PC), with a 30 μm orifice tube, gave a mean size (equivalent spherical diameter) of 4.1 μm, again with a very narrow distribution. The latter determination was for oocysts from a bovine source, whereas the former were of human origin and the difference in size between the two samples may be genuine. However the difference may possibly be due to an incorrect calibration of the Coulter Counter orifice tube in the former case. Published electron micrographs of oocysts are consistent with a diameter close to 4 μm. The more recent particle size distribution is shown in Figure 1. This was obtained by diluting 50 μl of stock oocyst suspension (containing about 2×10^9 oocysts per ml) into 20 ml of 2% filtered NaCl solution. Counts were taken over 128 channels of the Elzone 280PC and the results plotted in Figure 1

represent the number of particles in each channel. The particle size corresponding to each channel was determined by a prior calibration with two latex suspensions of known particle size. The parameters determined from this distribution are:

Mean size: 4.15 μm

Mode size: 4.03 μm

Standard deviation: ca. 0.4 μm

Particle counting and sizing indicate that doublets or higher aggregates are not present in significant amounts. Such aggregates may be present in the concentrated suspensions supplied, but undergo break-up during dilution. The fact that the diluted samples contain almost entirely single oocysts indicates that adhesion forces between oocysts are relatively weak.

Sedimentation Rate

Despite several attempts, it has not been possible to determine the settling velocity of oocysts directly. Microscopic observation of sedimentation in an electrophoresis cell was hampered by convection effects, despite the fact that the cell was in a water bath. Heating of the sample by the light beam may have been responsible. There were insufficient oocysts to measure settling rates by, for instance, the rate of fall of the boundary between suspension and clear supernatant.

However, it can be assumed that the settling rate is very low - previous estimates have been of the order of 2 mm/hr or less. This value is consistent with the behaviour in sample vials containing stock oocyst suspension, where complete sedimentation, through about 4 cm, occurred in 1-2 days.

Optical Properties and Monitoring

Oocysts in water, like other particles, scatter light and, in sufficient concentration, give measurable turbidity. Measurements of extinction values in a spectrophotometer enable estimates of the light scattering coefficient, Q, to be made over a range of wavelengths. Q is the ratio of light scattered by a particle to that incident on the particle. For oocysts, Q values at visible wavelengths are in the range 0.5 - 1, which are rather lower than expected for particles of about 4 μm diameter. The reason for the low scattering power of oocysts must be that they have a rather low refractive index. This can be calculated from light scattering theory and turns out to be only about 2% higher than that of water. With such a low refractive index, oocysts are, to a large extent, transparent to light and so cannot be easily detected by optical methods.

Because of their low refractive index, oocysts will not register in the "correct" size range using optical particle counters, where the passage through a light beam causes a pulse at a photodetector, the height of which is related to particle size. Such instruments are usually calibrated with mono-sized latex particles, which have quite high refractive indices and so scatter more light than biological particles of the same size. Preliminary observations suggest that the pulse given by an oocyst is comparable to that produced by a latex particle of only 2 μm diameter. Recently, Lewis & Manz (1991) have shown that *Giardia* cysts are counted as particles in the 1-5 μm range on a Hiac counter, despite being approximately 12 μm in diameter.

For essentially the same reason, a treated water with a very low turbidity, say 0.1 NTU, could still contain many thousands of oocysts per ml. Nevertheless, turbidity is still by far the most widely used measurement for assessing the particulate impurities in water after filtration and low values are assumed to indicate a high degree of particle removal. While this may well be the case in practice, it should be recognized that conventional turbidimeters, based on light scattering (nephelometry), are more sensitive to particles around 1 μm in size than to larger particles (Gregory, 1989). Removal of particles in the size range which includes *Cryptosporidium* and *Giardia* cysts (about 4 -12 μm) will not necessarily be reliably monitored by turbidity measurements.

Particle counting would be a much more reliable method of monitoring the quality of filtered water, but this would be an expensive option if applied on-line to every filter. A compromise would be to employ a technique which is more sensitive than nephelometry to particles in the appropriate size range, such as the method based on measurements of turbidity fluctuations (Gregory, 1989). Some results using this technique are shown in Figure 2. Suspensions were prepared with concentrations from about 350 to 7500 oocysts per ml and flowed through the particle monitor at rates of around 60 ml/min. The monitor readings showed a linear dependence on the square root of oocyst concentration, as expected theoretically. The intercept (about 50 units) at zero concentration represents the intrinsic noise in the instrument, which sets a lower limit for detection. An oocyst concentration of about 200 per ml would give a reading about twice the noise level and so could be reliably detected. Measurements of turbidity (Hach Ratio Turbidimeter) gave very low readings (always below 0.06 NTU over the whole concentration range and showed little correlation with concentration.

Of course, an oocyst concentration of 200 per ml (2×10^5/l) is far higher than any that would be encountered in treated water. Concentrations of only a few oocysts per litre are significant and need to be monitored. It is highly unlikely that any physical method could be developed in the foreseeable future that would be capable of specifically detecting oocysts at such low levels. The main problem is that there will always be very many other particles present, of about the same size (typically hundreds or more per ml). A purely physical method, whether optical or otherwise, would not be able to distinguish a few oocysts in the presence of a vast majority of other particles. Concentration, followed by a specific, biological, test procedure has to be used to detect oocysts at very low levels.

However, this does not seriously weaken the argument in favour of more sensitive particle monitoring being applied routinely to treated water. If it can be shown that a substantial reduction in particulate contamination has been achieved, then it would be reasonable to infer that pathogenic organisms in the same size range have also been removed to a large extent. Similarly, an increase in the monitor response could provide an early indication of the deterioration in filtered water quality and the possibility of oocyst contamination.

Electrophoretic Mobility

Particles in water generally have a surface charge, which can be important in influencing the adhesion of particles to surfaces (e.g. of filter grains) and to other particles. The processes of coagulation and filtration may thus be dependent on the surface charge carried by oocysts. Experimentally, the simplest approach is to determine the electrophoretic mobility, i.e. the velocity of oocysts in an electric field, divided by the field strength. This enables the zeta potential to be calculated, which is the potential at the plane of shear between the moving particle and the suspending liquid.

Electrophoretic mobilities were determined using a Rank Brothers Mark 2 Particle Electrophoresis apparatus, equipped with a flat cell and at a field strength of about 6 volts/cm. Simple statements of mobility or zeta potential results are of little value without specifying solution conditions, such as ionic strength and the concentration of specifically-adsorbing ions, which can have a great influence on surface charge.

In solutions of sodium chloride, which probably acts as an "indifferent" electrolyte mobility values from about -0.7 to -1.4 $\mu ms^{-1}/V\ cm^{-1}$ were found for salt concentrations in the range 0.1 to 10 mM. Corresponding zeta potentials are in the range -9 to -18 mV. In London tap water, containing a significant level of dissolved calcium (about 2 mM), the zeta potential of oocysts is about -15mV. In common with many biological particles, oocysts show a pH-dependent surface charge, with zeta potentials becoming less negative as pH is lowered and a reversal of charge occurring at some characteristic pH value. For oocysts the isoelectric point appears to be at about pH 4, although the very low mobilities in this region make a precise determination difficult.

It is of some interest to investigate the effect of typical water treatment additives on the electrophoretic mobility of oocysts. In this work, the additives chosen were aluminium sulphate ("alum") and a cationic polyelectrolyte. The latter was a low molecular weight, high charge density polymer, Percol 1697 (Allied Colloids Ltd.) In the case of alum, increasing concentrations in London tap water cause a gradual reduction in mobility and then a charge reversal (Figure 3). These results were obtained without pH control, so that there would be a reduction in pH as the alum concentration is increased. Also shown in Fig. 3 are corresponding results for latex particles (about 4.5 μm diameter) and yeast cells, which are potential surrogate particles for *C. parvum* oocysts in laboratory studies. The behaviour of yeast cells is very similar to that of oocysts. Latex particles have a much higher mobility than oocysts in tap water, but the addition of alum causes a rapid reduction and, at concentrations greater than about 0.1 mM, the mobility of latex particles becomes similar to that of oocysts and yeast cells.

Figure 4 shows the effect of pH on the mobility of oocysts and latex particles in solutions of a fixed alum concentration (0.1 mM). Although the latex particles show a higher positive mobility at low pH, both types of particle show a marked reduction in mobility and then a reversal of charge as the pH is increased. The isoelectric points are in the pH range 6.5 - 7.

The effect of low concentrations of cationic polymer on oocyst mobility are shown in Figure 5, for tap water and 1 mM NaCl solutions. In both cases reversal of charge occurs at very low polymer concentrations (less than 1 μg/l).

These mobility results indicate that oocysts have a rather low negative surface charge density (which probably arises from the ionization of carboxyl groups) and that the surface adsorbs hydrolyzed aluminium species and cationic polymers, as is the case with many other negatively-charged particles. It is likely that oocysts treated with these additives would show similar surface-chemical behaviour to other particles treated in the same way.

