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Fate and transport of phage and viruses in UK Permo-Triassic sandstone aquifers

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Steve Killeen
Head of Science

Executive summary

This project investigated the application of bacteriophage as surrogates for viral pathogens in studies of their transport in UK Permo-Triassic sandstone aquifers. The report details laboratory studies with sandstone cores and field tests undertaken in the Birmingham Permo-Triassic sandstone aquifer. The project also produced a database tool to inform environmental management policy planning by allowing rapid hypothesis testing of phage transport properties. This in turn may allow inferences be drawn on the mobility of human enteric viral pathogens (adenovirus, poliovirus, norovirus and rotavirus) in groundwater systems.

This study is the first time in the UK that tracer tests involving bacteriophage have been carried out in the Sherwood sandstone group sequence and, possibly, the first time internationally that such tests have been completed in a continental red bed aquifer. This is important because viruses have recently been found in some urban groundwater systems.

The report outlines the use of bacteriophage and fluorescein as tracers in groundwater. Information is provided on how to determine suitable surrogate bacteriophages for human enteric viruses based on physicochemical properties such as size, shape and isoelectric point. A series of laboratory and field survival experiments were used to determine survival rates in groundwater. The bacteriophage showed long and consistent inactivation rates in both field and laboratory studies.

Attachment of the phage to sandstone was tested using core material. There were significant differences in the attachment of different bacteriophage but some removal was found in all cases.

Laboratory column experiments were carried out on four undisturbed cores of 4–6 cm in length using various phage solutions made up in groundwater. Breakthrough was observed in each case but with significant removal; peak concentrations were 0.99–22.9 per cent of injection concentrations. Simple scaling up to field system travel distances would suggest that no breakthrough should occur.

To obtain direct evidence of mobility at the field scale, six forced-gradient tracer tests were undertaken between two boreholes in the sandstone aquifer beneath the University of Birmingham. The boreholes were located seven metres apart. Three types of interval were tested:

- approximately 1-metre thick sequences of non-fractured sandstone isolated by packers (Tests 1–4);
- an approximately 1-metre thick sequence of fractured sandstone isolated by packers (Test 5);
- an approximately 50-metre thick sequence (full-length open borehole) (Test 6).

In the case of the 1-metre interval tests, no bacteriophage breakthroughs were recoded at the recovery borehole. This was despite extending the sampling time of each experiment well beyond the peak fluorescein breakthrough and employing virus-binding ‘Biocap 30’ filters to maximise capture by sampling several tens of litres of water. In the case of the unfractured interval tests, the lack of breakthrough was as expected from the results of the laboratory core experiments.

Unlike the earlier tests, the final open borehole test used a cocktail of four phage in addition to fluorescein. Both the recovery borehole spot samples and the Biocap 30

filters contained detectable numbers of all four phage. This indicated that pathways capable of allowing virus migration do exist in the sandstone. However, they appear to be relatively rare and are not easy to predict or identify.

These results suggest that, although the sandstone is capable of removing the vast majority of viruses from solution over very short distances, small but nevertheless potentially harmful numbers of viruses are mobile over longer distances. The pathways through which the viruses can move are not ubiquitous in the sandstones, and were only observed when the test interval was extended from 1 to 50 metres thick.

The results have major implications for our understanding of pathogen transport in the Sherwood sandstone aquifer and similar formations, which may be seen as less protected than traditionally thought. The results presented suggest that pathways do exist in the Sherwood sandstone that permit very rapid transport of viruses, albeit at low numbers.

The investigation of survival times indicated that the phage (MS2, ΦX174, PRD1 and H40/1) used in the core and field tests have half-lives in groundwater at 12°C of several months (12, 21, 21, 21 months respectively). Phage survival tests produced the die-off rates outlined in the table below.

Survival rates for bacteriophage used in core and field studies

Phage	Initial phage concentration (pfu/ml)	Survival study time (months)	Final phage concentration (pfu/ml)	Log/fold-reduction
MS2 laboratory	3.47×10^{11}	12 (377 days)	2.59×10^{5}	2.7log/ 501-fold
MS2 field	3.55×10^{11}	12 (377 days)	5.83×10^{4}	2.7log/ 501-fold
PRD1 laboratory	6.40×10^{7}	21 (652 days)	3.55×10^{4}	0.65 log/4.47-fold
PRD1 field	6.20×10^{7}	21 (652 days)	1.33	1.6 log/39.8-fold
H40/1 laboratory	1.09×10^{9}	21 (652 days)	7.43×10^{5}	0.55 log/3.55-fold
H40/1 field	1.09×10^{9}	21 (652 days)	1.60×10^{1}	1.5 log/31.6-fold
ϕX174 laboratory	1.17×10^{8}	21 (652 days)	9.13×10^{4}	0.8 log/6.31-fold
ϕX174 field	1.09×10^{8}	9 (198 days)	3.13×10^{4}	0.48 log/3.02-fold

pfu/ml = plaque forming unit per millilitres

The survival rates indicate that the chosen phage are appropriate for use as microbial tracers. In addition, the data suggest that phage – and hence some viruses – may remain viable in aquifers for considerable lengths of time, perhaps up to 1–2 years. This is rather longer than the 50 days presently assumed by aquifer protection policies for microbiological pollutants.

The results of the research suggest that:

- bacteriophage are readily available that have similar sizes and isoelectric points to a range of human pathogenic viruses;
- the availability of bacteriophage, combined with their appropriately long survival times, indicate that they are suitable surrogates for human pathogenic viruses when carrying out laboratory and field experiments;
- the Birmingham Triassic sandstone aquifer is effective in removing a large proportion of phage, and therefore by implication other viruses, from contaminated recharge within the first few tens of centimetres of travel;
- the remaining small proportion is mobile over distances of at least seven metres and, by analogy with previous observations in the aquifer, much further than this;
- viruses may remain viable up to around two years from the point of entry into the aquifer.

The database developed during the project contains information about previous laboratory and field tracer test studies using bacteriophage and conservative tracers such as fluorescein. This database will make it easier to assess the potential of different bacteriophage as a surrogate for a particular virus of concern in a UK aquifer.

Contents

1 Introduction

1.1 Background and aims

Recent studies of bacterial and viral indicators of faecal contamination have highlighted a lack of knowledge on the fate and transport of pathogens in groundwater systems. In addition, recent work in urban areas of the UK has produced evidence that deep porous systems are not virus-free (Cronin and Pedley 2002, Powell *et al.* 2003, Taylor *et al.* 2004, Rueedi *et al.* 2005).

These findings may have implications for public health especially with regard to groundwater protection and the leakage or disposal of sewage, as well as potential contamination from other sources such as landfills. For example, current groundwater protection zones are based on particle tracking and die-off of bacterial species; this may be inaccurate for viruses. There is thus a need to understand the fate and transport characteristics of human enteric viruses in the environment to help assess any future risks from viral contamination of the subsurface.

It is expected that viruses will be transported by groundwater in a manner different to dissolved substances. They differ from solutes in their larger size and therefore potentially have access to a smaller pore volume, being then possibly restricted to zones of greater average groundwater velocity. Being larger, their diffusion coefficients will also be rather lower than those of solutes. The viruses will also be expected to show characteristic surface properties which will affect their interactions with each other, the rock and with other suspended particles. Finally, they are expected to be susceptible to degradation and predation.

The majority of existing information on virus survival and transport in groundwater is based on laboratory work with limited studies at the field scale internationally and particularly in the UK, where there has also been few laboratory studies. Accordingly, this project set out to investigate the mobility of viruses using field experiments, supported by laboratory work on survival times and virus/rock interactions. The work was undertaken with specific reference to sandstones of the Triassic Sherwood Sandstone Group.

The limited amount of field testing carried out previously elsewhere in the world often substituted microspheres of equivalent size for the virus of interest owing to the problem of introducing human pathogens into the environment. However, microspheres are likely to have very different attachment and transport properties compared with viruses in the subsurface environment.

To circumvent these problems, bacteriophages were used in this project as surrogates for pathogenic viruses in order to investigate their fate and transport characteristics in an important UK aquifer. Part of the project was concerned with justifying the use of phage for this purpose and, to this end, a database tool was produced which allows easy access to information on phage properties and hence their potential behaviour in groundwater systems.

1.2 Advantages of using bacteriophage

Bacteriophage have the following advantages:

- They are similar to human enteric viruses in relation to size, genetic makeup, isoelectric point (pI) and shape. They may therefore be expected to behave in a similar fashion.
- They are highly specific to the bacterial host and are inert to all other organisms. Hence they pose no risk to public health.
- They are easy to culture and prepare, and can be sensitively and rapidly detected – a single bacteriophage with a volume of 0.001 μm^3 will give rise to visible plaques of 2–3 mm diameter after approximately 12 hours of incubation on a lawn plate of host organisms.
- Their sensitive and rapid analysis, and minute size allows high concentrations –10^{11} to 10^{13} plaque forming unit per millilitre (pfu/ml) – to be grown without a concentrating step. Centrifugation at 20,000–30,000*g* results in concentrations of 10^{13} to 10^{14} pfu/ml.

1.3 Project objectives

The research addressed a number of important unknowns.

The travel paths of human enteric viruses to significant depths in the subsurface are not understood. Possible pathways include fissure flow, abandoned wells that were not properly sealed or boreholes that may provide rapid vertical mixing of contaminants, or a combination of these factors. There are limited published data on this topic for UK aquifers, especially for the Triassic sandstone aquifer.

The project produced a database tool for easy reference to information on phage properties and behaviour in the subsurface.

The approach used data from both laboratory and field studies. This complemented laboratory and field data, which supplemented information collected through a literature review.

Phages exhibit a range of inter-related physicochemical characteristics, which may be expected to affect their transport in aquifers. As a result they are not transported in a similar fashion to solute tracers such as fluorescein or chloride. These physicochemical characteristics are outlined in Figure 1.1. The simultaneous injection of chemical and microbial tracers allows a powerful comparison between the two different classes of tracers. This is main value of using a microbial tracer as it can best inform the potential transport characteristics of viruses in the subsurface. As noted above, current groundwater protection zones are based on particle tracking and die-off of bacterial species, which may be inaccurate for viruses.

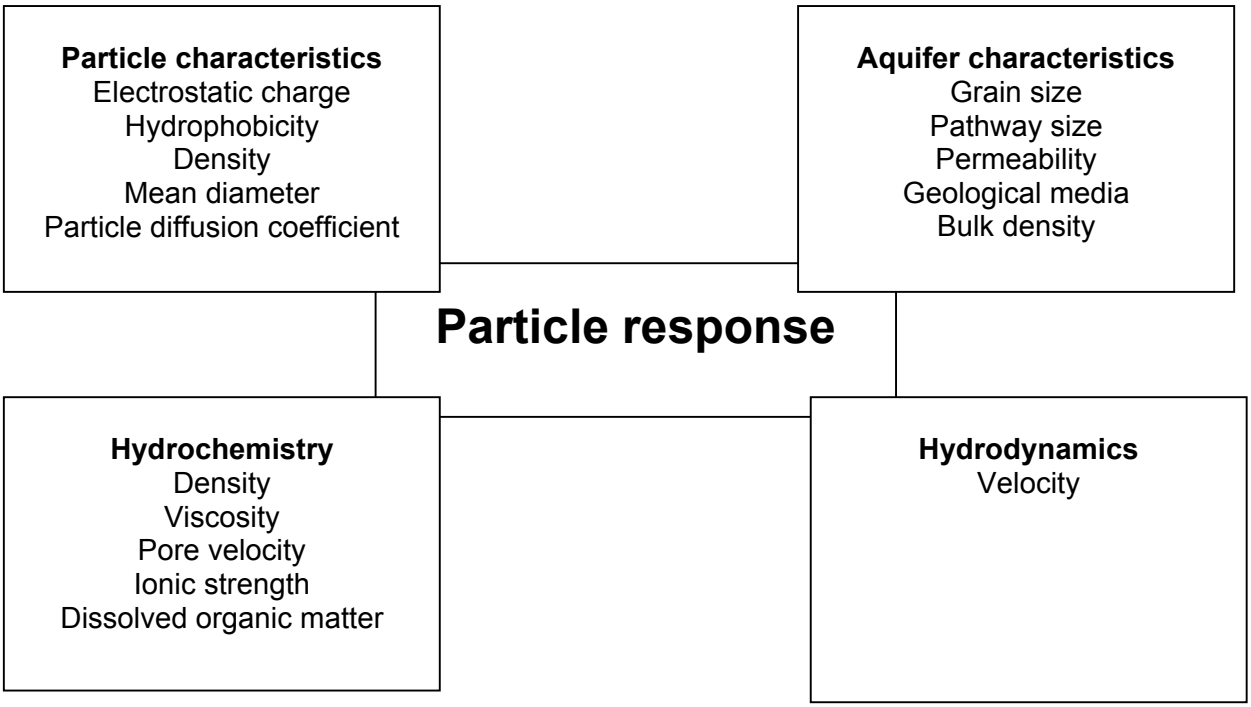


Figure 1.1 Characteristics affecting phage migration in aquifers

1.4 Report structure

Chapter2 describes bacteriophage selection and properties, while Chapter 3 gives details of the site chosen for the field tests. Chapters 4 and 5 describe the experimental and interpretation methods used. Chapter 6 presents the results and interpretations. Conclusions are presented in Chapter 7 and suggestions for further work are given in Chapter 8.

The appendices provide:

- details of literature surveys
- method details;
- the rationale for field site selection;
- a manual for the Environment Agency Virus Database.

2 Bacteriophage selection and properties

This chapter describes the selection of bacteriophage for the field experiments. The selection process involved consideration of the similarities between the phage characteristics and those of relevant pathogenic viruses as well as the practicalities of preparation and growth.

2.1 Literature review

A literature review was carried out to determine:

- the best procedures for the culture and analysis of the host and phage;
- the current state of knowledge of the effects that environmental factors (pH, temperature, ionic strength, sediment surface properties) and bacteriophage properties have on the migration of the phage in columns and field tests;
- if the indigenous microbial population and any faecal indictors have influenced phage mobility in other studies;
- the present state of knowledge of the physical processes involved in virus attachment;
- current knowledge of the survival and inactivation rates for pathogens and potential bacteriophage surrogates.

To this end, an 'Environment Agency phage database' was constructed based on the results of previous laboratory and field tracer test studies that had employed bacteriophage and conservative tracers, irrespective of aquifer matrix. This allowed better planning of the fieldwork and phage selection.

The review also considered theoretical modelling approaches to explain virus sorption (e.g. DVLO, filtration theory).

2.2 Phage selection for tracing work

The enteric viruses of concern in this study were:

- poliovirus
- rotavirus
- adenovirus
- norovirus.

The database constructed from the literature survey was interrogated to determine which bacteriophages might be the most appropriate surrogates for these pathogens. The bacteriophages used in the tracer studies were chosen on the basis of being the most suitable surrogates for the following enteric viruses in relation to size, genetic makeup, isoelectric point (pI) and shape.

Using this approach, four phages with varying properties were chosen for the laboratory and field experiments:

- MS2 (surrogate for poliovirus)

- PRD1 (surrogate for rotaviruses and adenoviruses)
- ΦX174 (surrogate for norovirus)
- H40/1 (surrogate for rotaviruses and adenoviruses)

The properties of these four bacteriophages are much more extensively documented than the others used in experimental work. In addition, three of the bacteriophage (MS2, PRD1 and ΦX174) are currently being used in a parallel laboratory analysis of virus transport – a National Environment Research Council (NERC) project being carried out jointly by the Robens Centre for Public and Environmental Health (RCPEH), the Health Protection Agency (HPA) and the University of Birmingham. For these reasons it was felt that these choices would be the most suitable for the field tracer tests.

The main properties of the chosen phages are given in Tables 2.1–2.3.

Table 2.1 Bacteriophage, host supplier details and culture media

Bacteriophage	Supplier and code	Host	Supplier and code	Media
MS2	NCIMB-10108	*Escherichia coli*	NCIMB-9481	Luria agar and broth
PRD1	ATCC-769-B1	*Escherichia coli*	ATCC-BAA-769	Tryptone soya agar and broth
ΦX174	NCIMB-10382	*Escherichia coli*	NCIMB-12416	Luria agar and broth
H40/1	Dr R Flynn, University of Neuchatel, Switzerland	*Pseudoalteromonas gracilis*	Dr R Flynn, University of Neuchatel, Switzerland	Sea water agar and broth

2.3 MS2

The taxonomy of MS2 is Levivridae, Levivirus, Enterobacteria phage MS2.

MS2 is a linear, single-stranded ribonucleic acid (ss-RNA) icosahedral F-specific bacteriophage, 27 nm in diameter with an isoelectric point of 3.5.

MS2 has been recommended as a surrogate for poliovirus due to the similarities in size. It is employed as a conservative tracer for enteric virus transport because, in the majority of soil types, the adsorption of MS2 is low compared with many other viruses (Jin and Flurry 1997, Schijven *et al.* 1999). However, similarities of removal rates for MS2, Hepatitis A virus (HAV), poliovirus 1 and echovirus 1 were observed in columns packed with clay loam at a pH of 6–8 (Sobsey *et al.* 1995).

2.4 PRD1

The taxonomy of PRD1 is Tectiviridae, Tectivirus, Enterobacteria phage PRD1.

PRD1 is a linear, double-stranded deoxyribonucleic acid (ds-DNA) icosahedral bacteriophage, 62 nm in diameter with an inner lipid membrane (Bales *et al.* 1991) and an isoelectric point of 3–4 (Loveland *et al.* 1996).

PRD1 has been used as a worst case surrogate for survival due to its low rates of inactivation at temperatures between 10 and 23°C. PRD1 is also more stable at elevated temperatures than MS2 (Yahya *et al.* 1993, Blanc and Nasser, 1996).
PRD1 has been recommended as a surrogate for rotaviruses and adenoviruses due to the similarities in size (Sinton *et al.* 1997). Adenovirus has a similar structural design to PRD1 though their isoelectric point values are different (Belnap and Stevens 2000). PRD1 is transported less conservatively than MS2, possibly because it more hydrophobic than MS2 (Bales *et al.* 1991, Dowd *et al.* 1998).

2.5 ΦX174

The taxonomy of ΦX174 is Microviridae, Microvirus, Coliphage ΦX174.

ΦX174 is a circular, single-stranded deoxyribonucleic acid (ss-DNA) icosahedral somatic coliphage, 27 nm in diameter with an isoelectric point of 6.6–6.8. ΦX174 shows the least electrostatic and hydrophobic areas of all the bacteriophages (Lytle and Routson 1995).

ΦX174 has been recommended as a surrogate for norovirus due to similarities in size. ΦX174 has a high rate of attachment to columns packed with Ottawa sand (Jin and Flurry 1997). This rate is higher than that of MS2 and may be due to the different pI values of the two bacteriophages. The isoelectric point of ΦX174 and poliovirus 1 are similar. However, when Funderberg *et al.* (1981) investigated ΦX174, poliovirus 1 and reovirus 3 in eight different soil types, they found that die-off of ΦX174 was more affected by residence time and distance than was the case for the two enteroviruses, whose die-off was more dependent on soil type.

Field studies conducted by DeBorde *et al.* (1999) showed that ΦX174 was very stable and its inactivation was minimal over a period of half a year. ΦX174 under high hydrophobic conditions would be the surrogate of choice compared with MS2 and PRD1, mainly due to is highly stability and low hydrophobicity. ΦX174 behaviour varies greatly with pH due to changing net surface charge; at pH 6, surface charge is positive and at pH 8 it is negative.

2.6 H40/1

The taxonomy of H40/1 is Siphoviridae (B1 morphotype).

The H40/1 phage was isolated from the Atlantic Ocean and was supplied by Dr R Flynn and Dr P Rossi, University of Neuchatel. H40/1 is a double-stranded deoxyribonucleic acid (ds-DNA) icosahedral bacteriophage with a tail. It is 85–82 nm in diameter and has a tail length of 39–42nm. The isoelectric point for H40/1 is >4.5, although this has not been clearly defined (Rossi 1994).

H40/1 has been recommended as a surrogate for rotaviruses and adenoviruses due to the similarities in size. Adenovirus has a similar structural design to H40/1, although their pI values are different.

Table 2.3 compiles information from literature reviews to allow users to determine the most suitable bacteriophage surrogates for the human enteric viruses to be studied. This table provides information relating to:

- commercial costs of the bacteriophage;
- physical characteristics (size, shape, morphology and isoelectric point);
- specific host;

- media type;
- method and duration of analysis;
- biosafety level risks to the user.