Adhesion

It has already been mentioned that oocysts show little evidence of aggregation in the suspensions used, after dilution from more concentrated samples. This could imply that particle-particle attraction is rather weak and that any aggregates present in the concentrated samples are easily disrupted by the agitation employed in dilution and mixing. Once in the diluted state, particle-particle encounters would be very infrequent, so that there would be little opportunity for aggregate formation.

Another aspect is the adhesion of oocysts to other surfaces, such as sand. This is clearly relevant to the removal of *C. parvum* by deep-bed filtration in water treatment. Some preliminary trials using a laminar flow technique were conducted. Oocysts were allowed to settle in thin glass cells and these were mounted on a microscope stage. By flowing water through the cell at various rates detachment of oocysts, caused by fluid shear, could be observed. The flow rate at which significant detachment occurs gives an idea of the strength of adhesion of oocysts to the glass surface.

Experiments have been conducted to compare the adhesion of oocysts to that of polystyrene latex particles of about the same size, in various aqueous solutions. The results are subject to some uncertainty, but a number of points have emerged. Adhesion is greater in tap water than in deionized water, as would be expected from the difference in ionic strength (at higher salt concentration, electrical repulsion between charged surfaces is reduced.) In tap water, adhesion becomes stronger as the particles are allowed to settle for longer periods (several hours) before flow is commenced. There is no consistent difference between the observed behaviour of oocysts and latex particles, except for the response to the passage of an air bubble through the cell. In the case of latex particles, complete detachment occurred, but some oocysts remained adhering to the glass surface after the bubble had passed. This may be due to the difference in hydrophobicity of the two types of particle. The more hydrophobic a particle, the more readily it will attach to an air bubble. This observation indicates that oocysts are less hydrophobic than latex particles, because they are less easily removed by an air bubble.

It is worth mentioning here that further work on oocyst adhesion is now in progress at University College London (as part of a study funded by SERC). This will provide more systematic information, using an improved laminar flow technique and an apparatus that enables the adhesion of individual oocysts to be measured directly.

REMOVAL OF OOCYSTS

Assay Technique

In order to determine the removal of *C. parvum* oocysts by various water treatment processes, it is essential to have a reliable means of enumerating oocysts in water samples before and after treatment. It was initially intended to carry out some experiments, such as coagulation/sedimentation, using quite high oocyst concentrations and monitoring the removal by particle counting. By counting particles in a narrow size range, corresponding to the known oocyst size, it was thought that removal could be reliably measured. This proved not to be the case. Because of the limited availability of oocysts, concentrations in the initial samples could not be very high and, if large removals were achieved, concentrations in the treated water would be quite low (100 per litre or less). No physical method available can enumerate oocysts reliably at such low concentrations. In coagulation/sedimentation the addition of alum causes the precipitation of aluminium hydroxide particles, many of which are in the same size range as oocysts, so that particle counting gives very misleading results.

For the above reason, it is necessary to use a specific technique which responds only to oocysts of *Cryptosporidium*. For most of this work, an indirect membrane filter immuno-fluorescent antibody (MF-IFA) technique was used. Monoclonal antibody (primary serum, anti-*Cryptosporidium*) was supplied by Dr V McDonald. Secondary serum (polyclonal) fluorescein isothiocyanate (FITC) in 0.01 M PBS solution, pH 7.4, containing 1% bovine serum albumin and 0.1% sodium azide was supplied by Sigma Chemical Co. Ltd. Primary and secondary antisera were diluted for use 1:20 and 1:40 respectively.

Samples of water (100-200 ml) were filtered through polycarbonate membranes of 3 μm nominal pore size (Millipore) in Swinnex filter holders. After filtration, the membranes were rinsed with PBS solution and then incubated with a small volume of diluted primary serum for 40 minutes at 37° in a humid environment. After incubation, excess antibody was removed by rinsing with PBS solution and the filter was then incubated in the presence of diluted secondary serum for 30 minutes at 37°. The filter was then rinsed with PBS solution and removed from its holder (all of the previous operations were carried out with the membrane filter in the Swinnex holder.) The membrane was then transferred to a microscope slide, treated with a drop of mounting medium and covered with a cover slip.

The membranes were examined by epi-fluorescence microscopy at 500x total magnification. Oocysts were recognizable by bright apple-green fluorescence, characteristic of fluorescein, and, in some cases, by a characteristic fold or suture line. A size of about 4 μm was also used as a criterion. In this way it was possible to count oocysts in the presence of large amounts of other organisms and debris. Depending on the number of oocysts on the filter, counts were carried out along a linear traverse (a horizontal or vertical diameter) or over the entire membrane surface. Provided that the same procedure was used for samples before and after treatment, the percentage removal of oocysts could be calculated.

In some cases a direct MF-IFA technique has been used, using a diagnostic kit supplied by Northumbria Biological Ltd. This is designed for detection of oocysts in stool samples and uses a monoclonal antibody (anti-*Cryptosporidium*) which is tagged with FITC, so that fluorescence can be observed without the use of a secondary serum. In order to detect oocysts on a membrane filter by epi-fluorescence microscopy, only limited dilution (up to 10-fold) of the supplied reagent can be used, which makes the technique quite expensive. Also the stained oocysts lose their fluorescence more rapidly than those prepared by the indirect technique. It will be seen in the results for rapid sand filtration experiments that the direct MF-IFA method gives rather erratic results.

Coagulation and Sedimentation

A limited number of jar test trials have been carried out, to investigate the effect of coagulant (alum) dose and the action of a coagulant aid in removal of oocysts from tap water. Water samples were prepared with about 2 x 10^5 oocysts per litre. These were stirred in 1 l beakers in a conventional jar test apparatus, dosed with different amounts of aluminium sulphate ("alum") during a brief period of rapid stirring, and then slowly stirred (30 rpm) for 15 minutes. Stirring was then stopped and the samples were allowed to settle for 1 hour. 200 ml of water from each beaker was then withdrawn for oocyst enumeration, as described above, and for turbidity measurement.

Some preliminary trials with alum alone indicated that the optimum dosage was around 10^{-4} molar or about 5 mg/l as Al, at pH 7.3. Under these conditions, more than 99% removal of oocysts could be achieved. As a coagulant aid, a high molecular weight anionic polymer (Percol 155 - Allied Colloids Ltd) was used. This was added at quite low concentrations - up to 0.1 mg/l, 60 seconds after alum addition. The results shown in Table 1 are for two different alum concentrations and two levels of polymer. The number of oocysts per litre, based on counts over the whole membrane filter are shown, together with an estimate of the percent removal. The latter is subject to some uncertainty because of the lack of complete recovery by the assay technique. The results are based on the assumption of 20% recovery. Also shown, for comparison, are the turbidity values of the settled samples.

The two alum dosages without polymer give very different numbers of oocysts in the settled water, illustrating the dramatic effect of increasing the dosage from sub-optimal levels to about optimum (10^{-4} M). Addition of 50 μg/l anionic polymer gives some improvement at the lower alum dose, but there is a worsening of performance when the polymer dosage is

TABLE 1

Removal of Oocysts by Coagulation and Sedimentation

Alum (M)	Polymer (µg/l)	pH	Oocysts per l.	Removal %	Turbidity (NTU)
2×10^{-5}	-	7.98	5160	87	0.41
2×10^{-5}	50	7.95	1010	97.5	0.35
2×10^{-5}	100	8.00	8980	78	0.52
10^{-4}	-	7.44	80	99.8	0.30
10^{-4}	50	7.42	120	99.7	0.33
10^{-4}	100	7.39	330	99.2	0.30

increased to 100 µg/l. At the higher (optimum) alum dose the polymer appears to have a slightly deleterious effect, although this is not great. The turbidity values show no obvious correlation with the oocyst levels.

These results indicate that, under conditions where a precipitate of aluminium hydroxide forms, the great majority of oocysts can be incorporated into the precipitate and are removed with it. Such behaviour is not surprising, since it has been shown in our electrophoretic studies that oocysts become coated with aluminium species and this should facilitate their incorporation into alum flocs.

The lack of any dramatic effect of the added polymer may be partly due to the long sedimentation time allowed. It is well known that such polymers can produce stronger and larger hydroxide flocs, which should settle more rapidly. However, in 60 minutes, even quite small flocs would have sufficient opportunity to settle out.

A series of jar tests was performed in order to test the effect of turbidity on oocyst removal. Kaolin (20 mg/l) was added to tap water, giving a turbidity of about 11 NTU. Over a range of alum dosages the removal of oocysts appeared to be slightly less effective than in the absence of kaolin, although there was considerable variability in the results.

Slow Sand Filtration

Tests have been conducted using two laboratory columns, A & B, both 11 cm in diameter and containing sand to a depth of 60 cm, over a supporting layer of gravel. The two columns had different grades of filter sand, with effective sizes of 0.27 mm (A) and 0.14 mm (B). These were fed from a constant head tank of about 20 l capacity at a rate of 50 ml/min or 0.3 m/hr, which is a rather high rate for slow sand filters.

Initially, filtration was carried out through the clean sand, without a *Schmutzdecke* (biological layer), to test the efficiency of the units simply as depth filters operated at a low flow rate. Oocyst suspension was dosed continuously into the constant head tank to give a concentration in the feed of about 5000 oocysts per litre. Filtered water samples were collected at intervals over a period of 3 days and monitored for oocysts by the MF-IFA procedure described previously. Turbidity of the samples was also measured.

The trials for filter A gave rather erratic results, but the filtrate samples nearly always contained significant numbers of oocysts (up to about 200/l). Percentage removals ranged between about 95 and 100%, with no consistent pattern throughout the runs. The average removal over the length of the run was 98.4% Filtrate turbidity was always low (< 0.2 NTU) and showed no correlation with the oocyst counts.

In the case of filter B considerably higher removals were found and in several filtrate samples no oocysts were detected. Over the length of the filter run, the average removal was 99.7%. Again there was no consistent pattern throughout the run and no correlation between oocyst removal and filtrate turbidity (which was about 0.2 NTU or less).

The results for filters without *Schmutzdecke* at a rate of 0.3 m/hr should represent "worst case" removals for practical slow sand filters, where the biological layer may contribute to oocyst removal and flow rates are somewhat lower. Subsequent tests were undertaken on filters A & B with *Schmutzdecke* at a rate of 0.15 m/hr, to give an indication of the best results that could be expected from slow sand filtration.

Schmutzdecke seed was obtained from a slow sand filter at Coppermills water treatment plant (Thames Water plc) and growth was promoted by continuous addition of 3 mg/l each of glutamic acid and glucose. Low power microscopic examination revealed an extensive algal community, including filamentous species.

It was decided to use a much higher oocyst concentration in the feed (5.8×10^6 /l) in order to have sufficient oocysts in the filtered samples for reliable counting. Again, continuous dosing of oocyst suspension into the constant head tank was carried out. The average removals for the two filters are shown in Table 2, with and without *Schmutzdecke*.

TABLE 2

Removal of Oocysts by Slow Sand Filtration

Filter (Flow Rate)	*Schmutzdecke*	Mean Oocyst Removal (Percent)
A (0.3 m/h)	No	98.4
A (0.15 m/h)	Yes	97.6
B (0.3 m/h)	No	99.7
B (0.15 m/h)	Yes	99.6

As expected, the efficiency of B (0.14 mm sand) is higher than that for A (0.27 mm sand) in both cases. However, the removals observed in the presence of *Schmutzdecke* at a rate of 0.15 m/h are marginally worse than for the corresponding clean sand filters at 0.3 m/h. This is a surprising result and a possible explanation is that oocysts left in the beds from the earlier experiments survived chlorination (10,000 mg/l) and were subsequently released and detected in the filtrates of the later runs. Earlier trials had indicated that significant elution of oocysts from column A occurred, long after a single dose of oocysts had been applied to the filter.

In previous work, filter A has been shown to remove more than 99% of E. Coli, despite their small size (0.5 x 2 μm). However, some of this "removal" could be due to die-off during retention in the bed, a mechanism that should be unimportant for oocysts.

The results are also consistent with data for penetration of slow sand filters by algae (some of which are of similar size to oocysts) showing up to about 20,000 cells/l in filtrates (Collingwood, 1979).

Rapid Filtration

Objectives

Existing data on the removal of oocysts by rapid filtration through sand and other granular media are principally from some full-scale observations in the USA. It was felt desirable to conduct some controlled experiments to determine order-of-magnitude removal efficiencies of typical sand and activated carbon grain sizes, for oocysts alone and when flocculated with aluminium sulphate.

Apparatus and materials

Four identical, laboratory-scale filter columns were operated in parallel, two with BS Sieves 18/25 (0.71 - 0.85 mm) Leighton Buzzard sand and two with BSS 16/18 (0.85 - 1.00 mm) granular activated carbon (GAC). The columns were 50 mm internal diameter, with 105 mm depths of media, and all were operated at 5 m/h approach velocity.

For Series 1 (oocysts only), the columns were supplied from a 100 l header tank, which represented 2.5 hours capacity for all 4 filters operating. A smaller header tank (40 l) was used for Series 2 (oocysts plus alum), giving 1 hour capacity. The total head loss was read from manometers and sampling points were available at the common manifold supply to all filters and from the effluent (filtrate) from each column.

Concentrated oocyst suspensions were seeded into the header tank for Series 1 and in Series 2 a solution of aluminium sulphate was added to the header tank immediately after the oocysts. In Series 2 a stirrer in the header tank provided initial mixing, and maintained a uniform suspension with some flocculation.

In Series 1 (oocysts only) measurements of pH and turbidity (Hach 2100A) were made on all samples.

Table 3 gives the basic details of the filter columns, and Figures 6 and 7 show the experimental arrangement in photographic and diagrammatic form.

TABLE 3 Details of Rapid Filter Columns

Column No.	1	2	3	4
Media	sand	sand	GAC	GAC
Size (mm)	0.71 - 0.85	0.71 - 0.85	0.85 -1.00	0.85 -1.00
Bed depth (mm)	105	105	105	105
Internal dia. (mm)	48	47	49	49
Mass of media (g)	295	295	90.4	104
Calculated porosity	0.42	0.39	0.74	0.70
Filtration rate (m/h)	5	5	5	5

Operation and data

In both series the header tanks were filled with tap water (Thames-derived: TDS 450 mg/l, conductivity 650 µS/cm, hardness 280 mg/l as $CaCO_3$, pH 8.1 ± 0.1, turbidity 0.40 ± 0.1 NTU, temperature 19-20°C). Initial tests established that the clean head loss was proportional to flow rate (Darcy's Law) and that the columns were performing in the correct laminar flow regime. At 5 m/h the sand columns gave an initial head loss of about 40 mm, and the GAC columns about 20 mm. These were read by water manometer differences and accuracy was limited to ± 5mm.

Series 1 (oocysts only)

Columns 1 and 2, containing sand, were parallel replicates, as were 3 and 4, containing GAC.

The header tank (100 l) was seeded with 1000 *C. parvum* oocysts per litre and a recirculating pump mixed the seeded water for about 30 minutes. Time was allowed for the seeded tank water to displace the tap water occupying the connecting tubes and filter columns, and the first filtrate samples were taken, designated 0 min. Thereafter, every 30 min, inlet (common manifold) and outlet samples were taken, up to a maximum of 150 min. These samples were analyzed for pH and turbidity and 105-110 ml were stored in the refrigerator (0 - 4°C) for later oocyst counting. Head losses across the filter columns were also recorded at the 30 min intervals.

The head losses (sand 40 mm, GAC 20 mm), pH (ca. 8.1) and turbidities (ca. 0.4 NTU) did not change through the 150 min run. The results of oocyst counting are given in Table 4, where the asterisked (*) values were obtained using the Northumbria Biological direct IFA method described previously. All of the other values were obtained using the indirect IFA technique.

TABLE 4 Series 1 (Oocysts only)
Numbers of Oocysts Detected per Litre of Filtrate

Sample time (min)	1 sand	2 sand	3 GAC	4 GAC	5 influent
0	60*	30	30*	60	-
30	50*	20*	40*	40*	0*
60	100	70	330	20	300
90	350	110	130	20	210
120	110	60	480	100	290
150	100	150	120	150	310

Note: Column 5 data are the influent samples taken from the common manifold.

Series 2 (oocysts plus alum)

As before, columns 1 and 2 were sand replicates, columns 3 and 4 were GAC replicates.

The smaller header tank (40 l) was seeded with 10^3 *C.parvum* oocysts per litre and stirred vigourously. A concentrated solution of alum ($Al_2(SO_4)_3$ $16H_2O$) was added to give 5 mg/l as alum (0.43 mg/l as Al; 1.6 x 10^{-5} M Al). This was mixed by stirrer, then gently stirred to promote flocculation and to keep microflocs in suspension.

TABLE 5 Series 2 (Oocysts plus alum)
Numbers of Oocysts Detected per litre of Filtrate

Sample time (min)	1 sand	2 sand	3 GAC	4 GAC	5 influent
15	15	75	45	1405	250
45	15	5	20	30	371
75	10	15	5	10	231
105	50	20	10	45	320
135	15	10	0	15	220
165	10	15	10	5	260
195	45	100	70	35	233
225	35	35	60	70	340

Because of the small capacity of the header tank, which was necessitated by the requirement for a stirrer, a new tank with oocyst seed and added alum had to be prepared every hour, by adding to the nearly depleted tank. The filters were kept running continuously, but the changes at 60, 120 and 180 min can be seen on the head loss vs. time graph in Figure 8.

The filters were operated in direct filtration mode, with no prior sedimentation of the flocs. Samples of influent and the 4 filtrates were taken at 30 min intervals, starting at 15 minutes, up to 225 minutes. Approximately 110 ml samples were refrigerated for later oocyst counting. The results are given in Table 5.