Table 2.2 Bacteriophage and potential virus surrogates[1]

Virus / Phage		Size (nm)	Capsid symmetry	pI	Nucleic acid
Phage	MS2 (F-specific)	26	Icosahedral	3.5–3.9	ss-RNA
	PRD1 (somatic)	62	Icosahedral	4.2	ds-DNA
	H40/1 (marine)	82–85(B) 39–43(T)	Icosahedral and tail	<4.5	ds-DNA
	ΦX174 (somatic)	25–27	Icosahedral	6.6	ss-DNA
Viral pathogen	Adenovirus	80–110	Icosahedral	N/A	ds-DNA
	Astrovirus	27–30	Polyhedral	N/A	ss-RNA
	Coxsackievirus	28–30	Icosahedral	4.8	ss-RNA
	Echovirus	28–30	Icosahedral	5.0–6.4	ss-RNA
	Apthovirus[2]	27–30	Icosahedral	N/A	ss-RNA
	Norovirus	35–39	Icosahedral	5	ss-RNA
	Poliovirus	28–30	Icosahedral	4.5–8.2 (AV = 4.5)	ss-RNA
	Rotavirus	80	Icosahedral	3.9	ds-DNA

[1] Red, blue and green show the surrogate relationships used in this project.
[2] Foot and mouth disease (FMD) virus
AV = average

Table 2.3 Entries in the Environment Agency phage database

Bacteriophage	PRD-1 (similar size to rotavirus & adenovirus)	Phi X174	MS-2 (similar size to enterovirus & norwalk virus)	Marine phage H40/1
Strain ref no	ATCC-769-B1($150), BAA-769-B1(£195), NCA on NCIMB	ATCC-13706-B1, NCIMB-10382	ATCC-15597-B1($28)(£28), NCIMB-10108	NCA (University of Neuchatel)
Background	Somatic coliphages	Somatic coliphages	Male-specfic coliphage	N/A
Taxonomy	Tectiviridae, Tectivirus, Enterobacteria phage PRD-1	Microviridae, Microvirus, Coliphage phiX174	Levivridae, Levivirus, Enterobacteria phage MS2	Siphoviridae (B1 morphotype)
Size	D = 62nm	D = 24-26nm, d = 1.43 g/cm3	20-26nm (D), 3.7 million (MW) 1.42 g/cm3 (d)	85-82 (D), 39-42 (T)
Shape	Lipid-containing (14-15% NuA) double icosahedral capsid, 60nm(L) 10nm(W)	Lipid-containing icosaherdral capsid (31% NuA)	Icosahedral capsid	Icosahedral capsid, tailed
Morphology	Linear dsDNA	Circular ss-DNA	Linear ss-RNA	ds-DNA
Isoelectric point (pI)	pI 4.2	pI 6.6	pI 3.9	pI > 4.5 (not clearly defined)
Host	*Escherichia coli* ATCC-BAA-769 *Salmonella typhimurium* LT-2 ATCC-29476	*Escherichia coli* NCIMB-12416 ATCC-25312/13706	*Escherichia coli* NCIMB-9481 ATCC-15597 *Salmonella typhimurium* WG49	*Pseudoalteromonas gracilis* (University of Neuchatel)
Technique	DAL (Tryptone Soya Broth & Agar)	DAL (Luria Broth & Agar)	DAL (Luria Broth & Agar)	DAL (Sea Water Broth & Agar)
Risk	BL-2	BL-1	BL-1	BL-1
Analysis Time	8 hr	7 hr	7 hr	7 hr
Bacteriophage	**fr**	**PM2**	**Qß (originate in human excrement)**	**T4**
Strain ref no	ATCC-15767-B1($150)(£195), NCA on NCIMB	ATCC-27025-B1($190)(£250), NCIMB-1913	ATCC-23631-B1($190)(£250), NCIMB-11289	ATCC-11303-B4($28)(£28), NCA on NCIMB
Background	Male-specfic coliphage	GTI	Male-specfic coliphage	Somatic coliphages
Taxonomy	Leviviridae, Levivirus, Enterobacteria phage fr	Corticoviridae, Corticovirus Type Phage PM2	Leviviridae, Allolevivirus, Enterobacteria phage Qß	Myoviridae, "T4-like Viruses", Enterobacteria phage T4
Size	21nm (D), 4.1 million (MW)	60nm (D)	24-26nm (D), 1.439 g/cm3 (d), 4.2 million (MW)	B: 95nm(L) 65nm(D), T: 110nm(L) 25nm(W), 120 million (MW)
Shape	N/A	Isometric capsid (13% NuA), spikes	Icosahedral capsid (31% NuA)	Polyhedral capsid, tailed (48% NuA)
Morphology	ss-RNA	Circular ds-DNA	ss-RNA	Circular ds-DNA
Isoelectric point (pI)	pI 9	pI 7.3	PI 5.3	PI 4.2
Host	*Escherichia coli* ATCC-19853	*Pseudoalteromonas espejiana* ATCC-27025 *Alteromonas espejiana* ATCC-2725	E. coli ATCC-23631 NCIMB-11288	*Escherichia coli* ATCC-11303 NCIMB-10360
Technique	DAL (Yeast Tryptone Broth & Agar)	DAL (AMS)	DAL (Nutrient Broth & Agar + 0.8g/l NaCl)	DAL (Nutrient Broth & Agar + 0.5% CaCl2)
Risk	BL-1	BL-1	BL-1	BL-1
Analysis Time	7 hr	7 hr	7 hr	7 hr
Bacteriophage	**R17**	**f2**	***Enterobacteria phage* X S24VA**	**PBS1**
Strain ref no	ATCC-25868-B1($190)(£250), NCIMB-13035	NCA on ATCC or NCIMB	NCA on ATCC, NCIMB-10645	NCA on ATCC or NCIMB
Background	Male-specfic coliphage	Male-specfic coliphage	UTI, CF & burn inf	Somatic coliphages
Taxonomy	Leviviridae, Levivirus, Enterobacteria phage R17	Leviviridae	Inoviridae, Inovirus	Myoviridae, "PBS1-like Viruses", Bacillus phage PBS1
Size	26nm (D), 1.46 g/cm3 (d)	20-30nm (D)	760-1950nm (L) 6-8nm (D)	B: 95-111nm(L) 65-80nm(D), T: 80-455nm(L) 16nm(W)
Shape	Tailed or nontailed	Icosahedral capsid, no tail, small capsomeres (30%NuA)	Isocahedral, Virions not enveloped (6-21 %NuA)	Virions not enveloped, tailed, head
Morphology	ss-RNA	Linear ss-RNA	Circular ss-DNA	1 mol. linear double stranded DNA (48% NuA)
Isoelectric point (pI)	pI 3.9	N/A	N/A	N/A
Host	*Escherichia coli* ATCC-25868	*Escherichia coli* K13 ATCC-15766	10644	Bacillus subtilis ATCC-15563 NCIMB-10715
Technique	DAL (Tryptone Soya Broth & Agar)	DAL (Nutrient Broth & Agar)	DAL (PGP)	DAL (Tryptone Soya Broth & Agar)
Risk	BL-1	BL-1	BL-1	BL-1
Analysis Time	7 hr	7 hr	8 hr	7 hr
Bacteriophage	**Phi MWD 1**	**Species Bacteroides fragilis phage B40-8**	**M1**	**N/A = not available**
Strain ref no	NCA on ATCC or NCIMB	ATCC-51477-B1($190)(£250), NCA on NCIMB	N/A	**NCA = not commerically available**
Background	Somatic coliphages	Phage infecting obligate bacteria	Somatic coliphages (heat resistant)	**F = family**
Taxonomy	Myoviridae, P2-like phage, Phi MWD 1	N/A	Myoviridae	**G = genus**
Size	N/A	N/A	B: 110nm(L) 85nm(D), T: 110nm(L) 25nm(W), 120 million (MW)	**Sp = species**
Shape	Icosaherdral capsid, tailed	N/A	Icosahedron, T-even phage with head, tail, base plate & tail fibers	**D = diameter**
Morphology	ds-DNA	N/A	ds-DNA	**d = density**
Isoelectric point (pI)	N/A	N/A	PI 4-5	**T = tail**
Host	*Escherichia coli* H2S+ K13 ATCC-15766	*Bacillus fragilis* HSP40 ATCC-51477	*Escherichia coli* ATCC-15597	**B = body**
Technique	DAL (Tryptone Soya Broth Agar + CaCl2)	PCR / Bateroides Phage Recovery Medium (BPRM)	N/A	**W = width**
Risk	BL-1	BL-1	N/A	**L = length**
Analysis Time	7 hr	11 hr	N/A	**MW = molecular weight**
				NuA = Nulceic acids
				DAL = Double agar method
				BL = Biosafety level

3 Site selected for field tests

3.1 The site selection process

A number of potential sites for carrying out the field tracing work were evaluated (Appendices 1 and 2) and a borehole array at the University of Birmingham was chosen.

The rest of this chapter describes the physical and hydraulic characteristics of the site and presents some initial interpretation of water quality.

3.2 Site background information

3.2.1 Investigation methods

Three 50-metre deep boreholes were drilled during autumn 2001 at the University of Birmingham campus to investigate sediment-filled fractures in the Triassic Sherwood sandstone (Bouch *et al.* 2006). These three boreholes were cored continuously (6-inch/15.24cm diameter) to a depth of 50 metres below ground level.

Groundwater levels measured in an existing well during drilling indicated no significant changes resulting from the new drilling suggesting that:

- no induced pathways were created during construction;
- rapid hydraulic interconnections were not expected.

The boreholes were logged using resistivity, natural gamma, calliper, flow and optical televiewer tools.

Bouch *et al.* (2006) used constant head and Temco Inc. MP-402 mini-permeameters to measure permeabilities on core taken from the three boreholes. They also took petrographical samples from all three boreholes for detailed analysis.

Thin sections, polished sections, rock chips, and powdered samples were analysed using:

- standard microscopy examination (including point counting);
- energy dispersive X-ray analysis (EDXA);
- scanning electron microscopy (SEM);
- ack-scattered electron microscopy (BSEM);
- cathodoluminescence (CL);
- particle size analysis (PSA);
- X-ray diffraction (XRD).

3.2.2 Sedimentology

The sandstones beneath the University of Birmingham campus have characteristics typical of the Wildmoor Sandstone Formation. The sandstones are typically silty and often relatively poorly cemented (Bouch *et al.* 2006).

Sedimentological logging indicated that the formation comprises:

- massive sandstone;
- planar laminated sandstone;
- ripple cross-laminated silty sandstone;
- pebbly sandstone;
- wind-ripple laminated sandstone;
- siltstone or claystone;
- mudstone with dolocrete nodules.

These lithologies are interpreted to be the result of sedimentation in a moderate to low-sinuosity braided, fluvial environment within which associations of both fluvial and subaerial facies occur (Bouch *et al.* 2006). The mudstone with dolocrete nodules represents a palaeosol facies.

Optical televiewer data indicate the bedding predominantly dips south-west with a minor component to south-east, though these logs are unable to resolve the dips of cross-bedding foresets.

Hydraulic conductivities range from about 10^{-11} m/s for clay layers to about 10^{-4} m/s for sandy layers.

3.2.3 Fractures

Due to the relatively soft nature of the recovered material, cores from all three boreholes contain very abundant drilling-induced or drilling-exaggerated discontinuities, which tend to form horizontal to low angle fractures. These artificial fractures are mainly recognised at lithological boundaries and fail to be imaged on the televiewer logs, indicating tight fits across fractures. In core specimens, they show no coatings on fracture surfaces.

In contrast, natural fractures are relatively scarce but are readily identified in cores and are typically imaged clearly on the televiewer logs. Various types of fractures are observed including granulation seams, cemented/filled and uncemented/unfilled fractures. Bouch *et al.* (2006) indicates that all these natural fractures are high-angle to vertical (20–90º).

3.2.4 Petrography

The sandstones have average grain sizes from coarse silt to coarse sand. They are poorly to well sorted, and commonly display well-developed millimetre-scale lamination. A range of diagenetic events are recognisable from early post-deposition precipitation to recent groundwater circulation dissolution.

Petrographic analysis revealed two types of granulation seam in the sandstone (Bouch *et al.* 2006):

- those characterised by the presence of crushed and/or corroded dolomite cement;
- those characterised by the presence of comminuted detrital sediment material and clay.

The latter type is less common.

3.2.5 Impact of heterogeneities on fluid flow and contaminant transport

The three boreholes demonstrate a number of scales of heterogeneity within the Wildmoor Sandstone Formation. These need to be considered when modelling groundwater flow or contaminant transport.

At the largest scale, the abandoned channel and the palaeosol facies are the most likely features to influence both groundwater flow and contaminant transport. This is due to their high clay content and/or their dolomite-dominated compositions, and therefore low permeabilities. Characteristic dimensions of these features are thicknesses up to a metre and lateral extents of tens of metres. These features are likely to act as impediments to vertical fluid flow on the scale of tens of metres and, as such, they may locally support perched water tables (Bouch *et al.* 2006) – as can be seen in outcrop elsewhere in the Midlands.

At a finer scale, the distribution of early dolomite cement will also influence fluid flow and transport behaviour. The sandstones are typically lightly cemented and, overall, they have high moderate porosities (with modal analysis of uncemented samples indicating primary intergranular macropore volumes of up to 20 per cent). However, early dolomite and late calcite cements severely degrade primary macropore volumes locally.

Core observations indicate that:

- dolomite and calcite cements are significant within approximately 2–5 per cent of the cored intervals;
- these cements are most prominently developed in the coarser grained sandstones of the channel lag facies.

Consequently, although these cemented beds are relatively thin (typically 0.5–10 cm), their correspondence with channel lag facies suggests they may be laterally continuous over distances comparable with the extent of the bedforms (tens to hundreds of metres). These bands are likely to be readily bypassed by horizontal flow, but they may act to inhibit vertical flow.

A secondary calcite and dolomite coating on sandstone leads to a large increase in grain surface and thus to a higher number of attachment sites per volume. This can result in higher adsorption rates of charged colloids such as bacteriophages (Flynn 2003).

The sandstones, as measured on a core from a borehole located in the Wildmoor Sandstone Formation about 100 metres from the boreholes used here, have cation exchange capacities (CECs) ranging from ~ 1 to 20 meq/100 g (Mitchener 2003). Much of this is supplied by clay minerals (illite, chlorite and smectite), but there is also a component from the haematite and manganese oxides, and possibly from the carbonates.

Surface areas are expected to be up to ~7 m^2/g (Mimides 1981). Pore size distributions measured using the mercury injection technique on a range of samples from the boreholes indicate median pore sizes of tens of microns (Bloomfield J P, personal communication) with, on average, 5 per cent pore volume having pore diameters of less than 100 nm. Mudstone median pore diameters, however, are much smaller.

Almost ubiquitous carbonate (Bouch *et al.* 2006) buffers the pH of the groundwaters (Falb 2005).

3.2.6 Preliminary tracing results

The University of Birmingham ran a number of preliminary tests focusing on tracer transport through fractures. Half metre intervals of fractured regions were packered off based on geophysical log data.

The results showed the times of first tracer appearance ranging from just a few hours to more than a few weeks. The pumping rates were around 0.5 litres per second and produced head drawdowns of up to 20 metres.

The natural head gradient at the site is directed approximately towards the north-east but, in addition, there is an upwards-directed vertical head gradient of approximately 0.05. Although these head gradients are small compared with the drawdowns likely to be induced in the pumping well during tracer testing, care is needed to ensure that this is the case in any test.

3.2.7 Water quality

As part of the evaluation of the suitability of the Birmingham site, 100 ml samples were taken from Borehole 3 and analysed for a routine suite of viable faecal indicator bacteria and phage (Table 3.1).

Total coliforms (TC) and sulphite-reducing clostridia (SRC) were detected in 100 ml sample volumes using membrane filtration. They were selectively enumerated by culture on membrane lauryl sulphate broth (thermotolerant coliform bacteria), Slanetz and Bartley agar (faecal streptococci) and perfringens agar (SRC) (Anon 1994).

The thermotolerant coliform bacteria (TTC) and faecal streptococci (FS) data were validated by duplicate analysis using the IDEXX® Quanti-Tray™ procedure: Colilert® to analyse specifically for *E. coli* (rather than the TTC group as a whole) and Enterolert® to analyse for FS.

The results from all analyses were recorded as colony forming units (CFU) or plaque forming units (PFU) per 100 ml (membrane filtration), or as most probable number (MPN) per 100 ml (IDEXX® procedure). The total viable count (TVC) was also measured.

This analysis aims to grow all culturable heterotrophic bacteria at temperatures that reflect environmental conditions (22°C) and human body temperature (37°C). This can give an indication of the level of culturable indigenous microorganisms in the water.

Table 3.1 Microbiological analysis of groundwater from University of Birmingham Borehole 3 (24 February 2003)

Type	No. of organisms present
Phage (PFU/100 ml)	<1
Clostridia (CFU/100 ml)	38
Enterococci – MF (CFU/100 ml)	<1
Enterococci – QT (MPN/100 ml)	0
Total coliforms – MF (CFU/100 ml)	<1
Total coliforms – QT (MPN/100 ml)	4
Escherichia coli – MF (CFU/100 ml)	<1
Escherichia coli – QT (MPN/100 ml)	0
TVC at 22ºC (CFU/ml)	240
TVC at 37ºC (CFU/ml)	1,360

MF = membrane filtration; QT = Quanti tray™

The results do not suggest any likely problems in the microbial content of the water. The clostridia numbers are perhaps higher than expected, but it is not unusual to find this long-lived indicator in the subsurface. Indeed it was found regularly in previous monitoring in Birmingham and Nottingham groundwater (Powell *et al.* 2003) and during current groundwater monitoring of the Triassic Sandstone sequence in the Doncaster urban area (Morris *et al.* 2006). Total viable counts (TVCs) (in the range 100–1,000 CFU/ml) also correspond well with the results from Doncaster. The conclusion from this work is that the groundwater is representative of other Sherwood Sandstone Group aquifers and of suitable quality for bacteriophage tracer experiments; the background counts are too low to affect the introduced microorganisms significantly.

Table 3.2 shows the results of analyses carried out by the University of Birmingham on samples taken from a borehole array adjacent to that used in this study.

Table 3.2 Analyses of groundwater samples taken in November 2000 from a borehole site in the same formation and about 100 metres from the site used in this study

Parameter	Maximum	Minimum	Mean	No. of samples
pH	7.8	7.3	7.7	6
Conductivity (at 20°C)	569	500	540	6
Turbidity (NTU)	0.4	0.3	0.4	6
Ca (mg/l)	76	70	73	6
Mg (mg/l)	30	25	28	6
Na (mg/l)	14	10	13	6
K (mg/l)	2	0	1	6
Alkalinity (as $CaCO_3$) (mg/l)	225	219	222	6
SO_4 (mg/l)	50	38	47	6
Cl (mg/l)	29	21	28	6
NO_3 (mg/l)	19	16	17	6

NFU = Nephelometric turbidity units

The data show that the composition of the groundwater is not unusual for the Sherwood Sandstone Group. There are no high concentrations of divalent ions that could be problematic from the point of increased phage binding.

These data because they were produced some time ago by the University of Birmingham and it was not possible to retrieve standard deviation values.

4 Methods

4.1 Bacteriophage survival experiments

4.1.1 Laboratory bacteriophage survival experiments

Survival experiments were undertaken using MS2, PRD1, H40/1 and ΦX174 bacteriophage suspensions prepared in groundwater abstracted from the Birmingham well field.

The Birmingham groundwater was filtered through a 0.2 µm syringe filter to remove any bacteria or algae present. The phage were resuspended in the filtrate and placed in glass universals (glass bottles) stored at 12ºC in a temperature-controlled incubator. Three samples (triplicates) were taken and assayed at three-month intervals using the double agar method (Adams 1959).

4.1.2 Field bacteriophage survival experiments

Survival experiments were undertaken using MS2, PRD1, H40/1 and ΦX174 bacteriophage suspensions prepared in groundwater from the Birmingham well field.

The Birmingham groundwater was filtered through a 0.2µm syringe filter to remove any bacteria or algae present before the phage were resuspended. Equal volumes of phage suspensions were placed in polyvinylidene fluoride (PVDF) (Medicell International Inc) dialysis tubing, sealed with universal clips and inserted into the boreholes at a depth of either 24 or 28 metres below ground level (mbgl). Samples were removed at three-month intervals and assayed in triplicate. The PVDF tubing containing ΦX174 split and was lost down the borehole following the third sampling round, and hence no further data could be collected.

4.2 Sandstone core experiments

4.2.1 Experimental set-up

The sandstone cores were encased in a polymer with tubing and connectors attached. Table 4.1 gives details of the cores.

Figure 4.1 illustrates the temperature-controlled column flush rig used. The temperature was maintained at 12ºC (the approximate *in situ* groundwater temperature) by housing all the apparatus within a modified refrigerator. The unreacting tracer (fluorescein), flow rates, pressure and temperature were monitored online.

Table 4.1 Properties of the sandstone cores

Core No.	1	2	3	4
ID	18 KR	KR56 V	KR53.5H	BH1 47.42 H
Core mass (wet) (g)	101.59	97.01	107.18	117.4
Core mass (dry) (g)	89.04	86.25	97.49	102.98
Length (cm)	4.9	3.8	4	6.4
Diameter (cm)	3.3	3.8	3.7	2.9 and 3.2 (AV = 3.05)
Volume (cm^3)	41.91	43.1	43.01	46.76
Bulk density (g/cm^3)	2.423	2.251	2.491	2.511
Porosity (%)	29.9	25	22.5	30.4
Location	Kensington Reservoir BH, Merseyside	Kensington Reservoir BH, Merseyside	Kensington Reservoir BH, Merseyside	University of Birmingham BH1

AV = average; BH = borehole

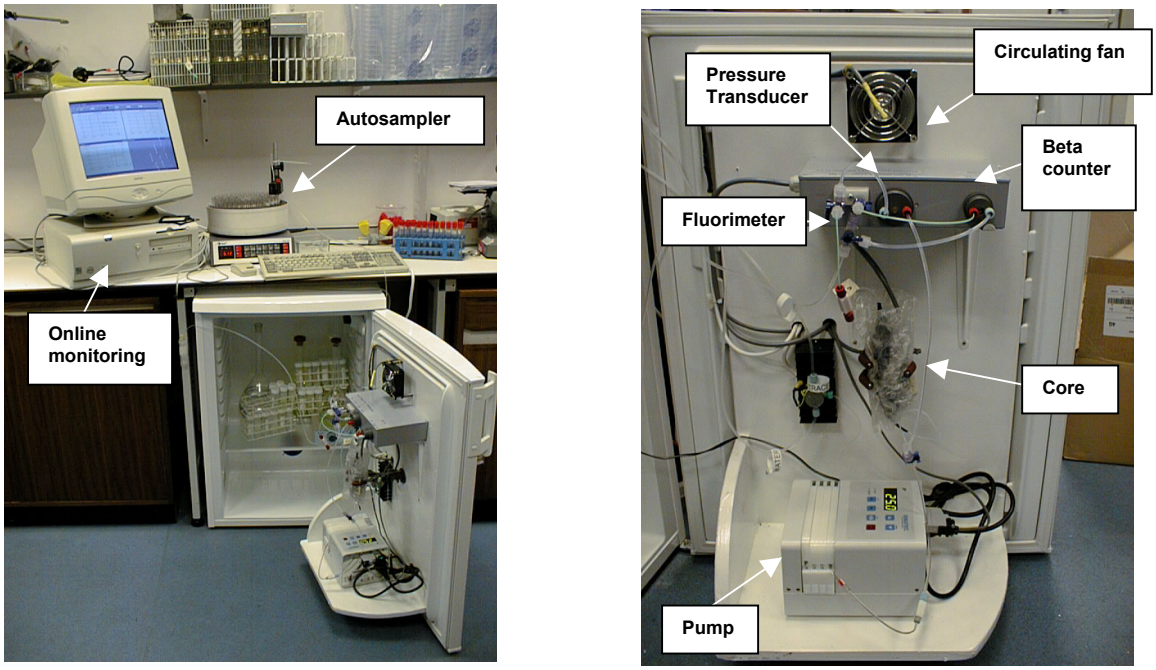


Figure 4.2 Laboratory (12ºC) set-up for column experiments

4.2.2 Laboratory experiments

Initial experiments were carried out to determine the optimum fluorescein concentration for detection. A further series of fluorescein (1 mg/l) tracer tests, each consisting of a short period of tracer injection followed by a flush of tracer-free solution, was then performed on the four experimental cores using two different flow rates (15 and 30 ml/minute) to determine the advective and dispersive properties of each core.