Data interpretation

Series 1 (oocysts only): With no significant changes in head losses during the 2.5 hour run, it can be assumed that no clogging occurred in the filters. Discounting the oocyst counts at 0 and 30 min (mainly using the Northumbria Biological reagent) as being inconsistent with the others, especially with no reliable influent counts, the mean overall removals from 60 to 150 min are as follows:

Column	Media	Total influent oocysts/4l	Total filtrate oocysts/4l	Percent removal (nearest 5%)
1	sand	1110	660	40
2	sand	1110	390	65
3	GAC	1110	1060	5
4	GAC	1110	290	75

These figures, considering the replicate columns, appear inconsistent. Further inspection of Table 4 shows three filtrate values greater than the influent: 1 (90 min) at 350, 3 (60 min) at 330 and 3 (120 min) at 480. If these values are eliminated, the table above becomes:

Column	Media	Influent	Filtrate	Percent removal
1	sand	900/3l	310/3l	65
2	sand	1110/4l	390/4l	65
3	GAC	520/2l	250/2l	50
4	GAC	1110/4l	290/4l	75

These figures are more consistent, but this does not mean that they are more reliable. Whatever caused the high values (inconsistent filter operation, disturbance during operation, variation in sampling procedure, unreliable sample counting) may also have affected the other values. There is no discernible trend with filter run time, although the influent count from 60 to 150 min is reasonably constant, varying from +30 to -70 about the mean of 280/l.

In overall terms there is little difference between sand and GAC. Some further discussion of these results will be given later.

Series 2 (oocysts plus alum): As shown in Figure 8, the head losses increased during the 240 min run, more significantly in the sand than in the GAC. These differences are to be expected due to the smaller grain size and lower porosity of the sand compared with GAC (see Table 3).

Table 5 presents the oocyst counts per litre in the 4 filtrates and the influent. The results show only one obvious inconsistency for column 4 (GAC) at 15 min. This value of 1405 oocysts/l will be discounted in further data analysis.

The mean overall removals from 15 to 225 min are as follows:

Column	Media	Total influent oocysts/8l	Total filtrate oocysts/8l	Percent removal (nearest 5%)
1	sand	2225	195	90
2	sand	2225	275	90
3	GAC	2225	220	90
4	GAC	1975[+]	210[+]	90

[+] Values for 7l, discounting 15 min sample.

These values are consistent to the nearest 5% removal, for both the sand and the GAC. They are markedly better, at 90%, than the removal percentages for oocysts only (mean 65%). They also show no significant difference between sand and GAC.

Discussion

The size of the sand and GAC represent the middle ranges of these media used in practice. Differences between these two media in the experiments given in Table 3 resulted, for GAC in lower initial head losses (sand 40 mm, GAC 20 mm) and lower rate of rise of head loss during clogging with alum (Figure 8). As the oocyst removal was not significantly different between the two media, the removal efficiency appears not to be greatly affected by small differences in grain size and packing. The flow rate was kept constant (5 m/h), so

variation in flow rate was not tested. This is likely to have a more significant effect on filter performance and an estimate of its influence is given below. Another factor mentioned in the Badenoch Report (1990) is the rate of change of flow rate, since increases may dislodge existing deposits of oocysts or alum floc containing oocysts.

The counting procedure, using the indirect MF-IFA method, produced reasonably consistent results after elimination of obviously wild data. The influent counts, between about 250 and 350 oocysts per litre, are only about one third of the nominal seeding of the supply tanks at 1000/l. The apparent losses cannot be accounted for, but they are most likely due to inefficiencies in the sampling and counting procedure. The procedure of measuring at the inlet manifold provides a measure of the actual load to the filters, and the filtrate samples were taken and tested in an identical manner. The counting of influent samples of oocysts with alum was difficult due to problems of passing the samples through the 3 μm membranes. It is worth noting that, even for this modest set of experiments, 70 samples had to be counted for oocysts, representing a large time demand on the Research Assistant.

The alum dose of 5 mg/l was chosen arbitrarily as typical for direct filtration and no attempt was made to optimize the dose. At 5 mg/l alum (0.43 mg/l as Al), it is approximately one twelfth of the dose required to give large, settleable flocs (5 mg/l as Al). The required optimum dose for removal of oocysts in jar tests was previously found to be 10^{-4} M alum or about 5 mg/l as Al. Large flocs would, however, be undesirable for direct filtration. At the low dosage employed here, it is very likely that significant amounts of hydrolyzed Al species are adsorbed on the oocysts, modifying their surface properties and their interaction with filter media.

The head loss data in Figure 3 show that both sand and GAC removed significant quantities of floc, although the higher porosity of the GAC reduced the clogging effects. By correcting the ordinate for initial head loss (i.e. head loss minus initial head loss), the two GAC curves become identical, but the sand curves diverge, particularly after 180 min. The cause of this behaviour is not known, but appears to be of little consequence in relation to oocyst removal efficiencies. The effect of preparation of fresh tank loads of seeded oocysts and alum, with the necessary short period of intense mixing, can be seen in Figure 3 as the steps in the curves. For this reason, the sampling times were chosen so as not to coincide with the refilling of the header tank. The head loss curves are approximately linear (only until 180 min for column 1), indicating in-depth removal of flocs and no significant surface mat formation. This observation lends some support to the theoretical calculations given below. No increases in head loss were observed in Series 1 (oocysts only), showing that removal of oocysts caused no significant clogging. This is to be expected considering their small size (about 4 μm) compared with the filter pores (≈ 200 - 500 μm).

As the turbidity values of the filtrates did not change during these experiments, no correlation could be established with the corresponding oocyst counts.

Analysis of results using filtration theory are speculative owing to lack of data from sampling at various depths, and over extended periods of many hours of filter run. The experiments for up to 3.5 hours represent only the initial part of a typical full filter run. For sand, initial head loss was 40 mm for 100 mm depth and head loss with alum rose at 40 mm/h. Corresponding GAC values were 20 mm/100 mm and 10 mm/h. Therefore for a 600 mm deep filter the following calculations can be made:

Media	Bed depth mm	Initial h.l. H_0 mm	Limit h.l.* H_L mm	Run time $(H_L - H_0)$/rise rate
Sand	600	240	2000	44 hours
GAC	600	120	2000	188 hours

*Typical value in practice.

These run times assume that the 600 mm depths of media are of uniform size as in the experiments. This would not be the case in practice, but more elaborate calculations are not justified, considering the reliability of the experimental data. The runs may be terminated before these head loss-controlled times, due to breakthrough. From the data, breakthrough times cannot be estimated; longer experimental runs would be needed.

There are no discernible trends in the filtrate oocyst counts in either Series to indicate whether a ripening process is operating, so it will be assumed for simplicity of analysis that the data represent constant removal efficiency.

It is generally agreed that for a uniform filter receiving a constant influent load (C_0) of particles, that the filtrate quality (C) for a given depth L is logarithmically related to depth:

$$\ln (C/C_0) = - \lambda L$$

where λ is the filter coefficient (mm^{-1})

$$\lambda = - [\ln(C/C_0)]/L$$

For Series 1 (oocysts only), $C/C_0 = 0.35$, L = 105 mm,

therefore $\lambda = 0.01\ mm^{-1}$

if L = 600 mm, $C/C_0 = \exp(-0.01 \times 600) = 0.0025$

and removal = $(C_0 - C)/C_0 = 0.9975 = 99.75\%$

This is 2.6 log removal

For Series 2 (oocysts plus alum) $C/C_0 = 0.1$, L = 105 mm

Therefore $\lambda = 0.022\ mm^{-1}$

if L = 600 mm $C/C_0 = \exp(-0.022 \times 600) = 0.000002$

and removal = $(C_0 - C)/C_0 = 0.999998 = 99.9998\%$

This is 5.7 log removal

If a high load of 1000 oocysts per litre were to challenge 600 mm deep filters of sand or GAC, without alum, 2.5 oocysts /l could pass, but with alum 0.002 oocysts/l could pass (i.e. 1 oocyst/500 l). The former would not be acceptable, the latter may be.

The advantages of using alum to flocculate oocysts during direct filtration are thus demonstrated for hypothetically uniform filters containing typical middle range sizes of sand and GAC. It may be assumed that similar results would be obtained with residual flocs from previous clarification/sedimentation processes which may behave similarly.

The results give no insight into the mechanisms of oocyst retention in the filter pores. For oocysts alone, at about 4 μm, they lie near the minimum efficiency for filtration removal (Badenoch, 1990, p.167), but in flocs of 10 - 100 μm size (so-called pinpoint flocs suitable for direct filtration) the removal efficiency approaches 100% due to gravity forces. The filter coefficient (λ) for oocysts alone, calculated above, is 0.01 mm^{-1}, which may be compared with 0.0042 mm^{-1} for Chlorella filtration through sand, given in Badenoch (1990, p. 168).