Experiments were then undertaken using bacteriophage to identify the transport and attachment characteristics of each phage. Table 4.2 summarises the phage and fluorescein concentrations passed through each core. A series of re-injections was completed on each core to determine whether the attachment rates varied with the concentration of phage injected.

Each tracer injection consisted of a 10 ml volume of phage and fluorescein, followed by unfiltered Birmingham groundwater. The fluorescein was monitored online and the phage were assayed using the double agar method (Adams 1959). The pH for all core experiments was not adjusted; the ambient pH at exit (~7.5) was very similar to that observed in the field.

Table 4.2 Phage and fluorescein tracer injection concentrations for each sandstone core experiment

Name	Size (nm)	pI	Core	Fluorescein concentration (mg/l)	Phage injection concentration (pfu/ml)
PRD1	62	4.2	1	1	3.57×10^4 1.37×10^9 4.10×10^4
H40/1	82-85(B) 39-43(T)	<4.5	2	1	3.03×10^8
ΦX174	25-27	6.6	3	1	1.26×10^4 1.54×10^6 4.10×10^4
MS2	20-26	3.9	4	1	1.49×10^4 2.77×10^8

B = body length; T = tail length

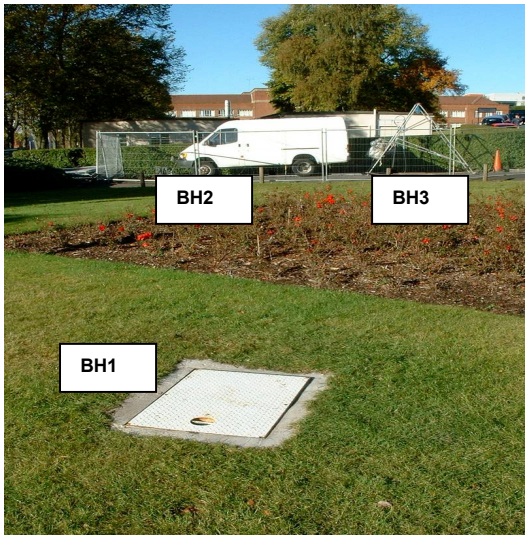
4.3 Sandstone field tracer experiments

4.3.1 Birmingham well field

The field test site was located at the University of Birmingham. The boreholes were drilled using wire-line coring. The design of borehole array consisted of three 50-metre boreholes each of ~15 cm diameter. Each borehole was solid-cased to consolidated rock, then left as an open hole to the base: Borehole 1 was cased to 12.44 metres, Borehole 2 to 15.70 metres, and Borehole 3 to 12.33 metres. The deviation from vertical at the base of the boreholes is 2.02, 1.88 and 1.37 metres respectively. Boreholes 2 and 3 were positioned with a separation of 7.55 metres at ground surface. Borehole 1 is approximately 20 metres away from the other boreholes.

The location of the boreholes and the basic arrangement of the packers and transducers are shown in Figure 4.2. The injection borehole was monitored for pressure, with transducers located above, below and in the packered interval. Samples were also taken from the

injection interval for analysis for fluorescein and phage concentrations. The pumped borehole was monitored online for particle concentrations, turbidity, temperature, flow and fluorescein concentration. Samples were taken using a fraction collector for laboratory analysis of phage (Figures 4.3–4.5).



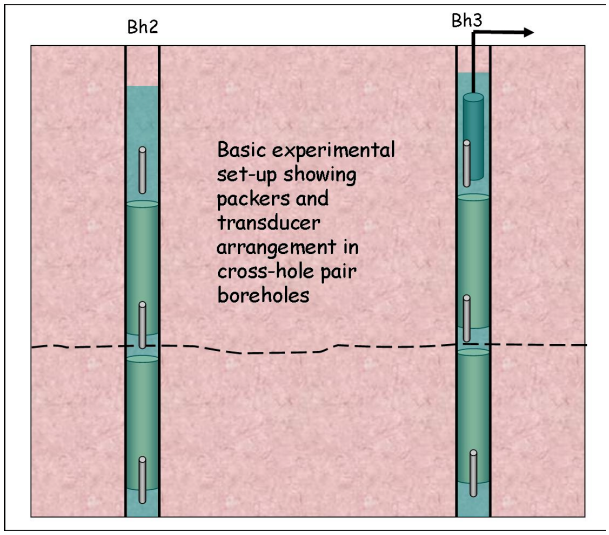


Figure 4.3 Location of Birmingham boreholes and packered interval field set-up

Figure 4.4 Field laboratory set-up for field tracer tests

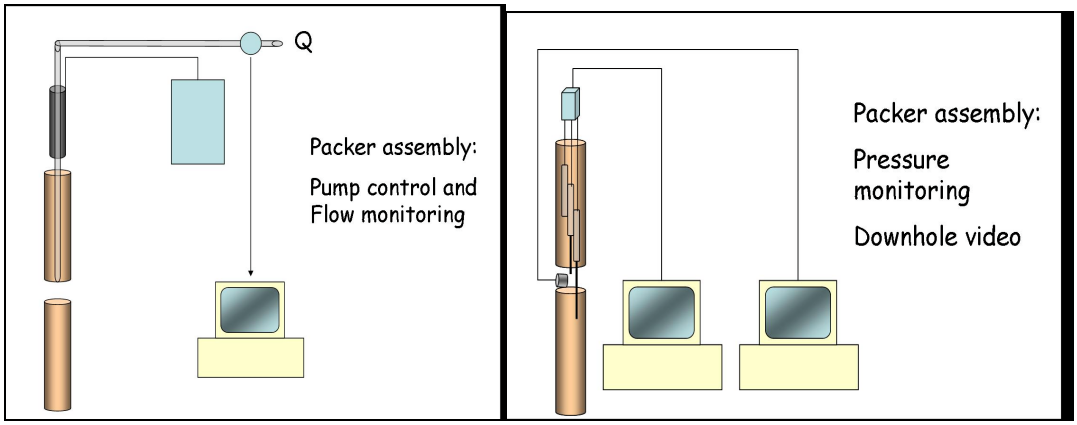


Figure 4.5 Experimental packered interval set-up illustrating the packer assembly: pump control, flow and pressure monitoring and CCTV

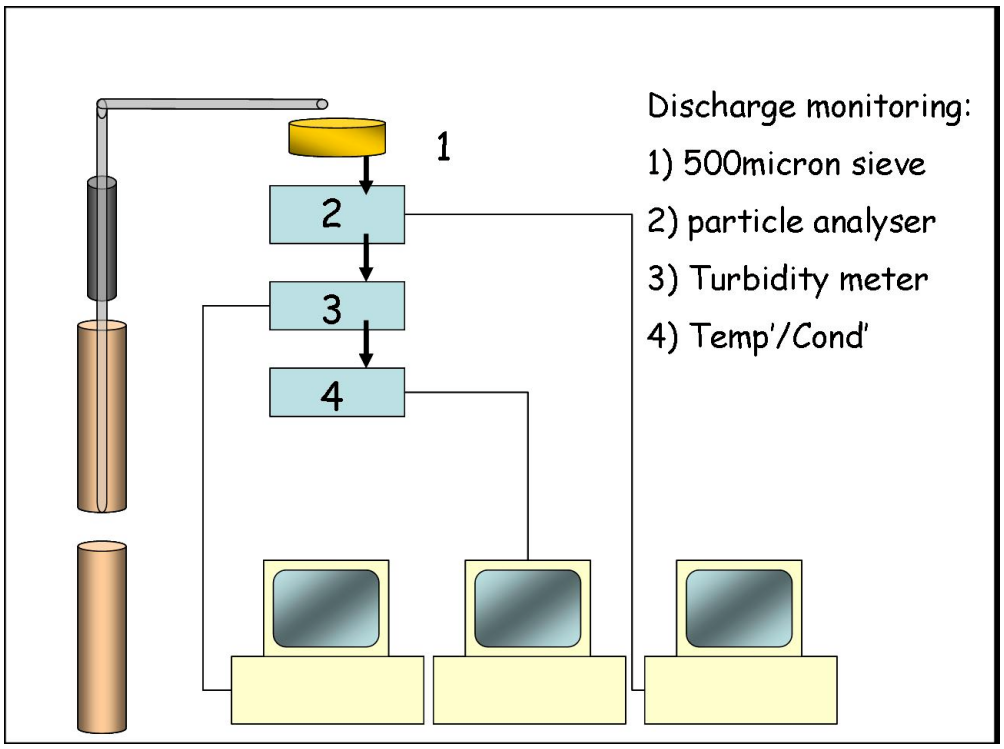


Figure 4.6 Experimental packered interval set-up illustrating the discharge monitoring: fluorimeter and fraction collector

4.3.2 Equipment for field tracing work

A list of the most important items for the injection and sampling of the bacteriophage in the field is given in Appendix 3.

4.3.3 Tracer test procedures

A series of field tracer tests was undertaken to determine the migration and survival of bacteriophage at a field-scale (Table 4.3). A conservative tracer (fluorescein) was added to all tests. The field tracer tests were carried out between July 2004 and August 2005.

The initial tracer tests focused on testing four different one metre packered intervals, with four different suspensions of bacteriophage in conjunction with fluorescein. This was intended to allow determination of individual bacteriophage migration and attachment in the Triassic sandstone aquifer.

Following the completion of the four individual injections, further tests using bacteriophage 'cocktail' suspensions containing the four bacteriophages (MS2, PRD1, H40/1, and ΦX174), were completed in one packered fracture interval test and over the whole length of the uncased boreholes.

The reason for using phage cocktails was to determine if there are any phenomena associated with interactions between the phage that might need to be considered in future tests. If no interactions occurred, the tests would also allow simultaneous testing of which phage are the most mobile.

These tests also highlighted how effectively phage of varying survival and attachment characteristics can be used in tandem and to achieve a suitable phage combination to assess 'worst-case scenario' of mobile persistent viral pathogens in the subsurface.

For the packered fractured interval test, a solution of 8 litres of phage suspension containing all four phage and fluorescein was used.

The final open borehole test was undertaken using 20 litres of suspension containing all four phage and fluorescein. Details of all the field tracer test injections and intervals tested are given in Table 4.3.

Each field test lasted between four to six weeks, with both the injection and pumping boreholes monitored for phage and fluorescein at regular intervals throughout the test period. For phage analysis, 15 samples were collected by an online autosampler. The frequency of sampling was varied based on results from the online fluorimeter.

The injection borehole was initially sampled every 30 minutes for the first six hours. This was reduced to two samples per day (morning and evening) for the first two weeks, then once per day until the numbers of phage (PFU/ml) and concentration of fluorescein (in parts per billion; ppb) had reduced to background levels.

The pumping borehole was initially sampled every 30 minutes, with every fourth sample analysed for phage (i.e. every two hours). If the two-hour samples gave positive results, a repeat analysis was completed to ensure the positive detects were not a result of contamination. The 30-minute sampling and two hour analysis regime was continued until the fluorescein tracer had arrived, peaked and returned to background levels. After this point, the sampling was reduced to every two hours with four-hour phage analysis, and this was continued until the end of the tracer tests. From the fourth test onwards, Biocap 30 filters (CUNO Ltd) were used to confirm findings from the autosampler data. These filters are placed online and trap any viruses present as the water passes through them, thus providing an estimate of total phage present.

Table 4.3 Summary of field test data

No.	Duration (days)	Date	Fluorescein mass	Phage	Volume (litres)	Phage concentration (PFU/ml)	Depth (mbgl)	Pumping rate (l/min)
Test 1	30	July 2004	2 g	ΦX174	2	10^8	19.1 (Unfractured sandstone)	7.3
Test 2	30	September 2004	2 g	H40/1	2	10^9	26.9 (Unfractured sandstone)	2.7
Test 3	30	February 2005	2 g	MS2	2	10^8	26.3 (Unfractured sandstone)	7
Test 4	40	March 2005	2 g	PRD1	2	10^8	19.5 (Unfractured sandstone)	6.8
Test 5	30	April 2005	2 g	**Cocktail**: MS2 PRD1 ΦX174 H40/1	 2 2 2 2	 1.6×10^{10} 3.97×10^6 7.43×10^7 2.93×10^{10}	44.2 (Fractured sandstone)	3.7
Test 6	45	August 2005	20 g	**Cocktail**: MS2 PRD1 ΦX174 H40/1	 2 5 2 2	 4.2×10^8 7.73×10^7 1.4×10^7 5.34×10^7	Full borehole test	145

Boreholes were pumped before beginning any tracer experiments to ensure injection occurred after the start of pumping and effects due to unsteady state flows at the start of pumping were avoided. Background analyses for chemical (major and minor ions) and microbiological (TVC, FC, FS, SRC and coliphage) components present in the groundwater were also completed before beginning the field tracer tests. Pumping rates, heads and particle concentrations were recorded throughout each tracer test.

4.4 Health and safety issues

Though not very hazardous, the nature of the work required care with respect to:

- transport to and from site;
- chemical sampling on site;
- microbiological sampling on site;
- other aspects of fieldwork;
- growth and storage of microbiological materials.

Accordingly, appropriate documentation was supplied to the Environment Agency including Control of Substances Hazardous to Health (COSSH) forms for:

- the growth and storage of bacteriophage ΦX174, MS2, PRD1 and H40/1;
- the growth of *Escherichia coli* (NCIMB-9481, NCIMB-12416, ATCC-BAA-769);
- the growth of *Pseudoalteromonas gracilis* for the culture or isolation of bacteriophage.

All testing was undertaken following permission from the Environment Agency's Regional Offices.

5 Modelling

5.1 Introduction

A model was developed to:

- aid interpretation of the laboratory and field data collected during the project;
- allow exploration of the sensitivities of the various processes represented.

A one-dimensional (1-D) model was used because:

- it was easier to solve analytically and numerically;
- calculation times could be reduced;
- more processes could be included and therefore studied.

An argument against a 1-D representation is that, in reality, the experiments take place in two-dimensions (Figure 5.1a) or even three-dimensions (Figure 5.1b). This means that some of the initially injected tracer will pass the observation point without being detected because hydrodynamic dispersion spreads out the plume with increasing transport distance. Such effects can be partly accounted for by using an unreacting tracer; this will indicate the magnitude of the breakthrough peak and the absolute recovery, which can then be used to model the phage transport. In the present case, all tracer tests were undertaken with combined phage and fluorescein injections.

However, hydrodynamic dispersion affects only the absolute recovery and the magnitude of the maximal concentration of the tracer. It does not affect the different parameters (e.g. attachment rate, decay rate, etc.) that are relevant to the experiments. Therefore, each experiment used a simultaneous injection of phage and fluorescein (conservative tracer). The latter corrects the effects of tracer loss due to dispersion. In other words, the conservative tracer is introduced to show the magnitude of the peak and the absolute recovery to be used to model the phage transport.

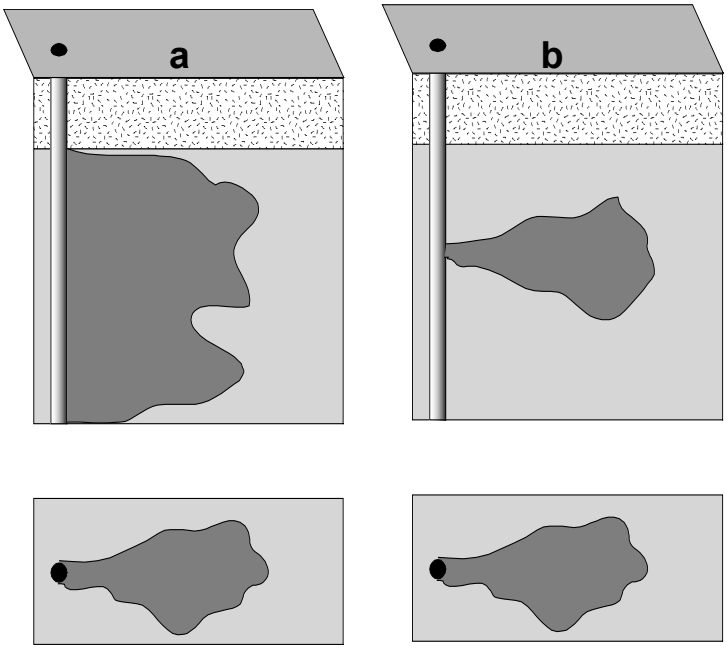


Figure 5.1 (a) Transport of tracer in the aquifer with injection over entire borehole depth. The top picture shows a cross-sectional view and the bottom one the view from top. (b) Transport of tracer in the aquifer with injection over a short depth interval only

Virus removal during subsurface transport is due to a complex interplay of processes, of which inactivation and attachment are of major importance (Yates *et al.* 1987). In sandy aquifers, attachment is normally reversible and kinetically limited. In addition, dispersion causes spreading of microorganisms and can affect their concentrations to a high degree (Schijven and Simunek 2002). Unreacting dissolved tracers are often assumed to move through all possible pathways, and thus represent the entire velocity spectrum. But in the case of particles, including bio-particles, those that break through are commonly observed to travel, on average, slightly more rapidly than conservative ones, indicating that the smaller diameter, slower pathways are not available to them.

5.2 Model approaches

Two different approaches often used in describing attachment in reactive solute movement in groundwaters are:

- equilibrium attachment
- kinetic attachment.

The general transport equation for attaching and decaying solutes is:

$$R \cdot \frac{\partial C}{\partial t} = D \cdot \frac{\partial^2 C}{\partial z^2} - v \cdot \frac{\partial C}{\partial z} - \lambda \cdot R \cdot C \qquad (1)$$

where:

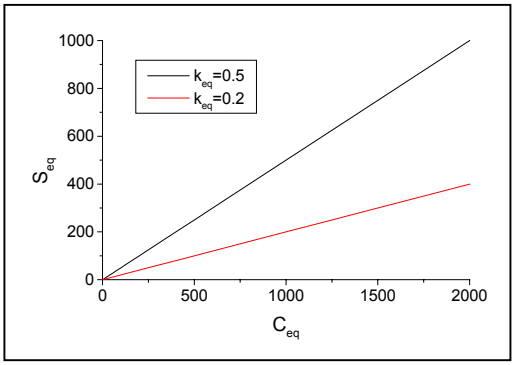
C = tracer concentration
t = time (days)
z = distance (m)
R = retardation coefficient (–)
D = dispersion coefficient [(m^2/day)
v = average groundwater velocity (m/day)
λ = degradation constant (day^{-1}]).

5.3 Equilibrium attachment

Equilibrium attachment has been assumed in several column and field experiments (Harter *et al.* 2000, Powelson and Mills 2001, Flynn 2003, Powelson *et al.* 1993). If used for dynamic processes such as phage transport through sandstone, it is assumed that attachment equilibrium is reached instantly – no matter how large the initial concentration. Batch studies, however, indicate that it takes a certain time to reach the steady state.

Three different types of isotherms are commonly used for attachment/detachment experiments.

5.3.1 Linear isotherm



$$S_{eq} = k_{eq} \cdot C_{eq} \qquad (2)$$

where:

S_{eq} = adsorbed tracer mass
C_{eq} = dissolved concentration in equilibrium
k_{eq} = equilibrium constant respectively.

Based on Equation 2, the parameters R and λ can be expressed as follows:

$$R = 1 + \frac{\rho_B}{n} \cdot k_{eq} \qquad (3)$$

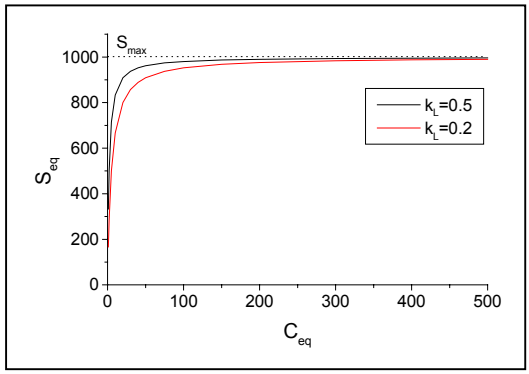
$$\lambda = \frac{\mu_l + \frac{\mu_s}{n} \cdot \rho_B \cdot k_{eq}}{R} \qquad (4)$$

where:

ρ_B = bulk dry density of the rock matrix
n = porosity
μ_l = inactivation rate in the solid phase
μ_s = inactivation rate on the solid phase.

Other non-linear attachment isotherms can be found in the literature, including a maximum sorption capacity and/or concentration dependency of attachment.

5.3.2 Langmuir Isotherm



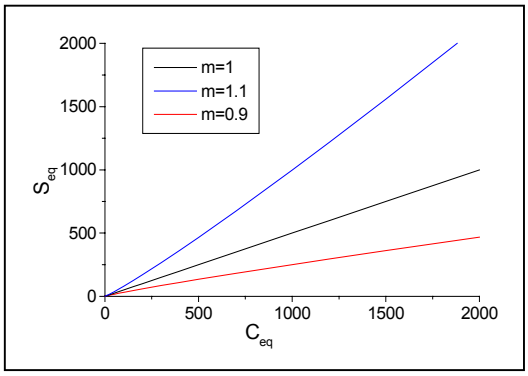
$$S_{eq} = \frac{S_{max} \cdot k_L \cdot C_{eq}}{1 + k_L \cdot C_{eq}} \qquad (5)$$

where:

S_{max} = attachment capacity of the adsorbent.