This indicates the possibility of Chlorella being used as a surrogate particle, with slightly worse filtration characteristics and therefore with an inherent "safety factor" when compared with Cryptosporidium.

These experiments do not allow calculation of dual or multiple layer filters, because the data for other coarser or finer media sizes are not available. It is possible to estimate the effect of other flow rates from the relationship $\lambda \propto v^{-a}$, where v is the approach velocity and a varies from 1 to 4. Using the data of Ives and Sholji (1965) which was for spherical pvc particles less than 2 μm in diameter, a = 1. If the filtration velocity were increased to 10 m/h , then the filter coefficient would be halved, compared to the values at 5 m/h obtained in this study. The values would become: Series 1, 0.005 mm^{-1}, Series 2, 0.011 mm^{-1} and removals would become, for 600 mm deep filters:

	Flow rate m/h	Removal %	Log removal
Series 1	5	99.75	2.6
	10	99.5	2.3
Series 2	5	99.9998	5.7
	10	99.864	2.9

Consequently, higher flow rates will result in significantly lower log removals. This has not been tested experimentally with C. parvum oocysts, so must be treated as a speculative conclusion.

There is very little data on the removal of oocysts with which to compare the results of the present study. Le Chevallier *et al* (1990) have published an evaluation of treatment processes with respect to parasitic cysts removal (*Giardia* and *Cryptosporidium*) in selected water treatment works in the USA. Many of the filters are proprietary designs, including mixed media (GAC-sand-garnet). None appeared to be in direct filtration mode. At one waterworks, sand filters 680 mm deep, sand size "conventional", operating at 4.5 m/h, received residual ferric flocs from a prior flocculation-sedimentation treatment. The average raw surface water contained 1.0 oocyst /litre, and the average settled water contained about 0.3/litre. The filtrate during the ripening period averaged 0.01/litre and during the principal run period of 24 h averaged about 0.001/litre. This represents about 1.5 log removal during the ripening period and 2.5 log removal during the principal run period. Filtrate turbidities averaged 0.07 NTU. Overall turbidity was not a good predictor of plant performance, but some individual units related parasite removals to turbidity and particle count (5 - 15 μm) removals.

The log removals in the US waterworks study were not as high as in the present experiments. However, the differences in scale and operating practice, and different flocculation procedures could account for this.

Conclusions

1 In small-scale experiments no significant differences in oocyst removals were found between sand and GAC

2 Suspensions of oocysts only in tap water were about 65% removed, but oocysts plus alum in direct filtration gave about 90% removal, through 105 mm depth of media.

3 Extrapolating these results to a more practical depth of 600 mm, the removals would be for oocysts only 99.75% (2.6 log), and for oocysts with alum 99.9998% (5.7 log)

4 Head losses with oocysts only did not change with time, indicating no clogging effect by those oocysts which were removed. With oocysts plus alum, approximately linear head loss increases with time indicated in-depth filtration of alum flocs.

5 Turbidity values were consistently low and were no indication of the presence of oocysts.

6 The removals of oocysts only were better than those for *Chlorella* under similar conditions. The filtration removals of oocysts plus alum were very significantly better than average waterworks results in the USA, where prior ferric flocculation and sedimentation was practised.

7 No data were produced for flow rates other than 5 m/h, nor for water temperatures other than about 20°C.

GENERAL CONCLUSIONS

Oocysts of *Cryptosporidium parvum* behave in many ways like typical aquatic particles, with a size of about 4 μm and a negative surface charge. Since the density of oocysts is quite low, they settle only slowly in water and so cannot be effectively removed by sedimentation alone. Their size makes removal by deep-bed filtration difficult since 4 μm is around the size giving minimum capture rate during flow through granular media. Also, because of their low density, oocysts will not be readily captured by sedimentation within pores of a filter, a mechanism which can be important for denser particles.

Standard water treatment processes should give a significant improvement in removal efficiency. Addition of a hydrolyzing salt such as aluminium sulphate causes adsorption of hydrolyzed species, as evidenced by changes in electrophoretic mobility, and oocysts are readily incorporated into precipitated hydroxide flocs and may then be removed by sedimentation. The efficiency of this process in laboratory jar tests can be of the order of 99% and better (more than 2-log removal). In practice, the removal of oocysts by coagulation and sedimentation would depend on the nature of the floc and the shear conditions, but these affect the removal of particulate impurities generally and are not specific to oocysts. In real waters, it is very unlikely that oocysts would be present in sufficient quantity to appreciably affect the properties of flocs. Optimising conditions (pH, alum dose, and perhaps polymer addition) to give the best overall removal of impurity particles (or reduction in turbidity), should also result in a high degree of oocyst removal.

For much the same reason it is likely that removal of oocysts by dissolved air flotation would also be effective, provided that optimum floc properties and flotation conditions for suspended solids removal had been achieved.

Removal of oocysts by filtration through granular media has been studied under conditions typical of slow sand filtration (fine sand, low flow rate) and of rapid filtration (coarser media, high flow rate). In the former case, removal by a 600 mm depth of sand was of the order of 98 - 99.6%, for untreated oocysts in London tap water. No significant effect of a biological surface layer (*Schmutzdecke)* was observed.

Rapid filtration through 100 mm depths of sand or granular activated carbon gave removals of 50 - 75% for untreated oocysts in tap water. Treatment with alum, at a dosage much less than that required for optimum removal by coagulation/sedimentation, gave a significant improvement in removal by filtration - to levels around 90%. This improvement

may be due simply to the adsorption of hydrolyzed aluminium species on the oocysts, giving improved adhesion to the filter grains. However the build up of head loss under these conditions points strongly to clogging of the filters by flocs, which may include oocysts.

In principle, it would be possible to use a cationic polyelectrolyte to improve the adhesion of oocysts to filter grains. It has been shown (Figure 5) that the negative charge of oocysts can be reduced and then reversed by quite low concentrations of a cationic polymer. An oocyst made positive in this way should have no difficulty in adhering to a negatively-charged sand grain. Because of the presence of many other particles which would also adsorb the cationic polymer, and uncertainties over relative rates of adsorption on different particles, it would be difficult to adjust polymer dosage to ensure adequate coverage of oocysts, especially if dosing occurred immediately prior to filtration.

Extrapolation of the rapid filtration data to depths of 600 mm, using standard filtration theory, predicts better than 2 log removal (99.75%) in the case of untreated oocysts and better than 5 log removal for oocysts treated with alum. These figures should be treated with some caution, since untreated oocysts are removed by a 600 mm deep slow sand filter only by about 99.7% at best. The fine sand and very low flow rate should give much better removal than coarse media operating at high flow rate and yet the removal predicted above for rapid filtration of untreated oocysts is of the same order as that observed for a slow sand filter of the same depth. Nevertheless, high removals can be expected for rapid filters, provided that the water is dosed with a suitable coagulant, as is usually the case for direct filtration.

For the case of rapid filtration following coagulation and sedimentation, it is likely that the residual oocysts, whether as single particles or incorporated in small flocs, would be removed with comparable efficiencies to those expected for alum-treated oocysts in rapid filters.

Despite the high removal efficiencies predicted for oocysts in water subjected to conventional treatment processes, it can never be assumed that **no** oocysts are present in the treated water. Even high-quality treated waters, of very low turbidity, invariable show suspended particles in the 1 - 10 μm size range when analyzed by a particle counter. Removal of these residual particles can only be reliably achieved by absolute filtration, such as by a membrane filter with pore size smaller than the particles to be removed.

In practice, reduction of particulate impurity levels by many orders of magnitude should ensure the virtual absence of oocysts in treated water. It may be acceptable to check the quality of a treated water by turbidity measurements, but these are not especially sensitive to oocyst-size particles. Some form of particle counting or sensitive particle monitoring would be preferable. Such techniques cannot detect oocysts specifically and so cannot guarantee their absence, but they can give an early indication of increasing particulate impurities in a treated water, so that remedial action can be taken quickly.

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FIGURE CAPTIONS

Figure 1 Particle size distribution of oocysts determined by Elzone 280 PC with a 30 μm orifice. Counts per channel vs particle size.

Figure 2 Response of UCL Particle Monitor to low oocyst concentrations. The monitor response (arbitrary scale) is plotted against the square root of oocyst concentration (number/ml).

Figure 3 Electrophoretic mobility (μm s^{-1}/V cm^{-1}) of oocysts, yeast cells and latex particles in London tap water containing different concentrations of aluminium sulphate.

Figure 4 Electrophoretic mobility of oocysts ([]) and latex particles (X) as a function of pH in solutions containing 0.1 mM aluminium sulphate.

Figure 5 Effect of added cationic polymer (Percol 1697) on electrophoretic mobility of oocysts in tap water and 1 mM NaCl.

Figure 6 Photograph of experimental rapid sand filters.

Figure 7 Schematic diagram of rapid filters (not to scale).