5.3.3 Freundlich isotherm

$$S_{eq} = k_{eq} \cdot C_{eq}^{\ m} \qquad (6)$$



Moore *et al.* (1981) applied the Langmuir equation to interpret attachment of poliovirus 2 to Ottawa sand where S_{max} appeared to be 2.5×10^{12} viruses per kg of sand. At lower surface loadings, attachment was successfully described by the Freundlich isotherm. Vilker and Burge (1980) showed that k_{eq} varies between 2 and 6.4×10^5 litres/kg. In some of these examples, S_{max} was shown to be very large (10^{14} to 10^{15} sites per kg of soil). It was concluded that virus attachment is saturation limited, i.e. the number of attachment sites is finite. Other examples of the application of the Freundlich equation to attachment experiments Gerba and Lance (1978).

5.4 Kinetic attachment

The second approach adds a one-site kinetic term to account for attachment and detachment during transport (Schijven *et al.* 2000).

$$\frac{\partial C}{\partial t} = D \cdot \frac{\partial^2 C}{\partial z^2} - v \cdot \frac{\partial C}{\partial z} - k_{att} \cdot C - \mu_l \cdot C + k_{\det} \cdot \frac{\rho_B}{n} \cdot S \qquad (7)$$

$$\frac{\rho_B}{n} \frac{\partial S}{\partial t} = k_{att} \cdot C - k_{\det} \cdot \frac{\rho_B}{n} \cdot S - \mu_s \cdot \frac{\rho_B}{n} \cdot S \qquad (8)$$

with:

$$D = \alpha_L \cdot v + D_0 \qquad (9)$$

where:

C = concentration of free microorganisms
S = concentration of attached microorganisms
α_L = longitudinal dispersivity (m)
n = porosity (–)
k_{att} = attachment rate coefficient (day^{-1}])
k_{det} = detachment rate coefficient (day^{-1}])
μ_1 = inactivation rate for free microorganisms (day^{-1}])
μ_S = inactivation rate for attached microorganisms (day^{-1}]
D_0 = diffusion coefficient (m^2/day)
ρ_B = dry bulk density (kg/m^3).

Equations 7–9 can be solved analytically with a set of numerical integrations. A numerical code was developed to solve the problem. The explicit code was tested using the analytical solutions for simplified cases.

5.5 Model including attachment kinetics

Figures 5.2–5.5 show the effect on the model results of changes of model parameter values. They indicate:

- what the model is able to represent;
- how standard attachment and detachment models affect the concentrations in solution and on the rock surfaces;
- what the applicability and limits of such models might be.

First consider the concentrations of a contaminant in solution at the column outflow for different detachment rates and constant but high attachment rates (500 h^{-1}).

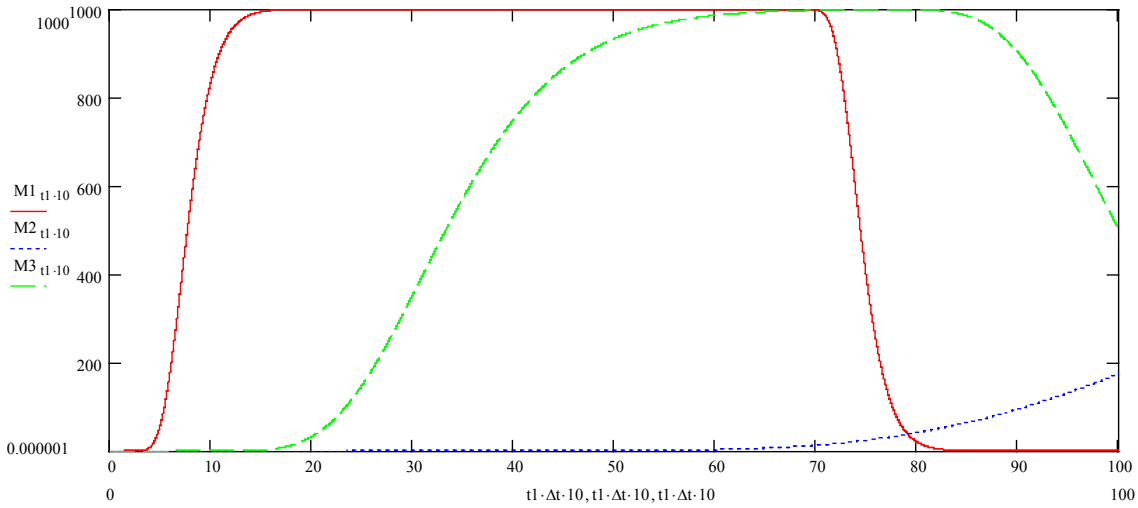


Figure 5.2 Model results for dissolved tracer concentrations at the base of a column for different detachment parameters and an attachment rate of 500 h^{-1}
The x-axis shows time (hours) and the y-axis shows concentration. The red curve shows the results for a detachment rate of 100 h^{-1}, the green curve for a detachment rate of 20 h^{-1} and the blue curve for a detachment rate of 5 h^{-1}.

It can be seen that decreasing the detachment rate leads to an increasing retardation of the observed tracer curve. Changing the detachment rate also changes the shapes of the breakthrough curves significantly.

The variation with time of dissolved and attached concentrations in the first cell of the column are shown in Figures 5.3 and 5.4.

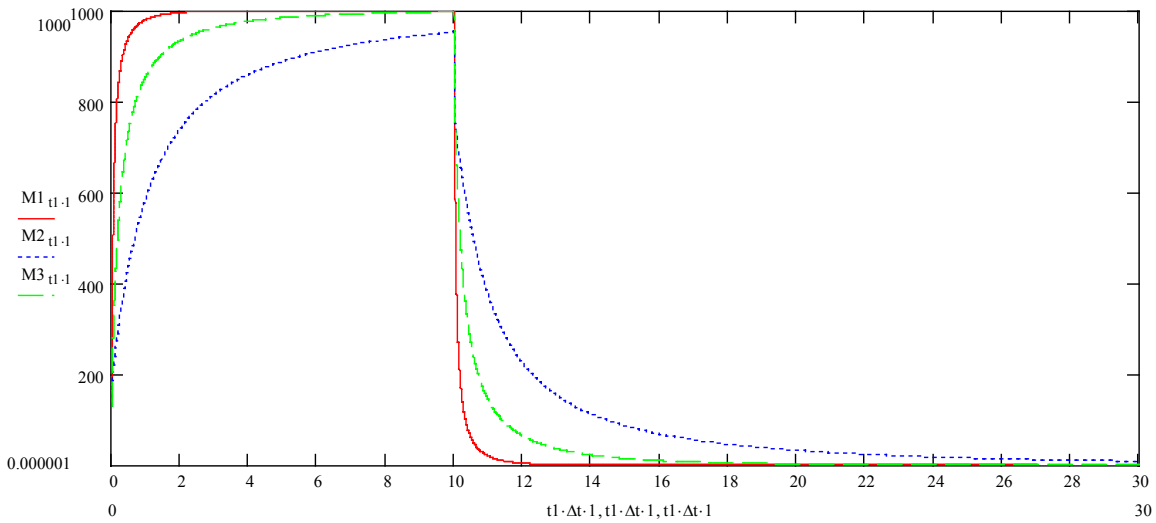


Figure 5.3 Model results for dissolved tracer concentrations in the first column cell for different detachment parameters and an attachment rate of 500 h^{-1}

The x-axis shows time (hours) and y-axis shows concentration. The red curve shows the results for a detachment rate of 100 h^{-1}, the green curve for a detachment rate of 20 h^{-1} and the blue curve for a detachment rate of 5 h^{-1}.

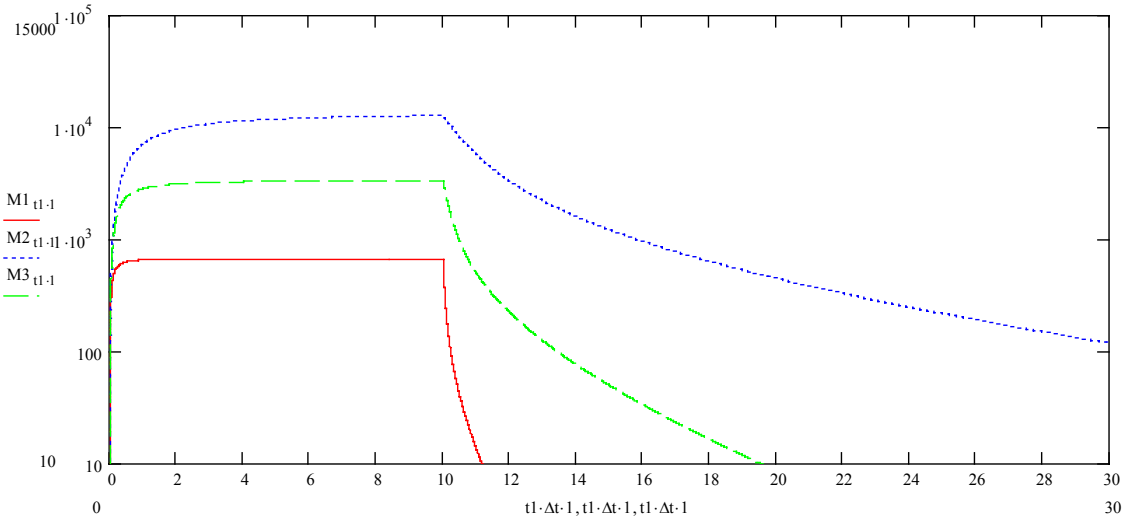


Figure 5.4 Model results for adsorbed tracer concentrations in the first column cell for different detachment parameters and an attachment rate of 500 h^{-1}

The x-axis shows time (hours) and y-axis shows concentration. The red curve shows results for a detachment rate of 100 h^{-1}, the green curve for a detachment rate of 20 h^{-1} and the blue curve for a detachment rate of 5 h^{-1}.

The concentrations in both liquid and adsorbed state will reach equilibrium when the rate of detachment of microbes from the rock equals the rate of attachment of microbes on the rock matrix. For linear attachment, this equilibrium can be calculated as:

$$K_{eq} = \frac{S}{C} = \frac{k_{att}}{k_{det}} \cdot \frac{n}{\rho_s} \qquad (10)$$

The time to reach equilibrium is linearly proportional to the ratio S/C (Figure 5.5), at least in the case examined here. The batch experiment (Figure 5.6) with a k_{eq} of 70 has a half life ($T_{1/2}$) of about 10 minutes, whereas Figure 5.5 suggests about 40 minutes (shown by the arrows).

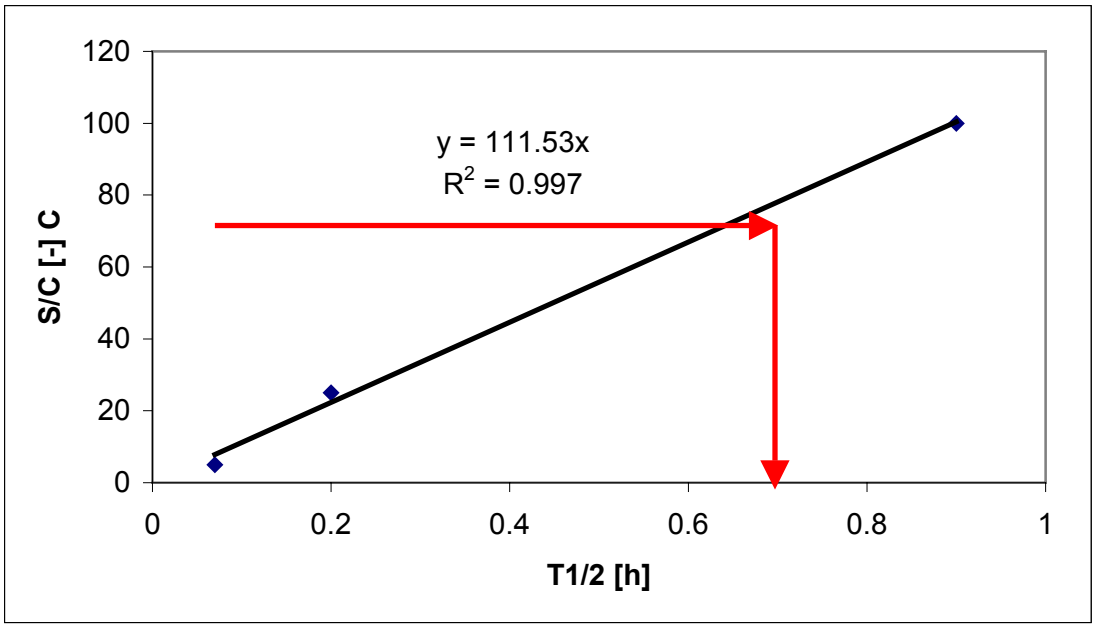


Figure 5.5 **Results of theoretical time required (expressed as half life) until equilibrium is reached assuming different equilibrium constants (S/C) of the kinetic model**

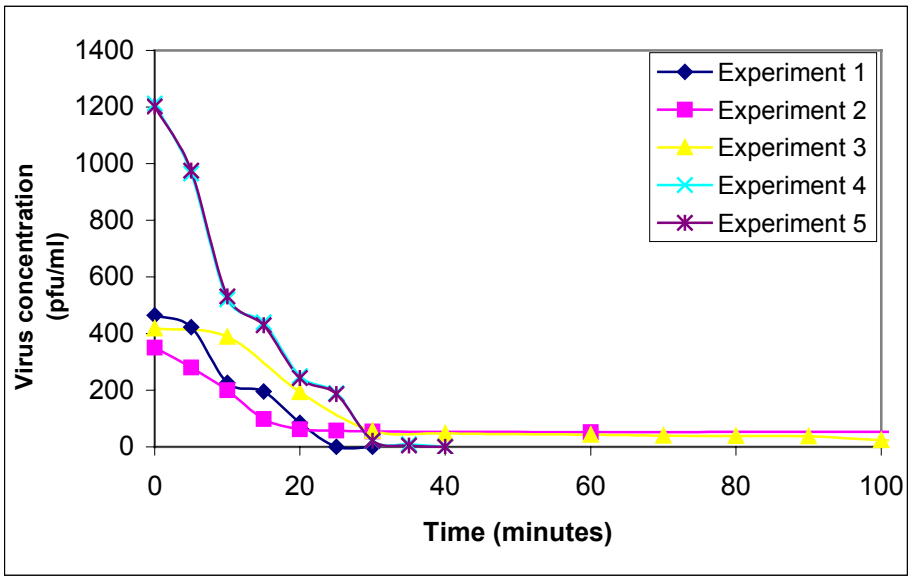


Figure 5.6 Results of the batch experiment performed for MS2 attachment
Experiment 1: 1 g rock and 20 ml water
Experiment 2: 0.1 g rock and 20 ml water
Experiment 3: 0.1 g rock and 20 ml water
Experiments 4 and 5: 1 g rock and 20 ml water.

5.6 Field experiments

The same approach can be used to estimate the tracer breakthrough in field experiments:

$$\frac{\partial C}{\partial t} = \frac{A \cdot \alpha_l}{r} \cdot \frac{\partial^2 C}{\partial r^2} + \frac{A}{r} \cdot \frac{\partial C}{\partial r} - k_{att} \cdot C - \mu_l \cdot C + k_{\text{det}} \cdot \frac{\rho_B}{n} \cdot S \qquad (13)$$

with

$$A = \frac{Q}{2 \cdot \pi \cdot b \cdot n} \qquad (14)$$

where:

r = radial distance from the well (m)
Q = pumping rate (m^3/dAY)
b = aquifer thickness (m).

5.6.1 Real groundwater systems

It was expected that introduced tracers would show multiple arrival peaks in the output well (Streetly *et al.* 2002). This is due to the heterogeneities in the sandstone, including fractures.

The simplest technique to represent multiple breakthroughs would be to simulate a number of fully independent layers without exchange (dispersion) between the layers. Since the approach ignores exchange between the layers, it simplifies the real situation. However, it does give an idea of transport parameters and contaminant behaviour in the different experiments (Streetly *et al.* 2002).

6 Results and discussion

6.1 Introduction

This project aimed to improve understanding of the potential factors that enable migration of viruses through sandstone aquifers (migration means transport over a certain distance in detectable amounts). Models have been used to quantitatively assess transport distances and times, and the importance of different parameters that can be used to predict virus migration in groundwater.

This chapter outlines the results obtained with nine sandstone cores and six-field tracer tests (four packered homogenous intervals, one packered fractured interval and an open borehole test) using four different bacteriophage (MS2, PRD1, ϕX147 and H40/1).

Previous chapters outline the following experimental investigations:

- survival studies
  - laboratory experiments
  - field experiments
- transport through sandstone columns in the laboratory
- transport through the rock mass in the field.

The models described in Chapter 5 were used to interpret the experimental data and to explore the effects and relative importance of the processes identified. The results of these investigations are presented and discussed below.

6.2 Field and laboratory survival studies

Field investigations of phage survival, supported by laboratory studies at 12^{o}C, were undertaken as described in Section 4.1. All four phage displayed a shorter survival in the field than in the laboratory (Table 6.1; Figures 6.1–6.4). The results suggest relatively long-term survival for bacteriophage in the aquifer. They also imply that enteric viruses may be similarly relatively long-lived.

Table 6.1 Reduction in numbers in field and laboratory survival studies

Phage	Field	Refrigerator	Figure
MS2	~2.7 log (501-fold)	~2.7 log (501-fold)	6.1
PRD1	1.6 log (39.8-fold)	0.65 log (4.47-fold)	6.2
H40/1	1.8 log (63.1-fold)	0.73 log (5.37-fold)	6.3
ϕX174	0.8 log (6.31-fold) after 5 months	0.48 log (3.02-fold)	6.4

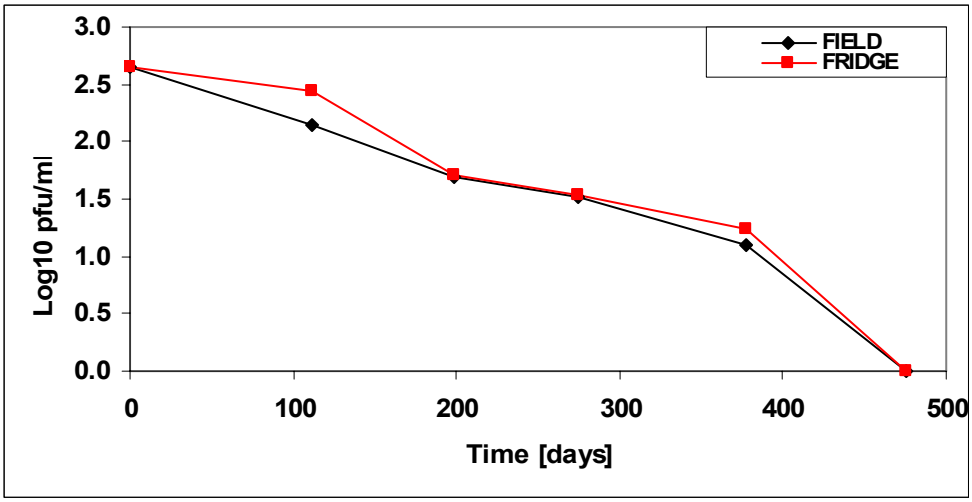


Figure 6.1 MS2 field and refrigerator survival studies

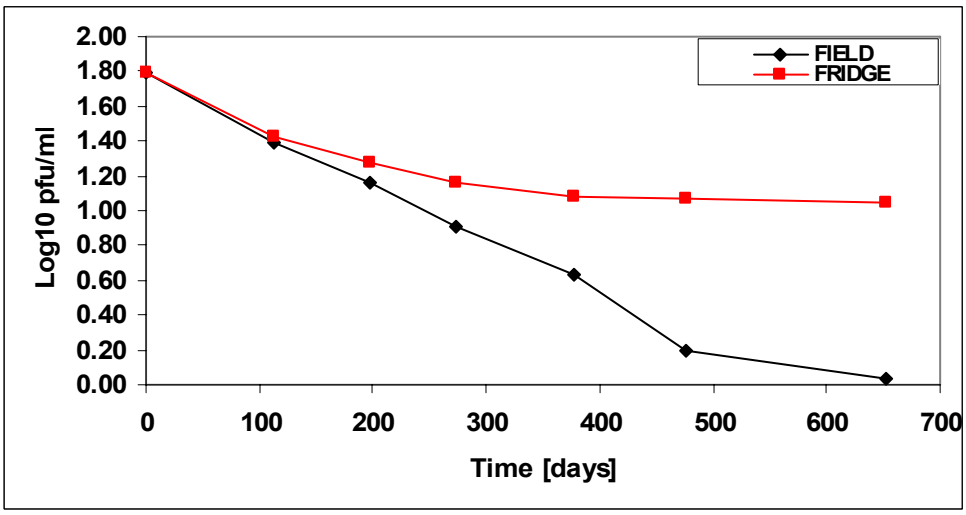


Figure 6.2 PRD1 field and refrigerator survival studies

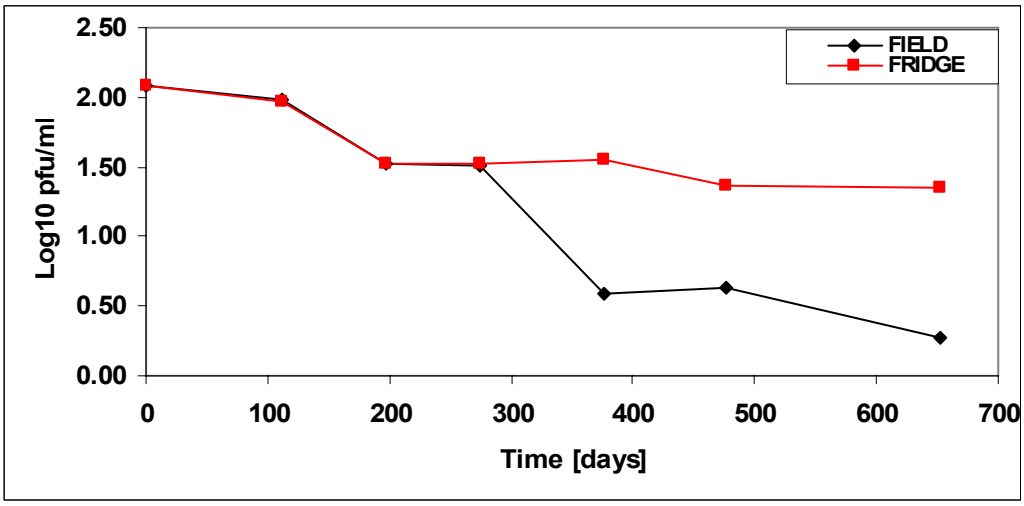


Figure 6.3 H40/1 field and refrigerator survival studies

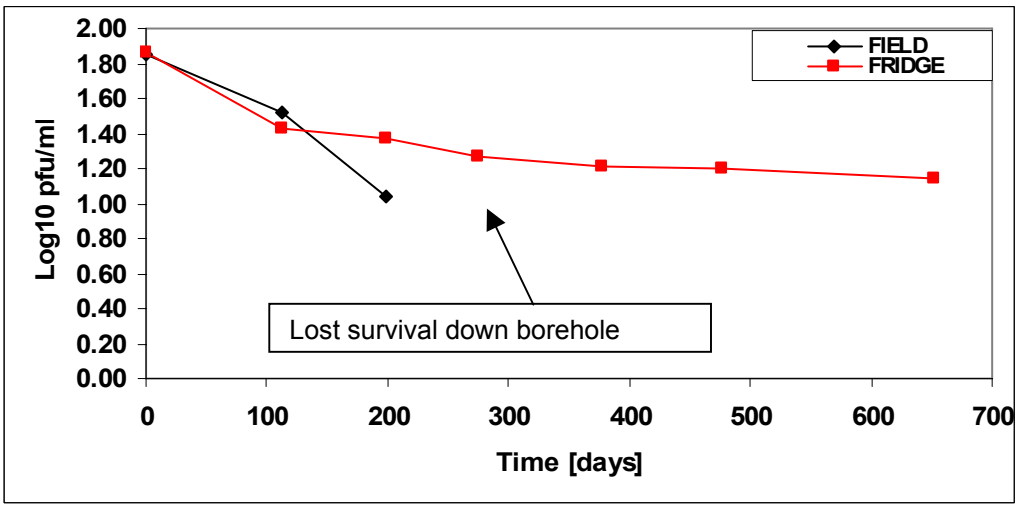


Figure 6.4 ΦX174 field and refrigerator survival studies

Results for all four phage indicate slightly higher inactivation rates in the field compared with laboratory studies. Over one year, MS2 showed a 2.7 log (501-fold) reduction, PRD1 a 1.6 log (39.8-fold) reduction, H40/1 a 1.8 log (63.1-fold) reduction and ϕX174 a 0.8 log (6.31-fold) reduction in numbers.

These results highlight the likelihood of long-term survival for bacteriophage in these aquifer systems and suggest similar persistence of enteric viruses in groundwater. The results also offer an explanation of why viable enteric viruses have been detected at depths previously thought to be free from viral contamination due to inactivation or attachment to the matrix (Table 6.2).

Table 6.2 Bacteriophage inactivation rates – decay constants and half lives for first order decay model

Phage	Type of inactivation study	Inactivation rate (day^{-1})	Half-life (days)	Correlation coefficient (R^2)
ϕX174	Field	0.0041±0.0073	200 (6 months)	0.97
	Laboratory	0.0009±0.0033	500 (16 months)	0.73
MS2	Field	0.0051±0.0024	300 (10 months)	0.95
	Laboratory	0.0052±0.0056	300 (10 months)	0.92
PRD1	Field	0.0028±0.0012	500 (16 months)	0.97
	Laboratory	0.0011±0.0030	280 (9 months)	0.75
H40/1	Field	0.0031±0.0058	320 (10.6 months)	0.92
	Laboratory	0.0011±0.0035	500 (16 months)	0.77

6.3 Sandstone column experiments

6.3.1 Experimental procedure

Combined phage and fluorescein solutions were passed through sandstone columns as described in Section 4.2. After conditioning the cores for around 20 pore volumes, 10 ml of a phage and fluorescein solution (Table 6.3) was injected followed by untreated, but thermally equilibrated, groundwater taken from the Birmingham University well site. A flow rate of 15 ml/hour was used. Between each experiment on the same core, the core was flushed with groundwater until phage and fluorescein concentrations fell below the detection limit (1 pfu/ml and 0 parts per million (ppm) respectively).

The fluorescein breakthrough curves were modelled using the model described in Chapter 5 assuming no chemical interactions with the rock (Streetly *et al*. 2002). This yielded the hydrodynamic property values used as a basis for interpreting the phage breakthroughs. The phage breakthroughs were modelled again by manual fitting using the code from Chapter 5, assuming there to be two types of attachment site. All rate and equilibrium partitioning coefficients were taken as calibration variables. Inactivation was also included in the model, with the rate for the suspended phase phage taken from the survival studies and the rate for the attached phage treated as a calibration variable.

Sections 6.3.2–6.3.6 present the results of the experiments on each of the four cores. Table 6.4 summarises the breakthrough characteristics of each experiment and Table 6.5 lists the model parameter values used to obtain the model fits shown in the figures in the following sections.

Table 6.3 Phage and fluorescein tracer injections for column experiments

Core test	Core	Fluorescein concentration (ppm)	Phage injection concentrations (pfu/ml)
Test 1: PRD1	1	1	3.57×10^4
Test 2: PRD1	1	1	1.37×10^9
Test 3: PRD1	1	1	4.10×10^4
Test 1: H40/1	2	1	3.03×10^8
Test 1: ϕX174	3	1	1.26×10^4
Test 2: ϕX174	3	1	1.54×10^6
Test 3: ϕX174	3	1	4.10×10^4
Test 1: MS2	4	1	1.49×10^4
Test 2: MS2	4	1	2.77×10^8

Table 6.4 Summary of core tracer experiments and respective recoveries

Phage	Experiment number	Core	Fluorescein concentration (ppm)	Fluorescein arrival (minutes)	Fluorescein peak (minutes)	Injected phage concentration (pfu/ml)	Phage arrival (minutes)	Phage peak (minutes)	% phage recovered
PRD1	1	1	1	20	60	$3.57x10^4$	30	90	22.9
PRD1	2	1	1	20	90	1.37×10^9	30	95	10.3
PRD1	3	1	1	25	60	4.10×10^4	30	120	2.9
H40/1	1	2	1	15	60	3.03×10^8	30	150	6.47
ϕX174	1	3	1	10	45	1.26×10^4	55	105	0.99
ϕX174	2	3	1	15	50	1.54×10^6	43	105	1.8
ϕX174	3	3	1	10	40	4.10×10^4	15	75	5.1
MS2	1	4	1	40	90	1.49×10^4	70	180	3.1
MS2	2	4	1	25	70	2.77×10^8	50	130	6.4

Table 6.5 Parameter values used in modelling column experiment data

Exp. No.	C_{inj}(ml^{-1})	v (cm/h)	Q (ml/h)	D (α) ($cm^2 \cdot h^{-1}$) (cm)	$K1_{att}$ (h^{-1})	$K1_{det}$ (h^{-1})	$K2_{att}$ (h^{-1})	$K2_{det}$ (h^{-1})	K_{eq}	λ_{exp} (h^{-1})	λ_s (h^{-1})
PRD1 (Core 1: length = 4.9 cm)											
1	3.57×10^4	6.6	15.9	3.4 (0.52)	50	50	2.2	0.0002	11,000	0.00127	0.03
2	1.37×10^9	4.9	11.8	1.7 (0.35)	50	45	2.7	0.0005	5,400	0.00127	0.03
3	4.10×10^4	7.9	19.2	1.5 (0.19)	50	33	6.3	0.005	1,260	0.00127	0.03
H40/1 (Core 2: length = 4.0 cm)											
1	3.03×10^8	4.6	11.7	4.2 (0.91)	50	20	3.4	0.0005	6,800	0.001	0.06
ϕX174 (Core 3: length = 4.0 cm)											
1	1.26×10^4	8.6	19.4	5.5 (0.64)	50	7	11	0.002	5,500	0.001	0.03
2	1.54×10^6	7.8	17.6	2.0 (0.26)	50	13	6.8	0.001	6,800	0.001	0.03
3	4.10×10^4	11	24.8	12 (1.09)	50	25	12.8	0.0015	8,533	0.001	0.03
MS2 (Core 4: length = 6.4 cm)											
1	1.49×0^4	6.7	14.8	0.7 (0.10)	50	50	2.3	0.0006	3,833	0.0017	0.06
2	2.77×10^8	6.8	15.0	1.7 (0.25)	50	30	5.8	0.006	967	0.0017	0.06

C_{inj} = injected concentration for phage
v = flow velocity in core
Q = volumetric flow rate through core
D = dispersion coefficient
α = dispersivity
$K1_{att}$ = phage attachment rate constant for fast sites
$K1_{det}$ = phage detachment rate constant for fast sites
$K2_{att}$ = phage attachment rate constant for slow sites
$K2_{det}$ = phage detachment rate constant for slow sites
K_{eq} = equilibrium partitioning coefficient for phages (isotherm)
λ_{exp} = inactivation rate constant for suspended phage (from survival studies)
λ_s = inactivation rate for attached phage (calibration variable)

6.3.2 Core 1: PRD1

Three core experiments were undertaken on Core 1 by injecting different concentrations of PRD1 in conjunction with 1 ppm fluorescein (Table 6.3). The concentration of the phage injections were 3.57×10^4, 1.37×10^9 and 4.10×10^4 pfu/ml respectively.

The breakthrough curves are shown in Figure 6.5a–c. The lower right plot in each case shows how the phage concentrations changed over the full flushing phase, extending well beyond the times when fluorescein concentrations were undetectable. The third PRD1 injection experiment (Figure 6.5c) encountered a problem with air entering the system and the phage concentrations are less repeatable. Recoveries for the three experiments were 22.9, 10.3 and 2.9 per cent respectively of the injected phage numbers, indicating effectively irreversible attachment of the phage to the sandstone under the conditions of the experiments. The first and third experiments used approximately the same concentrations of phage, but the recovery in the third experiment was only 10 per cent of that of the first. This suggests complexity and history effects not included in the current model of the system; further investigation is needed to identify the processes concerned.

Due to the high attachment capacity, the maximum recovery PRD1 concentrations were 1 log10 (10-fold), 1.5 log10 (31.6-fold) and 2 log10 (100-fold) lower than the injected concentration with a total recovery of 22.9, 10.3 and 2.9 per cent respectively of the injected phage (Figure 6.5a–c).

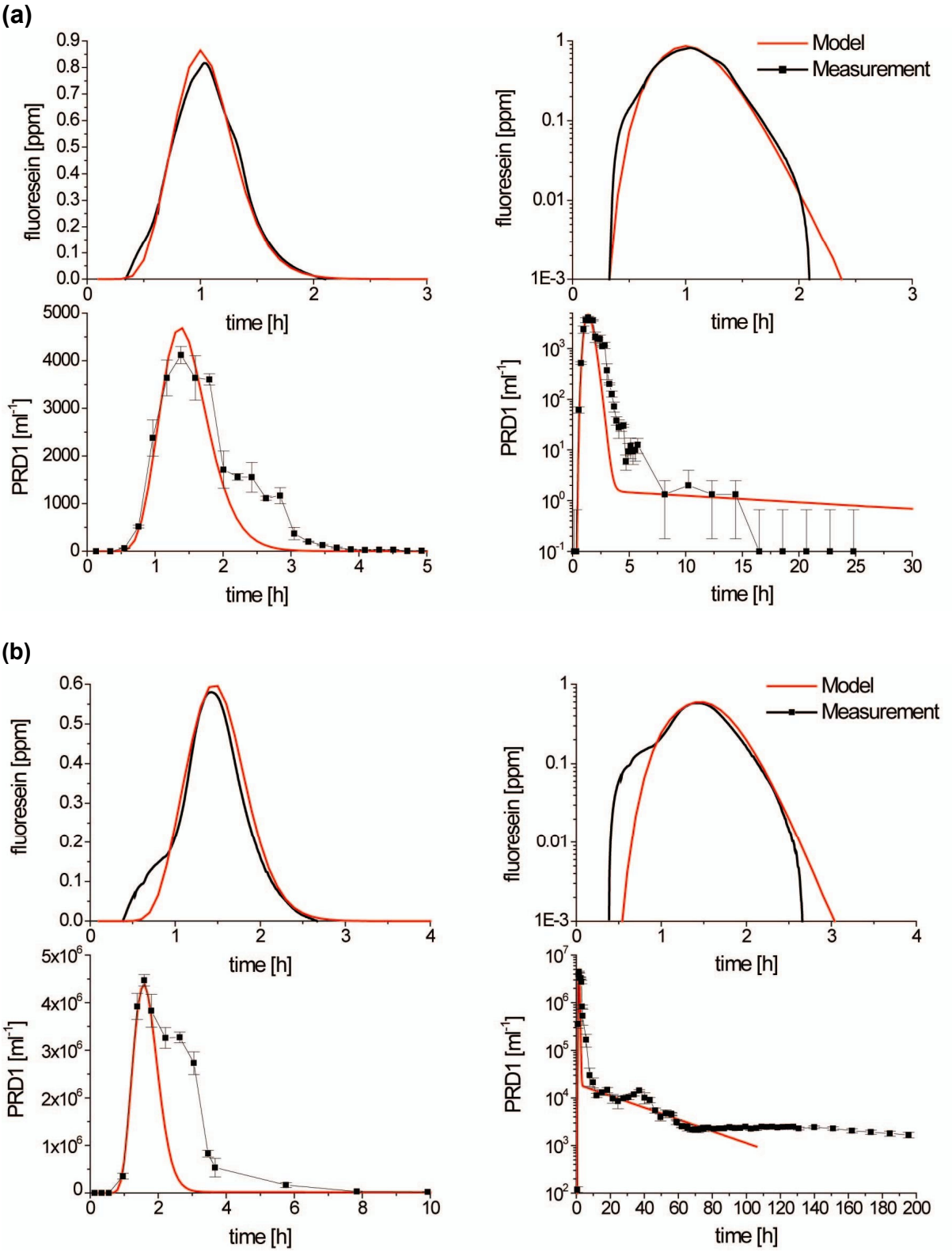


Figure 6.5 Measured and modelled PRD1 and fluorescein breakthrough curves for experiments on Core 1
(a) PRD1 (3.57×10^4 pfu/ml) with 1 ppm fluorescein
(b) PRD1 (1.37×10^9 pfu/ml) with 1 ppm fluorescein

(c)

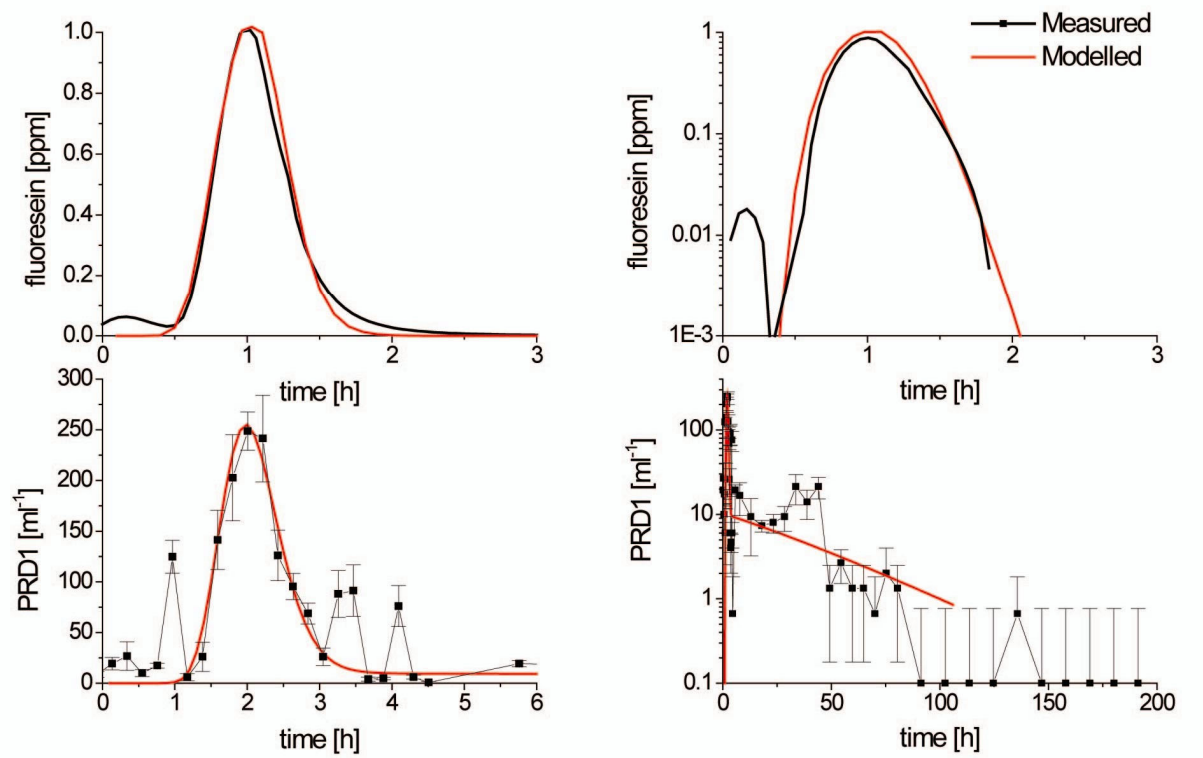


Figure 6.5 Measured and modelled PRD1 and fluorescein breakthrough curves for experiments on Core 1
(b) PRD1 (4.10×10^4 pfu/ml) with 1 ppm fluorescein

Figure 6.5a–c indicates that, in general, the fluorescein breakthrough curves could be successfully reproduced by the model. Early minor shoulders on the fluorescein curves suggest that, despite the flushing of cores between experiments, some fluorescein remained in the system. This may indicate a dual region process is occurring (Bashar and Tellam 2006) but, if so, this process is barely if at all seen in the flush-out phase of the curves where tailing might be expected. Dispersivities are approximately as expected for sandstone samples (Bashar 1997).

The PRD1 curves, however, proved to be more difficult to model. The rising limb of the curves can be reproduced reasonably well, but the falling limbs and the tails of the curves could only be reproduced poorly. The first two experiments show a distinct shoulder on the falling limb of the breakthrough curve that could not be matched. Slightly better success was achieved in matching the tails of the curves, but only by using attached phase inactivation rates more than an order of magnitude greater than the aqueous phase rates measured in the PRD1 survival experiments. A relatively rapid fall in concentration occurs up to 80–100 hours and this is followed by a much slower decrease.

Two possible explanations for these observations are as follows:

- High inactivation rates of PRD1 in the first few tens of hours, after which they drop to rates similar to those which can be explained using the rates observed in the survival experiments.

- Flow behaviour in the core does not follow a simple one region breakthrough but is affected by diffusive exchange with a less mobile zone. This exchange is manifest as a tailing in the phage breakthroughs. Although tailing is much less well developed in the fluorescein data (the fluorescein detection limits are too high), the presence of the small

shoulders on the rising limbs of the fluorescein breakthrough curves may result from the same process.

Although the modelling does not fully represent all the processes and further work is necessary, it appears that:

- the dispersive properties of the fluorescein and phage are similar;
- the phage are delayed relative to the fluorescein (though less so in experiment 2);
- phage attachment is a dominant process and cannot be described by a single site model;
- inactivation rates for the attached phase may be greater than for the aqueous phase.

In addition, the rather different breakthrough curves for the three experiments – and the consequent need to change model parameter values significantly from one experiment to the next – suggests that developments may need to take into account previous attachment.

6.3.3 Core 2: H40/1

The column experiment undertaken on Core 2 used H40/1 at a concentration of 3.03×10^8 pfu/ml and fluorescein at a concentration of 1 ppm. The experimental conditions were otherwise identical to those used for Core 1 (Section 6.3.2). Figure 6.6 shows the breakthrough curves and model fits: Table 6.4 lists the model parameter values used in the fitting.

Due to moderate attachment capacity, the maximum H40/1 breakthrough was 0.5 log10 (3.16-fold) lower than the injection concentration with a total phage recovery of 6.2 per cent of the injected phage. As time was limited for these tests, it was only possible to complete one core tracer test using H40/1 (Figure 6.6).

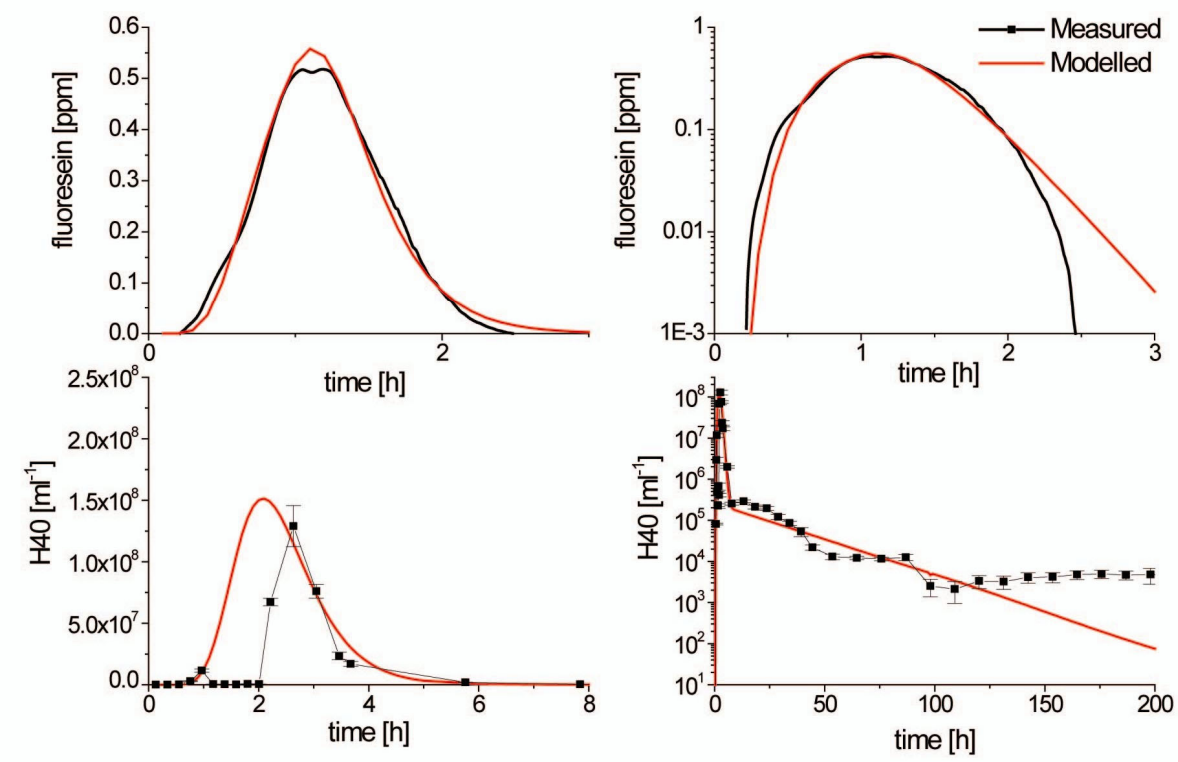


Figure 6.6 Measured and modelled H40/1 and fluorescein breakthrough curves for experiments on Core 2
H40/1 (3.03×10^8 pfu/ml) with 1 ppm fluorescein

Fluorescein breakthrough was reproduced reasonably well by the model though the falling limb drops slightly faster than the model predicts (Figure 6.6). The H40/1 breakthrough curve appears to have a double peak – the first after one hour and the second and much better substantiated peak three hours after injection. This double peak cannot be reproduced by the model and Figure 6.6 simply shows an example single peak breakthrough.