Figure 8 Head losses vs time for the four columns during rapid filtration of oocysts treated with alum.

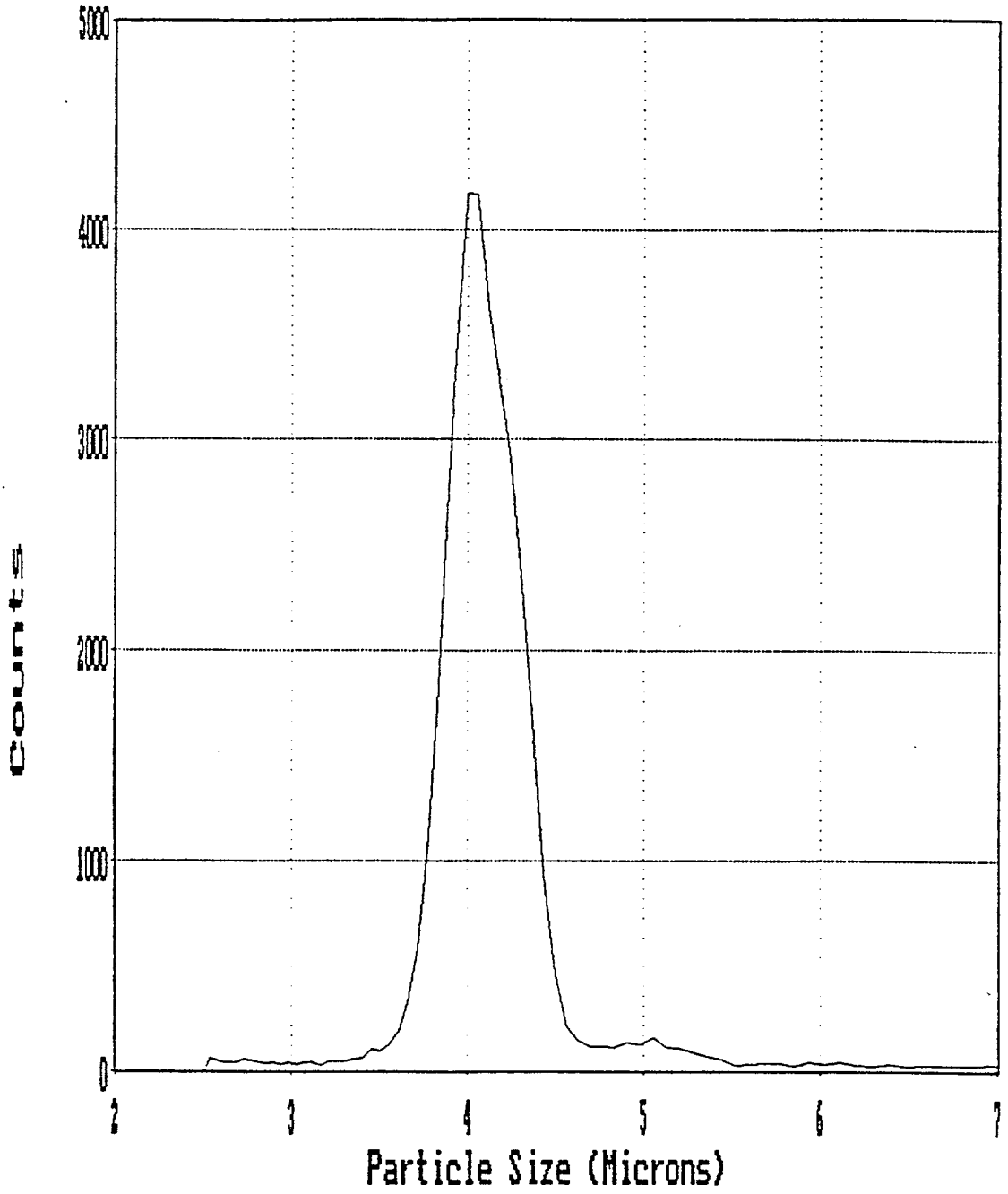

Figure 1 Particle size distribution of oocysts determined by Elzone 280 PC with a 30 µm orifice. Counts per channel vs particle size.

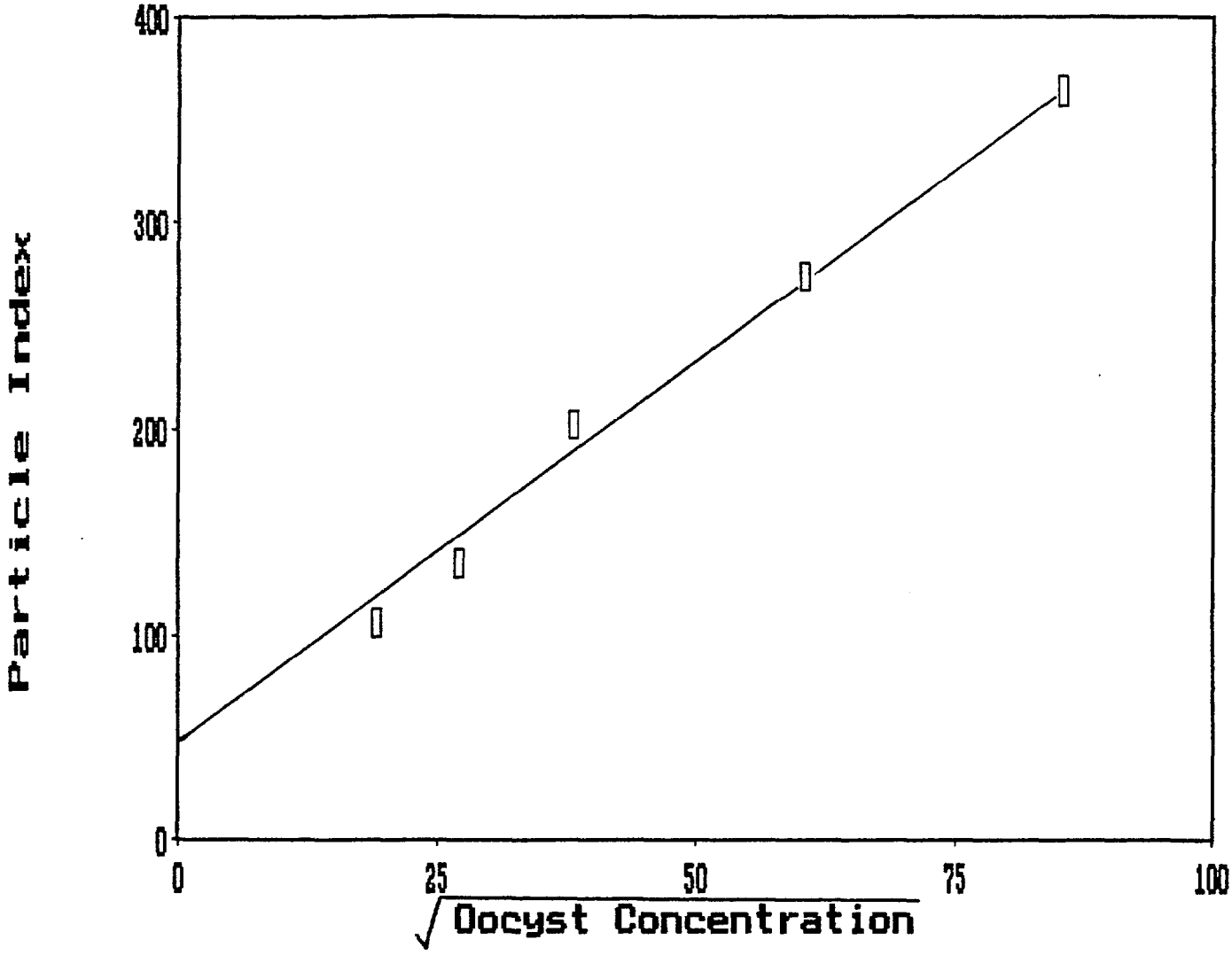

Figure 2 Response of UCL Particle Monitor to low oocyst concentrations. The monitor response (arbitrary scale) is plotted against the square root of oocyst concentration (number/ml).