Although little can be deduced from the data without further confirmation of the double peak, it is interesting that the apparent change in rate of concentration decrease at ~100 hours seen in Core 1 in the PRD1 experiments is also seen here.

6.3.4 Core 3: ϕX174

Three column experiments were undertaken using Core 3 and ϕX174. These used concentrations of 1.26×10^4, 1.54×10^6 and 4.10×10^4 pfu/ml respectively (Figure 6.7a–c). Due to the high attachment capacity, the maximum ϕX174 breakthrough was 2 log10 (100-fold) lower for all experiments than the injection concentration; total corresponding recoveries were 1.0, 1.8 and 5.1 per cent of the injected phage numbers (Figure 6.7a–c).

(a)

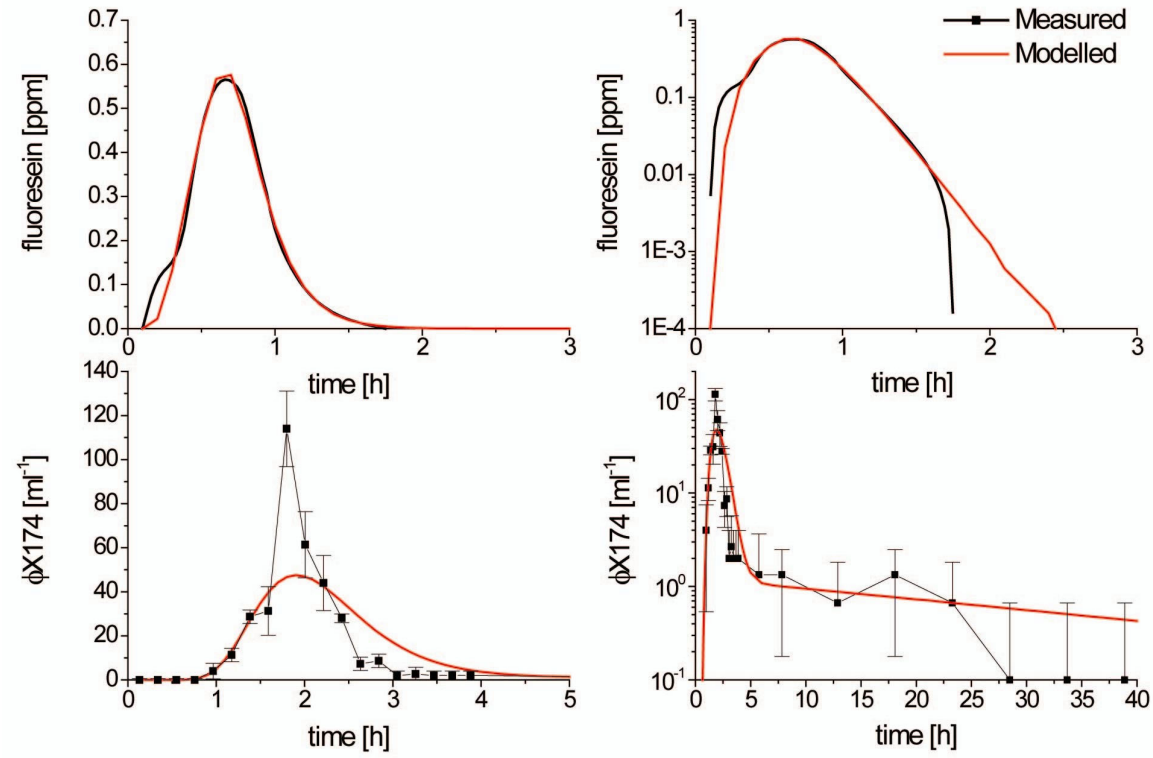


Figure 6.7 Modelled and measured ϕX174 and fluorescein breakthrough curves for experiments on Core 3
(a) ΦX174 (1.26×10^4 pfu/ml) with 1 ppm fluorescein

(b)

(c)

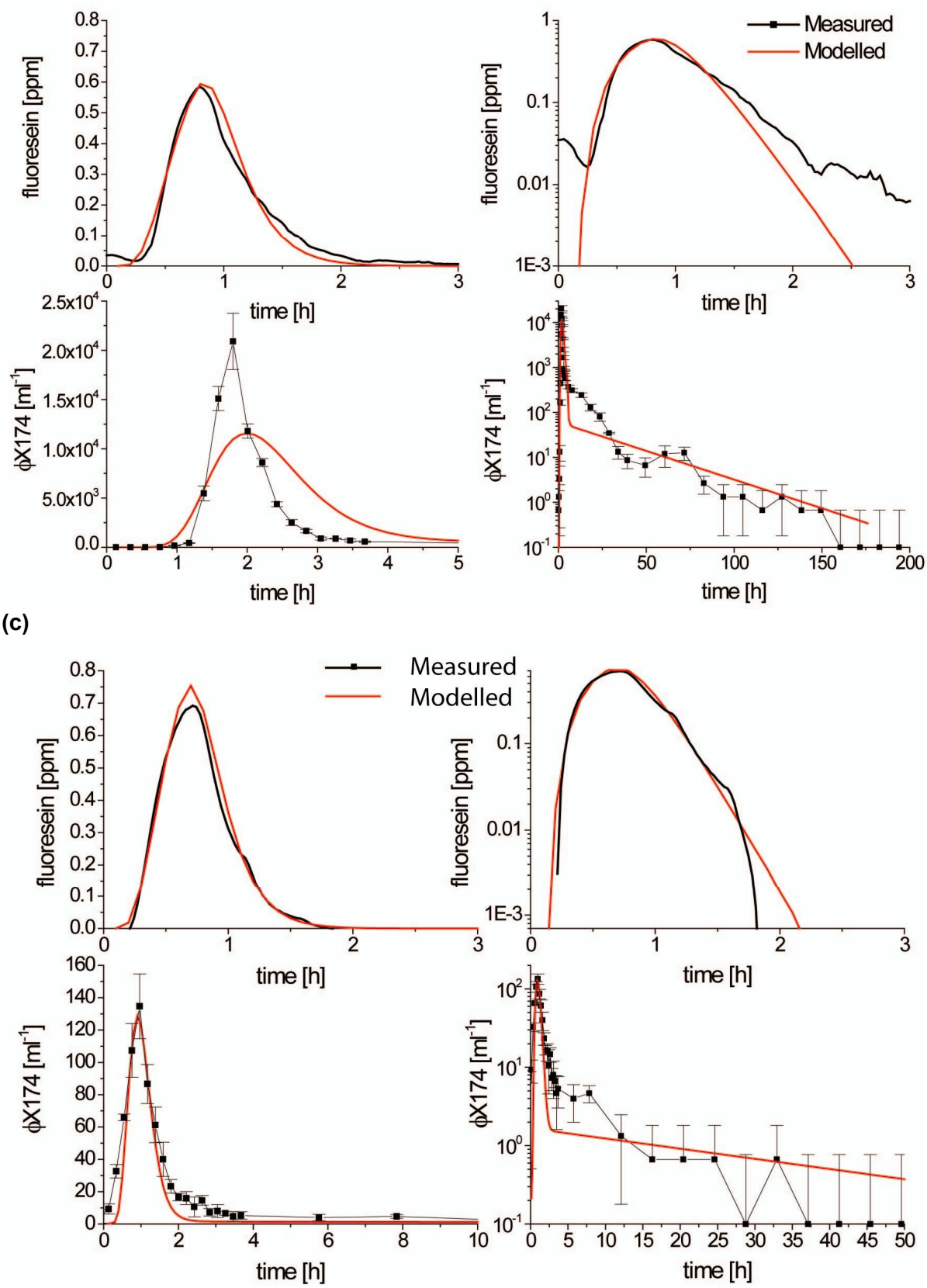


Figure 6.7 Modelled and measured ϕX174 and fluorescein breakthrough curves for experiments on Core 3
(b) ΦX174 (1.54×10^6 pfu/ml) with 1 ppm fluorescein
(c) ΦX174 (4.10×10^4 pfu/ml) with 1 ppm fluorescein

The fluorescein breakthrough curves for the first and third experiments were satisfactorily matched using the model (Figure 6.7). However, the model was less successful at reproducing the breakthrough curves for the second experiment due to the presence of tailing. The reason for this is uncertain, but it is possible that some fluorescein remained unflushed in the core following the first experiment.

Although the phage breakthrough curves cannot be reproduced entirely satisfactorily by the model (Figure 6.7), all phage breakthroughs are delayed relative to the fluorescein, considerable un-reversed attachment occurs and the late slow concentration decline is again observed.

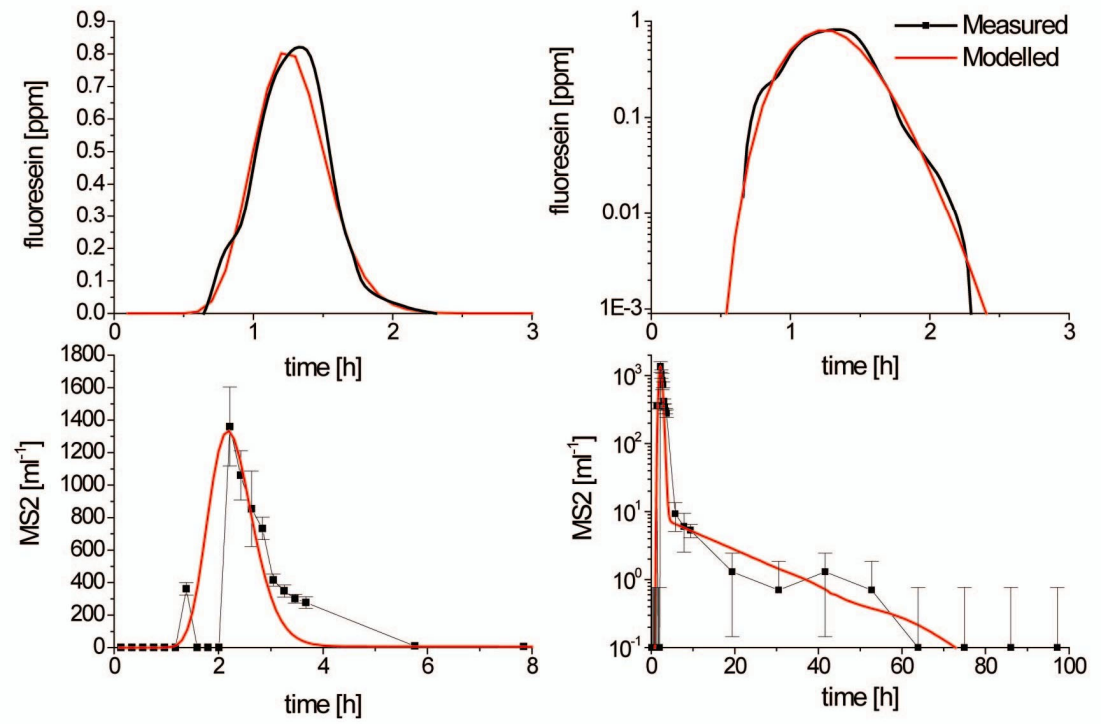
6.3.5 Core 4: MS2

Two experiments were undertaken using Core 4 and MS2 suspensions at concentrations of 1.46×10^4 and 2.77×10^8 pfu/ml respectively (Figure 6.8a and b). Due to the high attachment capacity, the maximum MS2 breakthrough was 1.5 log10 (31.6-fold) and 3.2 log10 (1584.9-fold) lower than the injection concentration with a total. Phage recoveries were 3.1 and 6.4 per cent of the injected phage numbers.

The phage recovery of the first experiment showed a double peak whereas the second one occurred as a continuous curve. Therefore, both recoveries were treated as one curve. As with the PRD1 results, it was not possible to reproduce the dropping limbs of the curve accurately. Furthermore, the tail of the curves (particularly for experiment 2) suggests higher attachment rates on the sandstone surface during the first 100 hours and a slight decrease of rates after that.

The fluorescein recovery curves were reasonably well reproduced by the model (Figure 6.8a and b). The phage breakthrough curves are possibly more complex, with a marked tailing and, a possibly, two peak morphology. The falling limbs were not reproduced by the model. Again, phage peaks were delayed relative to fluorescein peaks. The change in the rate of concentration decline seen previously at ~100 hours was again seen in experiment 2. Concentrations in experiment 1 were too low at 100 hours to determine any change in rate.

(a)



(b)

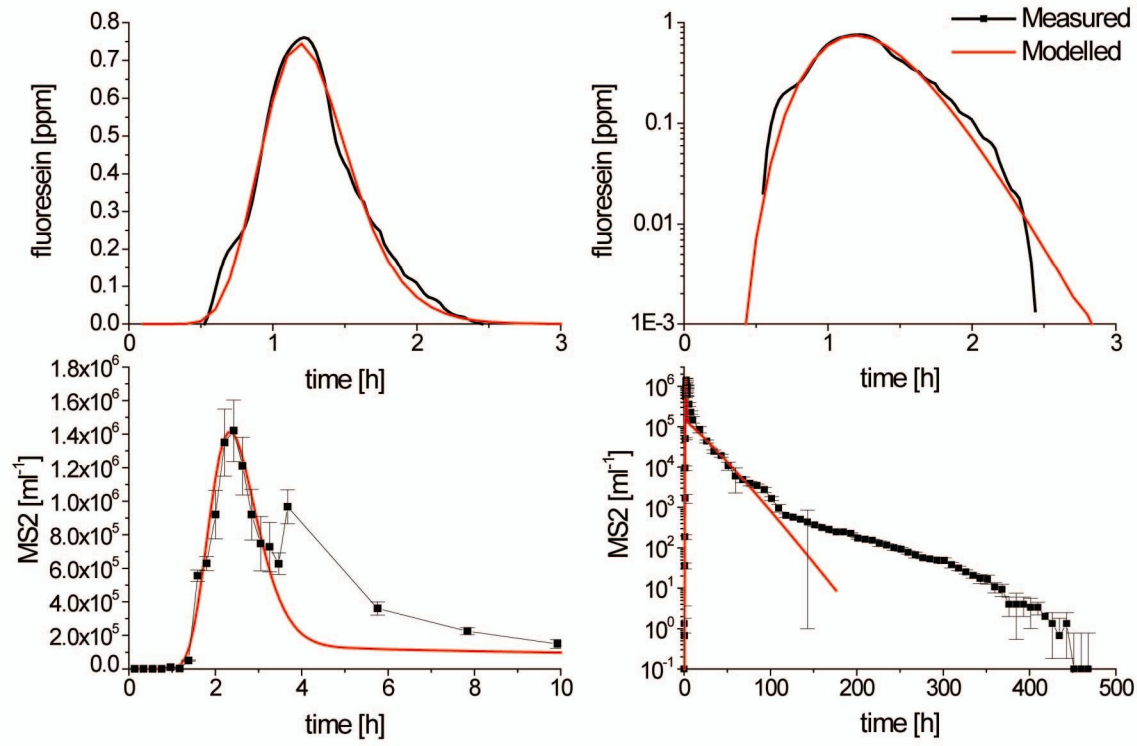


Figure 6.8 Modelled and measured MS2 and fluorescein breakthrough curves for experiments on Core 4

(a) MS2 phage (1.49×10^4 pfu/ml) with 1 ppm fluorescein

(b) MS2 phage (2.77×10^8 pfu/ml) with 1 ppm fluorescein

6.3.6 Detachment experiments

Following the ϕX174 experiments on Core 3, the core was flushed with groundwater adjusted to pH 8.5 until no phage were detected and then with groundwater adjusted to pH 9.5 until no phage were detected. Flushing rates were the same as for the initial breakthrough experiments.

Detachment experiments were carried out following injections of ϕX174 phage (1.26×10^4, 1.54×10^6 and 4.10×10^4 pfu/ml) plus 1 ppm fluorescein. The recoveries of ϕX174 for each injection were 0.99, 1.8 and 5.1 per cent respectively. The columns were run until no further phage transport was evident (i.e. all samples negative for phage). The initial elution at pH 8.5 resulted in a recovery of 8.6×10^3 pfu/ml (0.06 per cent of the injected phage); elution at pH 9.5 gave an additional recovery of 2.9×10^4 pfu/ml (0.2 per cent of the injected phage) (Figure 6.9).

Further work is necessary to investigate the relative importance of pH change, the period of the stopped flow and the change in flow rate, but it appears that:

- the attachment of the phage is reversible;
- under at least some conditions, phage can be released slowly over a considerable time period.

The low recovery rates indicate the phage are attached to the sandstone matrix rather than been filtered by the pores. It was not possible to interpret quantitatively the detachment experiments because no solution was found.

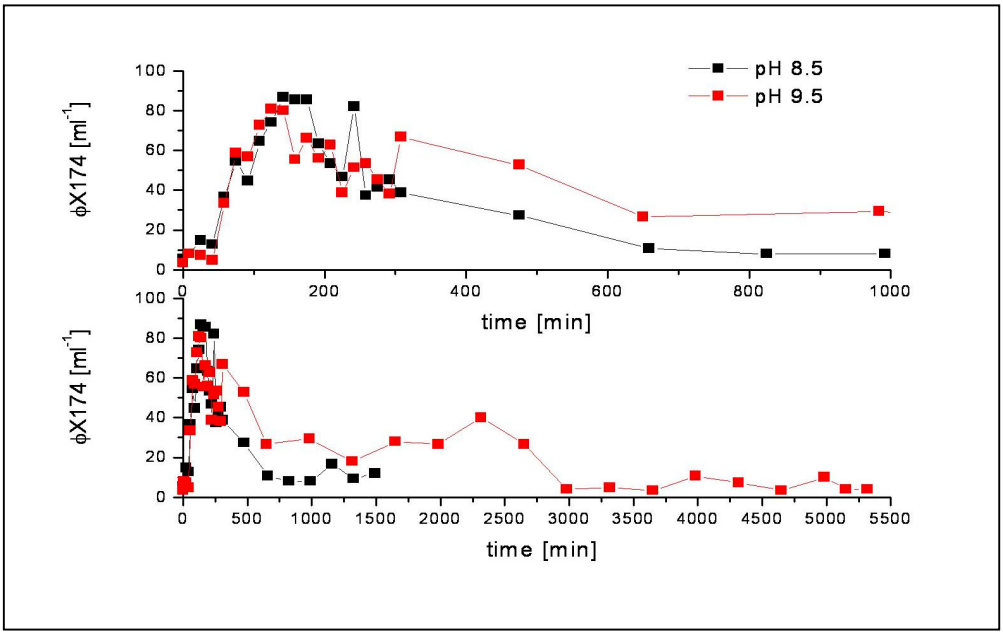


Figure 6.9 Flushing of Core 3 by groundwater at an adjusted pH of 8.5 and 9.5

6.3.7 Discussion

A semi-quantitative indication of phage/sandstone interaction can be obtained using Derjaguin–Landau–Verwey–Overbeek (DLVO) theory. Figure 6.10 shows the calculated interaction energy between a phage particle and a sandstone grain as a function of the distance between the particles based on existing information on zeta potentials of surfaces and phages.

Far away from a sandstone grain (> 50 nm), the interaction energy is close to zero (Figure 6.10) and hence there will be neither significant attraction nor repulsion. If a virus approaches the sandstone grain, it has to overcome a potential energy barrier if the particles are move closer.

- If the energy of the collision of the particles is less than this energy barrier, the phage will be repulsed.
- If the collision energy exceeds the energy barrier, the particles will approach each other stabilising at distances corresponding to the energy minimum shown on Figure 6.10.

The higher the energy barrier, the less likely the virus will attach to the sandstone grain.

Figure 6.10 suggests that MS2 and ϕX174 would be more susceptible to attachment to the sandstone than H40/1 and PRD1. Interestingly, the column experiment results do not reflect this difference and further work is necessary. Although useful in a semi-quantitative way, the theory upon which Figure 6.10 is based is far from precise – especially when dealing with particles of such contrasting characteristics.

Standard DLVO theory implies that attachment should be concentration-independent and that, once in the primary energy ‘well’, particles will not part again even if solute concentrations change. The column experiments appear to suggest that there is some detachment. This may reflect the presence of additional forces not accounted for in the

DLVO theory; various researchers have suggested forces arising from the structuring of the water surrounding the particles (Hunter 2001).

Another possibility is that a secondary energy well exists at greater distances from the particles (small differences in the assumptions used in constructing plots such as Figure 6.10 can result in the prediction of a secondary energy well). Secondary minima are less well developed than the primary minima, and are protected by lower energy barriers. Consequently particles can become attached more easily, but also become detached more easily (even through changes in the flow system). It is conceivable that the complex behaviour observed in the column experiments could ultimately be explained by invoking different types of attachment process. However, much more experimental and modelling work would be required to confirm this.

The behaviour of all four phages used is broadly similar despite their rather different physical properties. In all cases, the breakthroughs are severely attenuated over distances of a few centimetres.