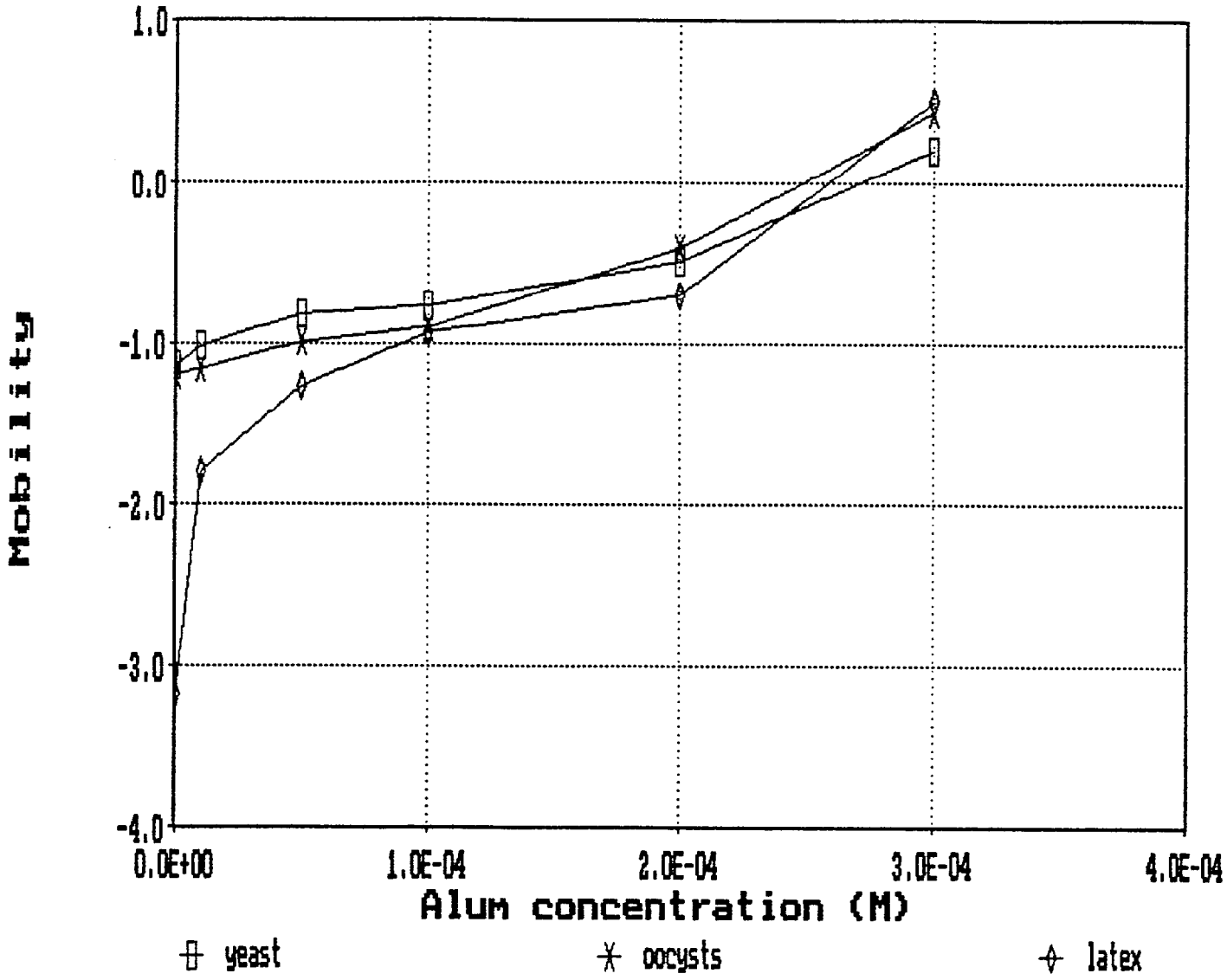

Figure 3 Electrophoretic mobility ($\mu m\ s^{-1}/V\ cm^{-1}$) of oocysts, yeast cells and latex particles in London tap water containing different concentrations of aluminium sulphate.

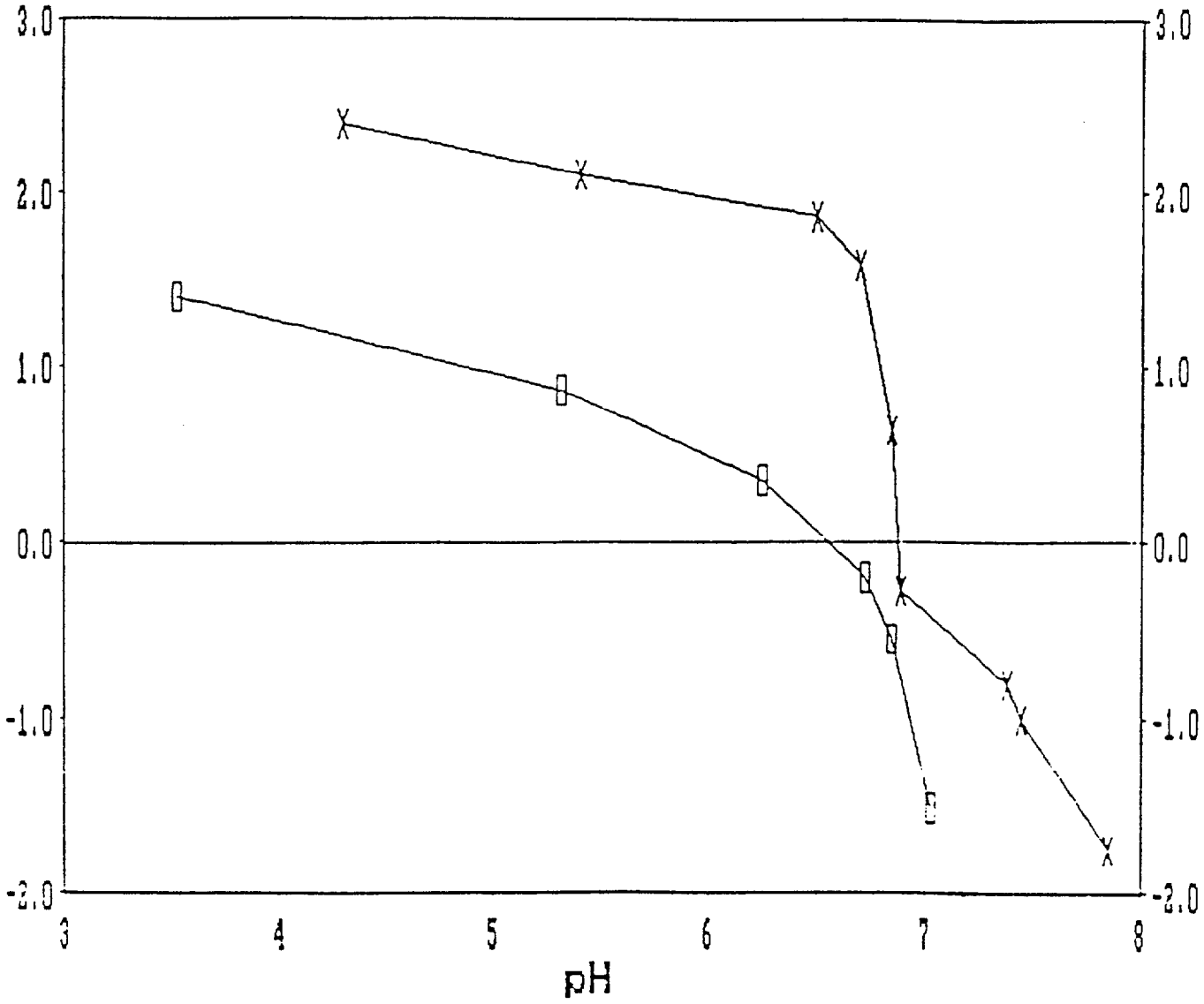

Figure 4 Electrophoretic mobility of oocysts (□) and latex particles (X) as a function of pH in solutions containing 0.1 mM aluminium sulphate.

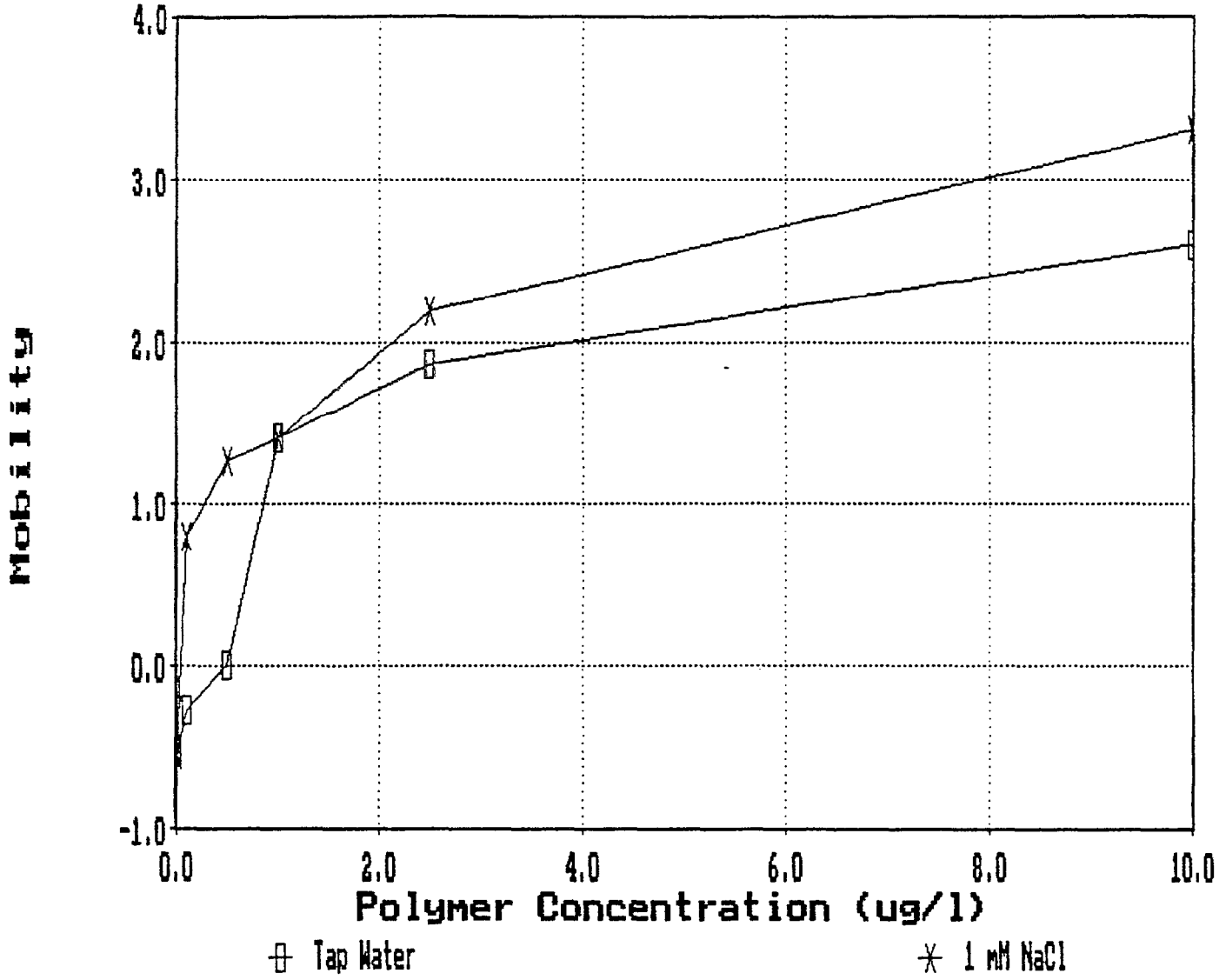

Figure 5 Effect of added cationic polymer (Percol 1697) on electrophoretic mobility of oocysts in tap water and 1 mM NaCl.

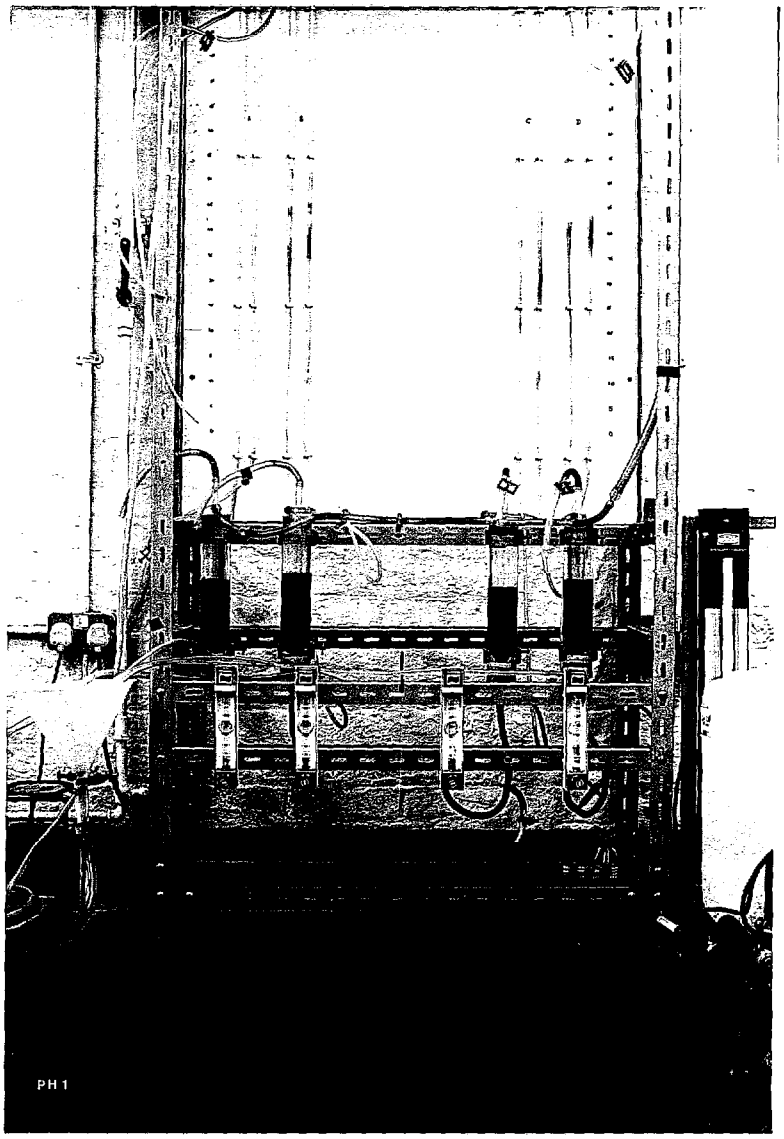

Figure 6 Photograph of experimental rapid sand filters.

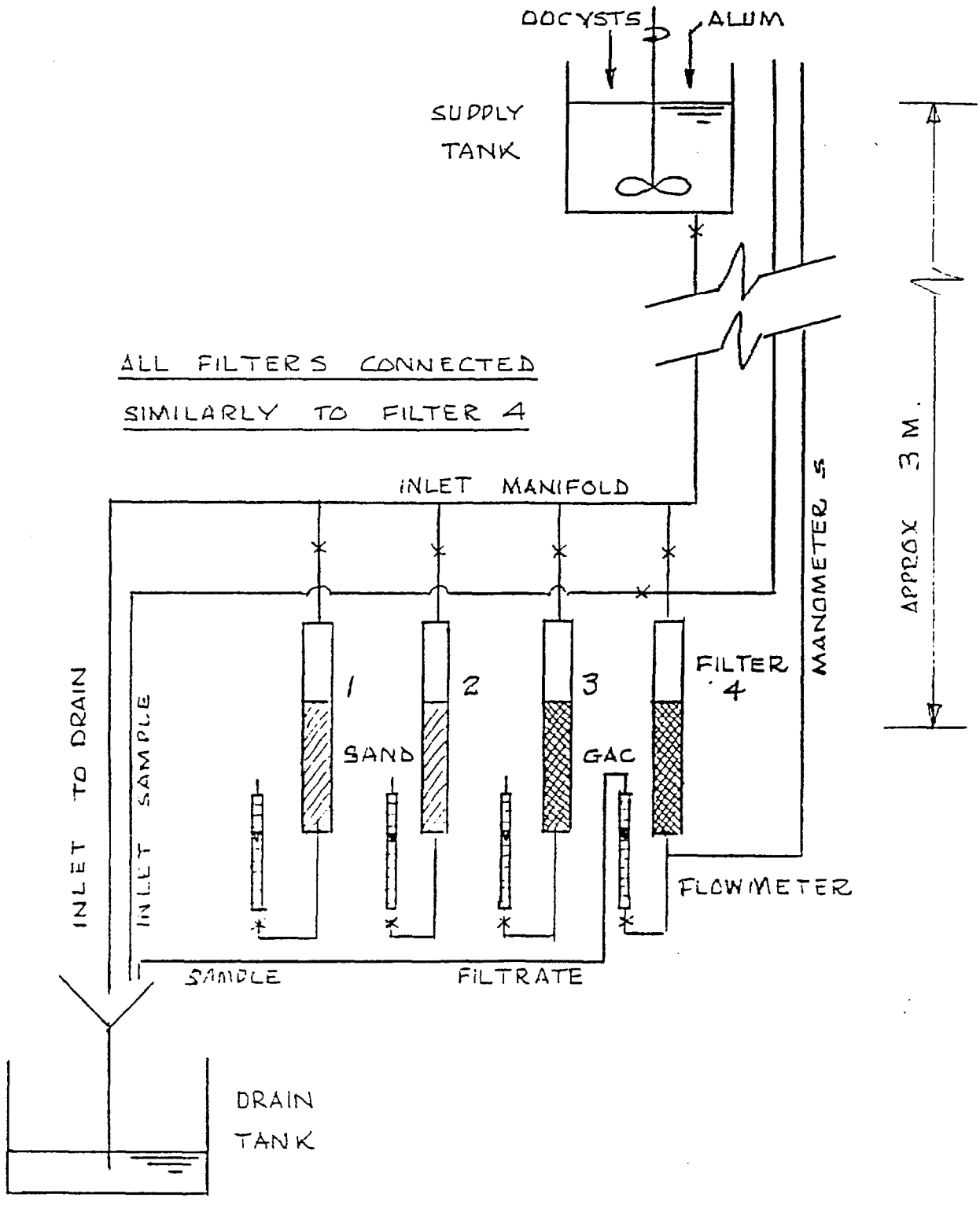

Figure 7 Schematic diagram of rapid filters (not to scale).