Although filtering may explain some of the removal, only a few volume percentage of the sandstone has pores small enough to restrict phage movement and breakthrough curve shapes are more complex than would be expected from simple filtering. The attenuation appears to involve some degree of concentration-dependence and is to some degree reversible, even if attachment is strongly favoured over detachment. This suggests that sandstone/phage interaction models based on DLVO theory alone will not be able to describe the observed breakthroughs. The modelling results suggest that deactivation of the attached phage are more rapid than that of the phage in the aqueous phase. Overall, the laboratory experiments suggest that the phage should in general not be mobile at the field scale.

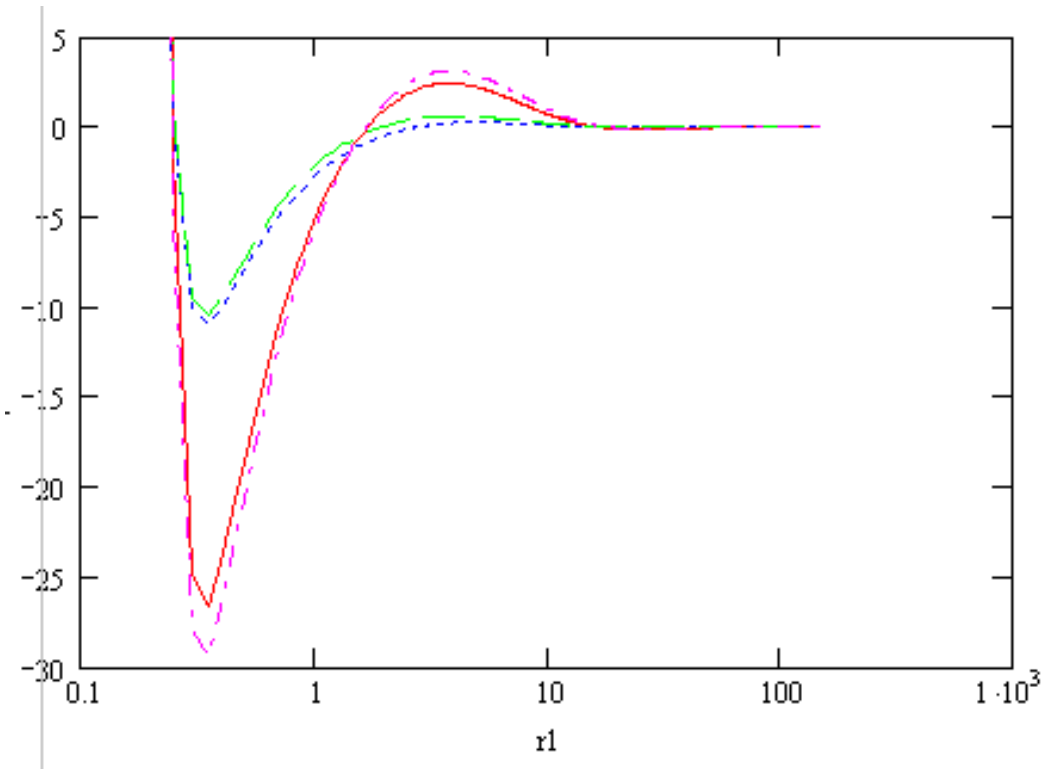


Figure 6.10 Semi-quantitative indication of the variation in interaction energies between a sandstone grain and a phage particle as a function of distance r1 (nm)

Energies expressed as mV – positive being repulsive and negative being attractive. H40/1: full line. PRD1: dash-dotted line. MS2: dashed line. ϕX174: dotted line.

6.4 Field tracer experiments

6.4.1 Introduction

Six forced gradient tracer tests and one pump-out test on an injection interval were carried out between two boreholes in the sandstone aquifer at the University of Birmingham. Each experiment was designed to test different characteristics of the viruses and/or features of the sandstone aquifer perceived to be potentially important for the fate and transport of viruses in these aquifer systems.

The boreholes are located 7 metres apart. Five of the six experiments were run on packered half-metre intervals of:

- non-fractured sandstone (Tests 1, 2, 3 and 4);
- fractured sandstone (Test 5).

The sixth experiment was undertaken to determine how much injected phage could be recovered from the injection interval of Test 5.

The final test, Test 7, used the entire uncased sections of both injection and pumping boreholes (~35–40m) (Test 6). Table 6.6 gives details of the experiments.

Fluorescein was used as a conservative tracer in each of the experiments and recovered in significant amounts from the extraction borehole (Table 6.6).

For the five packered interval experiments, no bacteriophage were detected in the recovery borehole despite extending the sampling time of each experiment and employing virus-binding Biocap 30 filters to extract viruses from tens of litres of pumped water in an attempt to increase the sensitivity of detection.

Test 7, the full-borehole experiment, used a cocktail of four phages in conjunction with fluorescein. All four phages were detected in both auto-sampler and Biocap 30 filter samples, demonstrating that pathways do exist between the two test boreholes. Details and further comment are provided in the following sections.

The fluorescein data were modelled using the one-dimensional (radial flow) code described in Chapter 5, providing some indication of the flow system present in the experiment. The interpreted hydraulic/dispersive properties were then used to confirm that no phage breakthrough would be expected assuming the laboratory-determined phage/rock interaction parameter values. The modelling is not intended to present a definitive interpretation of the datasets, but simply as scoping calculations to facilitate the discussion: the final interpretations are qualitative or semi-quantitative at most.

6.4.2 Test 1: ϕX174 with fluorescein at 19.1 mbgl in unfractured sandstone

The first field tracer test was undertaken in July 2004 and continued for 30 days. The packered interval was pumped at 7.3 litres/minute until steady state was achieved; the phage and fluorescein were then injected. A 2-litre suspension of ϕX174 (2.48×10^8 pfu/ml) and fluorescein (1 g/l) was injected into a ~0.5 m packered interval centred on 19.1 mbgl. The decreases in phage and fluorescein concentrations in the injection borehole (BH2) were similar, being rapid in the first 33 hours after injection, but becoming less so subsequently (Figure 6.11).

The fluorescein breakthrough in BH3 showed a double peak at 8 and 9.5 days after injection, with the first detected arrival being at six days. Total fluorescein recovery after 30 days of pumping was 47 per cent, although the majority was recovered within the first 14 days of the test. ϕX174 was detected in the observation borehole for a short period six days after injection. There were a number of other positive detections, although these were not reproduced. Since it is likely that these results were as a result of sample contamination, they were excluded (Figure 6.11).

Table 6.6 Summary of field experiment conditions and fluorescein recoveries

Tracer test	Duration (days)	Date	Fluorescein mass (g)	Injected phage concentration (volume, pfu/ml)	Depth (mbgl)	Flow rate (l/min)	Mass of fluorescein recovered (%)
Test 1	30	July 2004	2	ϕX174 (2 litres, 2.48×10^8)	19.1 (Unfractured sandstone interval)	7.3	47
Test 2	35	September 2004	2	H40/1 (2 litres, 5.4×10^{10})	26.9 (Unfractured sandstone interval)	2.7	53
Test 3	29	February 2005	2	MS2 (2 litres, 6.83×10^8)	26.3 (Unfractured sandstone interval)	7	81
Test 4	40	March 2005	2	PRD1 (2 litres, 1.32×10^8)	19.5 (Unfractured sandstone interval)	6.8	63
Test 5	30	April 2005	2	**Cocktail**: MS2 (2 litres, 1.6×10^{10}) PRD1 (2 litres, 3.97×10^6) ϕX174 (2 litres, 7.43×10^7) H40/1 (2 litres, 2.93×10^{10})	44.2 (Fractured sandstone interval)	3.7	72
Test 6	9	May 2005	0	Back pumping of injection borehole following Test 5	44.2 (Fractured sandstone interval)	1	0
Test 7	45	August 2005	20	**Cocktail**: MS2 (2 litres, 4.2×10^8) PRD1 (5 litres, 7.73×10^7) ϕX174 (2 litres, 1.4×10^7) H40/1 (2 litres, 5.34×10^7)	Entire borehole depth	145	76

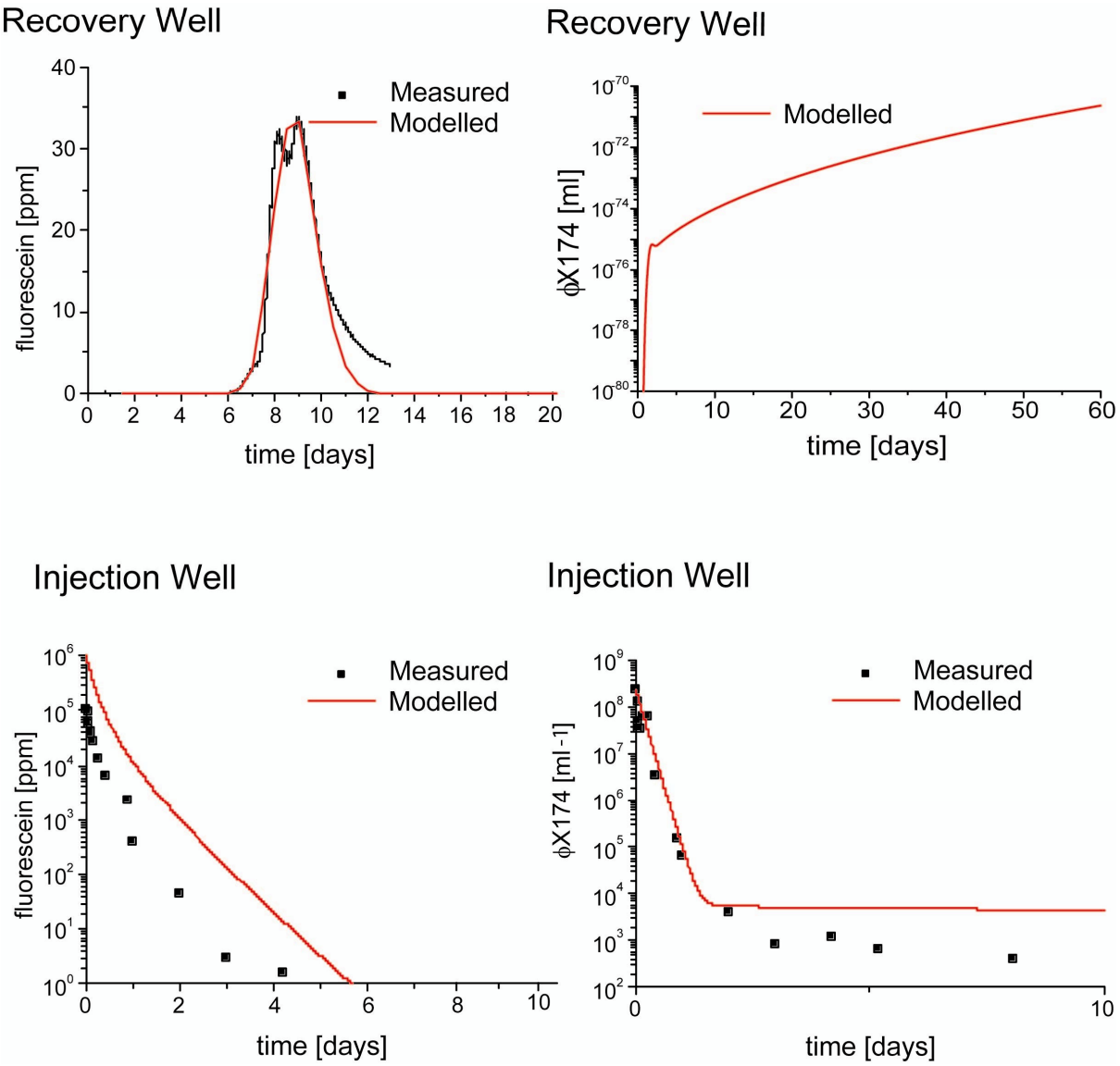


Figure 6.11 Measured and modelled results for Test 1 (ϕX174)

Fluorescein concentrations at the pumping well were reproduced approximately by a simple one pathway radial the model (Figure 6.13), but the observed concentration decline at the injection borehole was more rapid than suggested by the model. This suggests that flow past the injection borehole was slightly greater than the average flow towards the pumping well. The decrease in phage concentrations in the injection borehole was, however, modelled successfully. The model results for the phage concentrations at the recovery well show that, using the same parameters as found for the laboratory columns, ϕX174 should be undetectable (note axis on top right plot of Figure 6.11).

Thus it would appear that the laboratory and field experiments are consistent. This demonstrates that, as suggested previously, the occasional counts detected during this experiment were probably due to contamination.

After obtaining these odd results in this experiment, the sampling procedure was reviewed and improved to reduce the possibility of contamination.

6.4.3 Test 2a (Aug 2004): H40/1 with fluorescein at 26.9 mbgl in unfractured sandstone

The second test was undertaken in August 2004 and lasted for two days. The packered interval was pumped at 2.7 litres/minute to steady state before the phage and fluorescein were injected. A 2-litre suspension of H40/1 (1.04×10^9 pfu/ml) and fluorescein (1 g/l) was injected into the half-metre packered interval centred at 26.9 mbgl. Twenty-four hours after injection, it was observed that the fluorescein concentrations in the injection hole had decreased very quickly and, upon closer examination, it was clear that the injection well packer system had failed. The decision was taken to abandon the test, remove the packer system for repair, leave the site for one month, and then proceed with a re-injection of H40/1.

6.4.4 Test 2b (Sept 2004): H40/1 with fluorescein at 19.1 mbgl in unfractured sandstone

The second attempt at completing the second test was undertaken in September 2004 (Figure 6.12). The test lasted for 35 days. A half-metre packered interval, centred on 26.9 mbgl in BH3, was pumped at 2.7 litres/minute to steady state before a 2-litre suspension of H40/1 (5.4×10^{10} pfu/ml) and fluorescein (1 g/l) was injected in the equivalent half-metre interval in BH2.

Following injection, a problem was again experienced in the injection packer array; increased packer pressure corrected the problem within a day but some injectant undoubtedly leaked below the packer. The decreases of phage and fluorescein concentrations in the injection borehole (BH2) were similar, with a rapid decrease in the first 33 hours after injection which became much less rapid subsequently.

Recovery of fluorescein in the pumped borehole (BH3) resulted in a double peaked breakthrough curve (Figure 6.12). The first arrival of fluorescein was approximately four days after injection, peaking at approximately eight days. The first peak probably originates from the previous, aborted test (Test 2a). The second fluorescein breakthrough had a first detectable arrival after approximately 13–15 days and peaked at 21 days. Total fluorescein recovery at the end of the test (35 days) was 53 per cent. No H40/1 was detected in the observation borehole up to 35 days after injection.

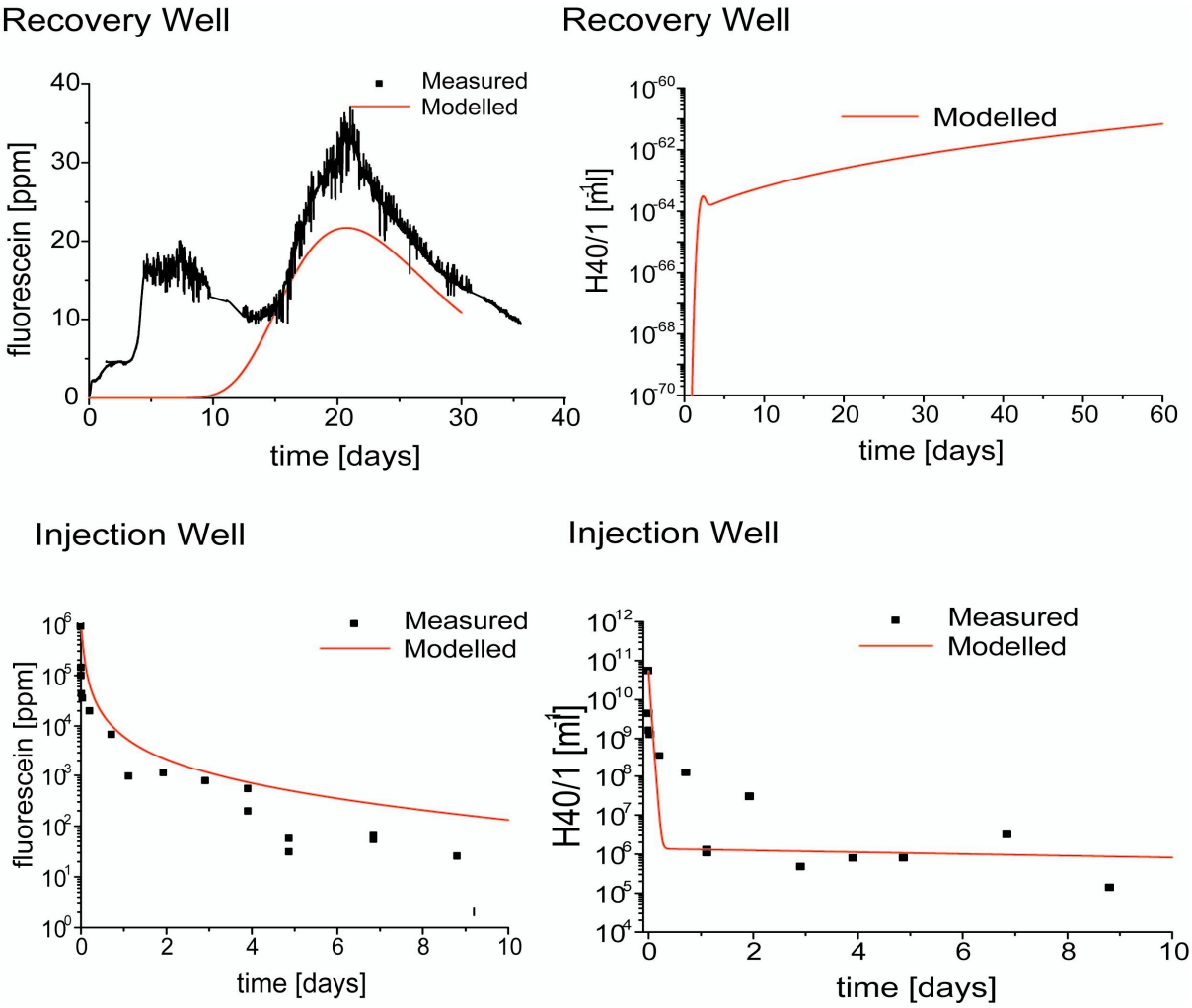


Figure 6.12 Measured and modelled results for Test 2b (H40/1)

The first fluorescein peak is assumed to originate from the August injection and therefore can be ignored in the interpretation. Although the amount of fluorescein remaining from Test 2a cannot be determined accurately, a suggested model fit for the Test 2b component of the breakthrough is given in Figure 6.12 along with the model-calculated concentration decrease in the injection borehole. The latter underestimates the rate of fall in concentration, as was also found to be the case in Test 1.

As was found in Test 1, the model again predicts a fall in phage concentration in the injection borehole which is too rapid. The model results for the recovery well show that, using the same parameters as found for the laboratory columns, expected H40/1 counts should be zero. The observed counts in the recovery borehole were therefore still the result of cross-contamination. This led to further improvements in the sampling set-up.

6.4.5 Test 3 (Feb 2005): MS2 with fluorescein at 26.3 mbgl in unfractured sandstone

The third test was undertaken in February 2005 and lasted for 29 days (Figure 6.13). The half-metre packered interval, centred on 26.3 mbgl, was pumped at 7 litres/minute to steady state before 2 litres of phage (MS2; 6.83×10^8 pfu/ml) and fluorescein (1 g/l) solution was injected. The decreases in phage and fluorescein concentrations in the

injection borehole (BH2) were similar, with a rapid decrease in the first 33 hours after injection followed by a much slower rate of decrease.

Recovery of fluorescein in the observation borehole (BH3) resulted in a double peaked breakthrough curve (Figure 6.13). The first detected arrival of fluorescein was approximately one day after injection, peaking at approximately 1.5 days. The second peak of the fluorescein breakthrough was estimated as having a first arrival approximately 10 days after injection; the maximum concentration occurred at 15 days. Total fluorescein recovery at the end of the test (29 days) was 81 per cent, although the majority of fluorescein was recovered within the first 20 days. No MS2 was detected in the observation borehole up to 29 days of sampling and analysis.

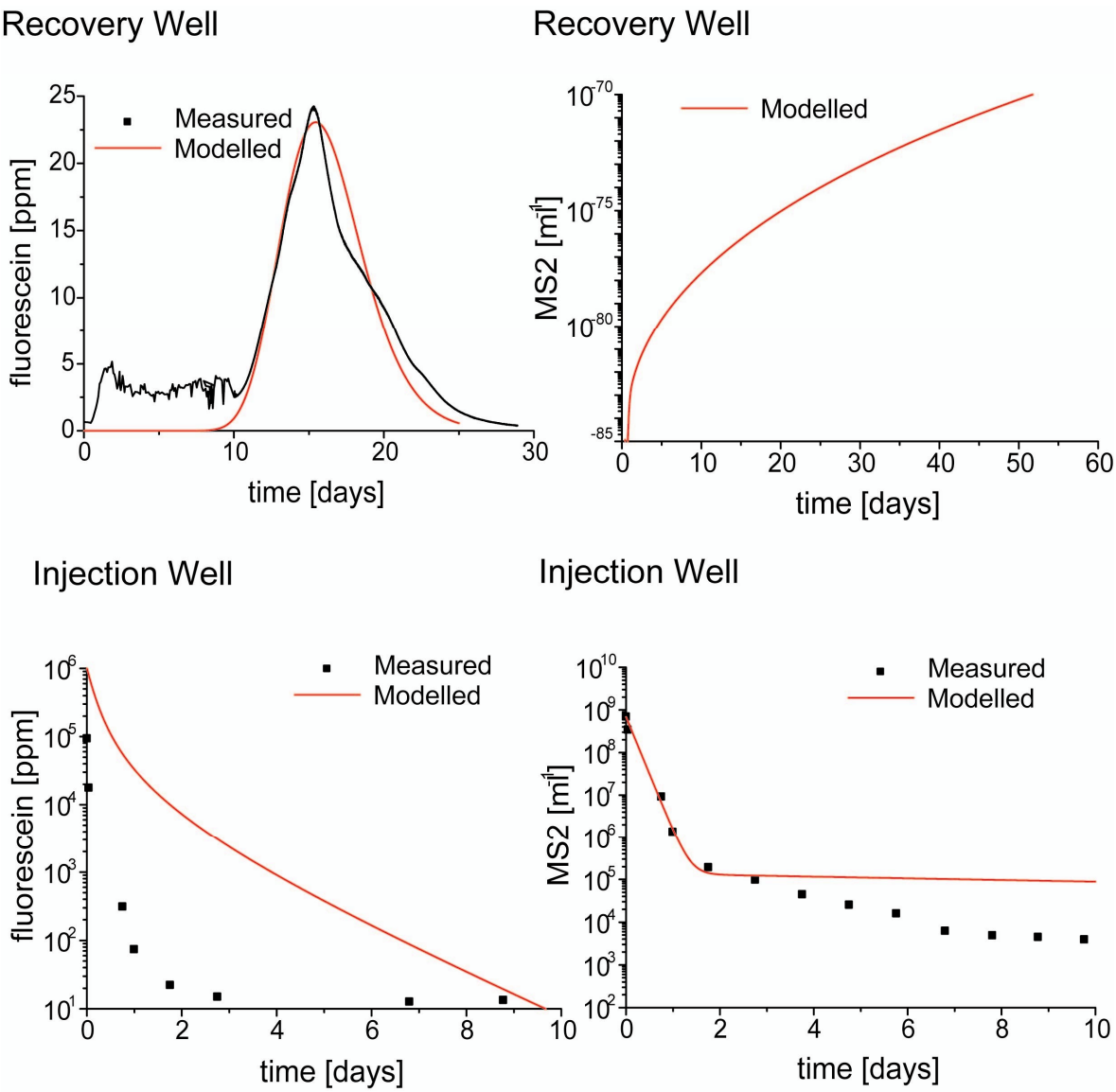


Figure 6.13 Measured and modelled results for Test 3 (MS2)

Although not capable of reproducing the subtler features of the fluorescein breakthrough curve, the model can reproduce the general shape of the main breakthrough. Model predictions of the fluorescein decay in the injection borehole were too slow, suggesting an unproportionately large flow through the part of the aquifer containing the borehole. It would appear that the model is not adequately representing the flow system.

The decay of the phage concentrations in the injection borehole was reproduced reasonably well for early times but, at late times, the decay is slower than seen in the field system. Despite the poor fluorescein model fit, the early time agreement – as in the case of Test 1 – suggests that the good fit may be fortuitous.

The model results for MS2 concentration in the recovery well show that, using the same parameters as found for the laboratory columns, expected MS2 should again be zero. Again the laboratory and field systems appear to be in agreement.

6.4.6 Test 4 (March 2005): PRD1 with fluorescein at 19.5 mbgl in unfractured sandstone

Test 4 was undertaken in March 2005 and lasted 40 days. The half-metre packered interval, centred on 19.5 mbgl, was pumped at 6.8 litres/minute to steady state before 2 litres of phage (PRD1; 1.32×10^8 pfu/ml) and fluorescein (1 g/l) test solution injected. Biocap 30 filters were used throughout in addition to the auto-sampling system. The decreases of phage and fluorescein in the injection borehole (BH2) were similar, having a rapid decrease in the first 33 hours after injection before subsequently becoming much less rapid (Figure 6.14).

Breakthrough of fluorescein in the pumped borehole (BH3) displayed a double peaked curve. The first detected arrival of fluorescein was 2.5 days after injection, peaking at approximately 7.5 days. The second fluorescein peak was at 14 days. Total fluorescein recovery after 40 days was 63 per cent, although the majority was recovered within the first 20 days. No PRD1 was detected in the pumped borehole up to 40 days.

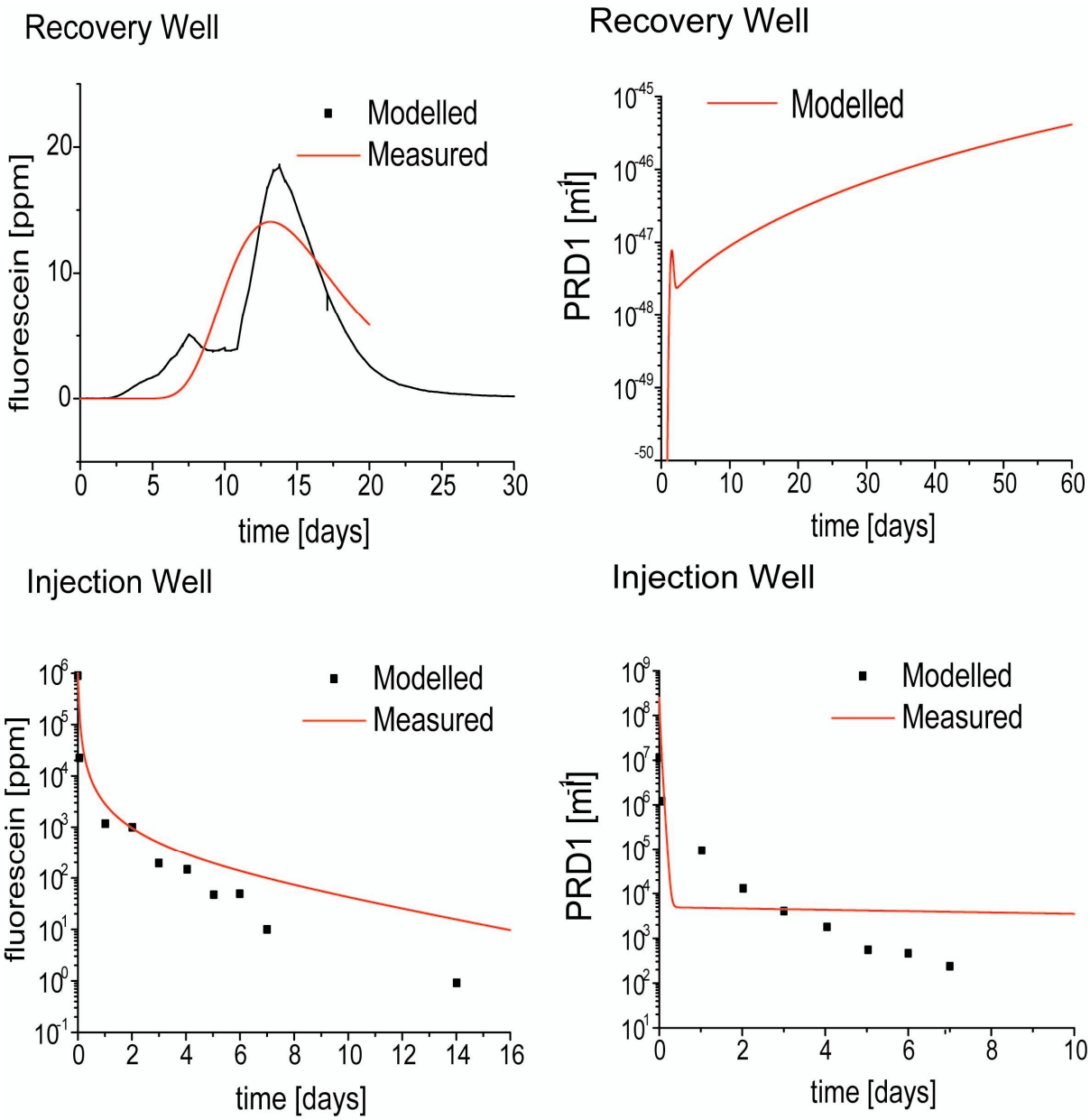


Figure 6.14 Measured and modelled results for Test 4 (PRD1)

The breakthrough curves and the model fits for Test 4 are similar to those for Test 2. In Test 4, the model fit for the fluorescein is intended to represent only the approximate timing of the main peak breakthrough; the pathways between the injection and pumping boreholes are strongly heterogeneous. The decay of fluorescein in the injection borehole is reasonably reproduced, if somewhat overestimated, and again the implication is that the system shows a significant departure from radial symmetry. The rate of phage decay in the injection borehole is very significantly overestimated by the model at early times: breakthrough at the pumping well is again predicted to be zero.

6.4.7 Test 5 (April 2005): MS2, PRD1, ϕX174, and H40/1 with fluorescein at 44.2 mbgl in fractured sandstone

The fifth test was undertaken in April 2005 and ran for 30 days (Figure 6.15). The half-metre packered interval, centred on 44.2 mbgl, was chosen because it contained a fracture – unlike all the previous test intervals. It was pumped at 3.7 litres/minute to steady state before the phage and fluorescein were injected. The injection solution

consisted of an 8-litre suspension of the four phages and fluorescein made up to have the following concentrations:

- 4.0×10^9 pfu/ml MS2;
- 0.99×10^7 pfu/ml PRD1;
- 1.86×10^7 pfu/ml ϕX174;
- 0.73×10^{10} pfu/ml H40/1;
- 0.5 g/l fluorescein.

Recovery of fluorescein in the observation borehole (BH3) resulted in a strongly double-peaked breakthrough curve (Figure 6.15). The first detected arrival of fluorescein was 1.5 days after injection. The peaks occurred at approximately 2.5 and five days. Total fluorescein recovery after 30 days was 72 per cent, although the majority was recovered within six days.

None of the four phages were detected in the pumping borehole during the whole 30 days of the test, even in a Biocap 30 filter attached to the pumping borehole discharge line.

This result suggests that, despite the presence of the fracture in the test interval, there was a considerable amount of contact between the phage and the sandstone matrix. This could result from one of the following reasons:

- The fracture is not continuous between the two boreholes, despite there being similar fractures observable in both borehole walls.
- The fracture is at least partly filled with sediment.
- The fracture is less permeable than the surrounding sandstone.

Drawdowns and pumping rates recorded for this test suggest that the fracture does not increase the permeability of the interval by any significant degree.

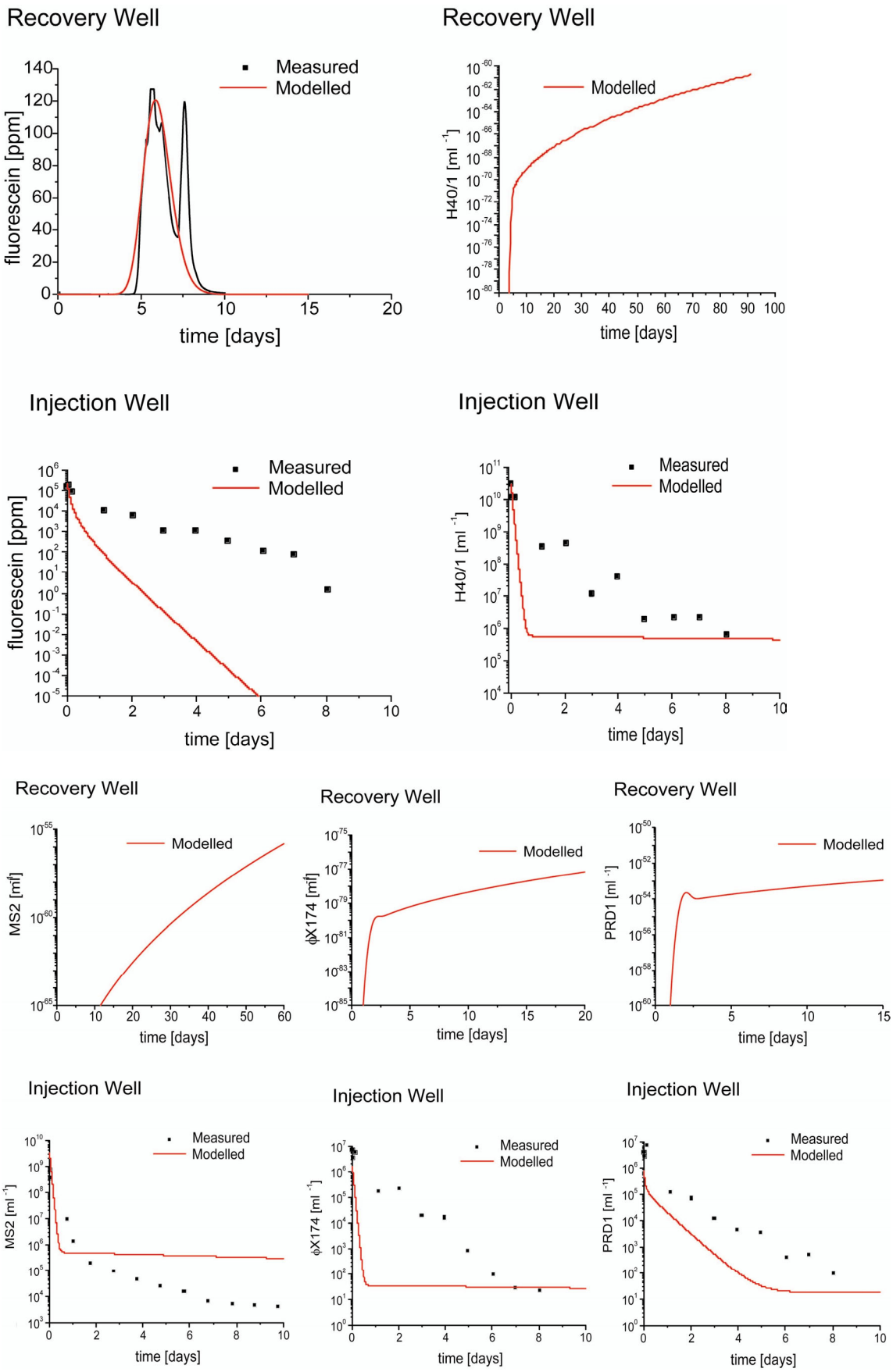


Figure 6.15 Measured and modelled results for Test 5 (phage cocktail, fractured sandstone interval)

The model used to interpret the field experiments assumes homogeneous flow conditions, an assumption that is violated for this experiment. This is because most of the flow will occur through the fracture whereas some flow will pass through the adjacent sandstone.

The model was, therefore, adapted to optimally reproduce the observations. Fluorescein concentrations in the injection and the recovery wells were reasonably well reproduced (Figure 6.15). However, modelled phage concentrations in the injection hole match with observations only to within an order of magnitude. The modelled phage concentrations in the recovery well show that MS2, ϕX174, H40/1 and PRD1 concentrations are expected to be, at most, 10^{-50} per ml. These concentrations are based on the assumption that attachment and detachment coefficients are the same as for the core experiments.

Although the first main peak of the fluorescein breakthrough can be modelled reasonably well, the decay rate of the fluorescein concentrations in the injection borehole is grossly overestimated. The decays in phage concentrations in the injection borehole are also not well reproduced by the model. MS2 differs in style of decay from the other phages yet, in the modelled decays, it is PRD1 that shows a different pattern.

The field and model results illustrate the potential importance of phage properties rather than flow conditions for decay in concentration at a source. In all cases, application of the laboratory-determined phage property values results in the prediction that no phage should breakthrough to the recovery borehole (Figure 6.15).

6.4.8 Test 6 (May 2005): back-pumping of Test 5 interval

The first five tests suggested there was no bacteriophage transport through either unfractured or fractured sandstone. To determine if the attached phage could be remobilised, the injection borehole for the Test 5, fractured interval, was pumped. The choice of this interval was influenced by the fact that all four phages had been used during Test 5. However, problems were encountered with the MS2 host and hence no MS2 analysis was possible for this test.

The back pumping test lasted nine days and was undertaken at 1.44 m^3/day. The results are shown in Figure 6.16; Biocap 30 filters were also used throughout the test and support the results from the auto-sampling presented here.

Phage numbers remained low throughout the test relative to the injection concentrations:

- maximum PRD1 concentrations were ~10^3 lower than at injection;
- maximum ϕX174 concentrations were ~10^6 times lower than at injection;
- maximum H40/1 concentrations were ~10^4 times lower than at injection.

The recovery curves showed an initial rapid drop in concentration followed by a more gradual decline, with log (concentration) decreasing approximately directly as time increased.

Analysis of the results indicates the phage are not filtered by, but attached to, the sandstone matrix. However, the previous test was pumped for one month so this may have influenced where the phage are filtered or attached within the 7-metre packered interval.

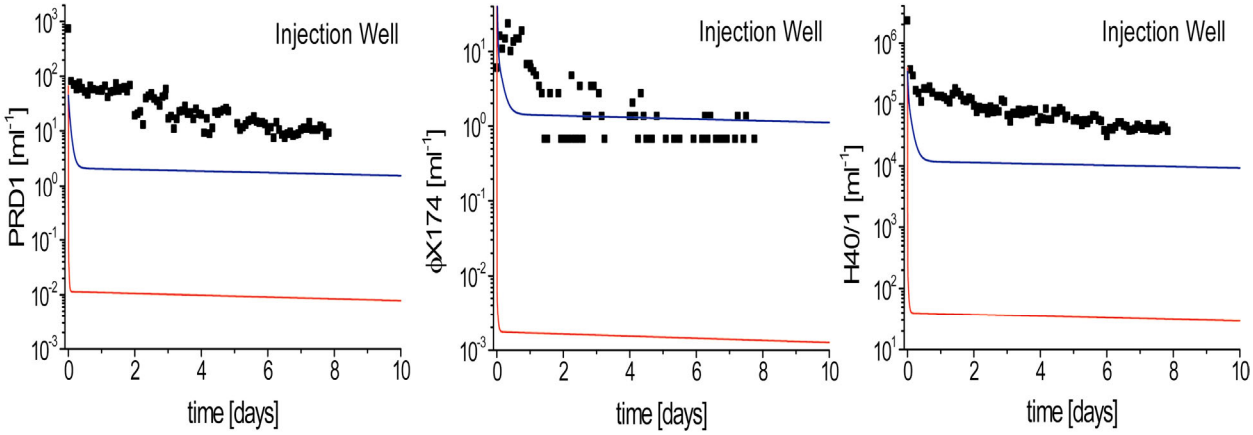


Figure 6.16 Measured and modelled results of back pumping experiment on the injection borehole of Test 5

The lower model (red) curve indicates model results with parameters taken from core experiments. The upper (blue) curves show model results with 10 times smaller attachment and detachment rates.

Using the laboratory values for attachment and detachment rates, modelling of the test grossly underestimates the release of the phages. Figure 6.16 shows the predicted release and the expected release if the attachment/detachment rates had been an order of magnitude smaller than indicated by the laboratory experiments. It is clear that the mechanisms are not well represented. The initial high concentrations – seen especially well in the PRD1 and H40/1 datasets – may indicate a filtering process. The role of the fracture in the flow system at this interval is unknown and may have influenced the results. This sort of 'push–pull' test may prove a useful means of evaluating attachment and detachment properties in the field.

6.4.9 Test 7 (August 2005): MS2, PRD1, ϕX174, and H40/1 with fluorescein at over full 50 m depth of boreholes

The final test was undertaken in August 2005 and ran for 45 days. It was designed to investigate a larger block of rock to determine if there were pathways allowing transmission of viruses which might have been too rare to be identified by the very limited volumes of rock tested in the previous experiments.

BH3 was pumped at a rate of 145 litres/minute until steady state was achieved. A cocktail of the four phages and fluorescein was then injected into the un-packered BH2. Heads and flow rate were monitored in BH3, and samples were taken for phage analysis every 30 minutes using an auto-sampler. In addition, Biocap 30 filters were used throughout the test, each being installed for one week.

The injection solution was made up using purified phage suspensions purchased from CREH Analytical Ltd, Leeds: 2 litres of MS2 (5×10^{13} pfu/ml), 5 litres of PRD1 (1×10^{10} pfu/ml), 2 litres of ϕX174 (1.5×10^{11} pfu/ml) and 2 litres of H40/1 (1×10^{11} pfu/ml).

The 20 litres of solution injected into BH2 contained:

- 4.20 x 10^{8} pfu/ml MS2;
- 7.73 x 10^{7} pfu/ml PRD1;
- 1.40 x 10^{7} pfu/ml ϕX174;
- 5.34 x 10^{7} pfu/ml H40/1;

- 1 g/l fluorescein.

The phage and fluorescein data collected are presented in Figure 6.17. The broad nature of the fluorescein breakthrough curve indicates that there are numerous pathways between the boreholes, with much overlapping of times of passage. The diurnally distributed troughs were identified as being due to photo-decay during passage of the fluorescein through transparent tubing during this period of unusually sunny days.

The first detected arrival of fluorescein was seven hours after injection, peaking after 2.5 days and then again at three and five days. Assuming a homogeneous system, breakthrough from BH2 would be expected in about 7.5 days (close to the centre of mass of the recorded breakthrough), but these early arrivals represent significantly faster routes through the system. Total fluorescein recovery after 45 days of pumping was 75.7 per cent, although the majority was recovered within the first 20 days.

All four phage were detected in both the auto-sampler samples (Figure 6.18) and the Biocap 30 filter samples (Figure 6.19). Concentrations were relatively low, being <10 pfu/ml in the former and <100 pfu/ml in the latter. These figures represent dilutions of around 10^4 to 10^6 fold relative to injection concentrations compared with ~10^2 fold dilution for the fluorescein.

The times of the first detected arrivals were at:

- 1 hour for MS2 and H40/1;
- 3 hours for PRD1;
- 9 hours for ϕX174.

These breakthrough times are even earlier than the earliest of the fast fluorescein breakthroughs. This may be because of some pore-size exclusion mechanism, but is possibly simply because phage detection limits are significantly lower than fluorescein limits. Fluorescein can be detected to a dilution of about 10^4 to 10^5 under the conditions of the present experiment, whereas phage can be detected down to dilutions of around 10^6 to 10^8. Interestingly, breakthroughs continued up to at least 35 days with only a relatively small decline in concentrations.

It is clear that pathways exist through the sandstone able to allow passage of a small proportion of the viruses. These pathways are relatively infrequent and have properties not seen in the pathways available to the phages in the laboratory or smaller-scale field tests. The determining factor would appear to be properties associated with the pathways, though it may be that even with these ‘special’ pathways only a small proportion of the phage possess properties suitable to allow them to be mobile.

Since phage were detected in both the samples and the eluent from the Biocap 30 filters, it is clear that pathways do exist between the two boreholes that are not easy to predict or identify through closed circuit television (CCTV) imaging of the boreholes. The low numbers of phage recovered from the observation borehole confirm that transport through either the matrix or fractures is occurring. But since high numbers of phage were not recovered, attachment to or filtration by the sandstone matrix must also be considered.

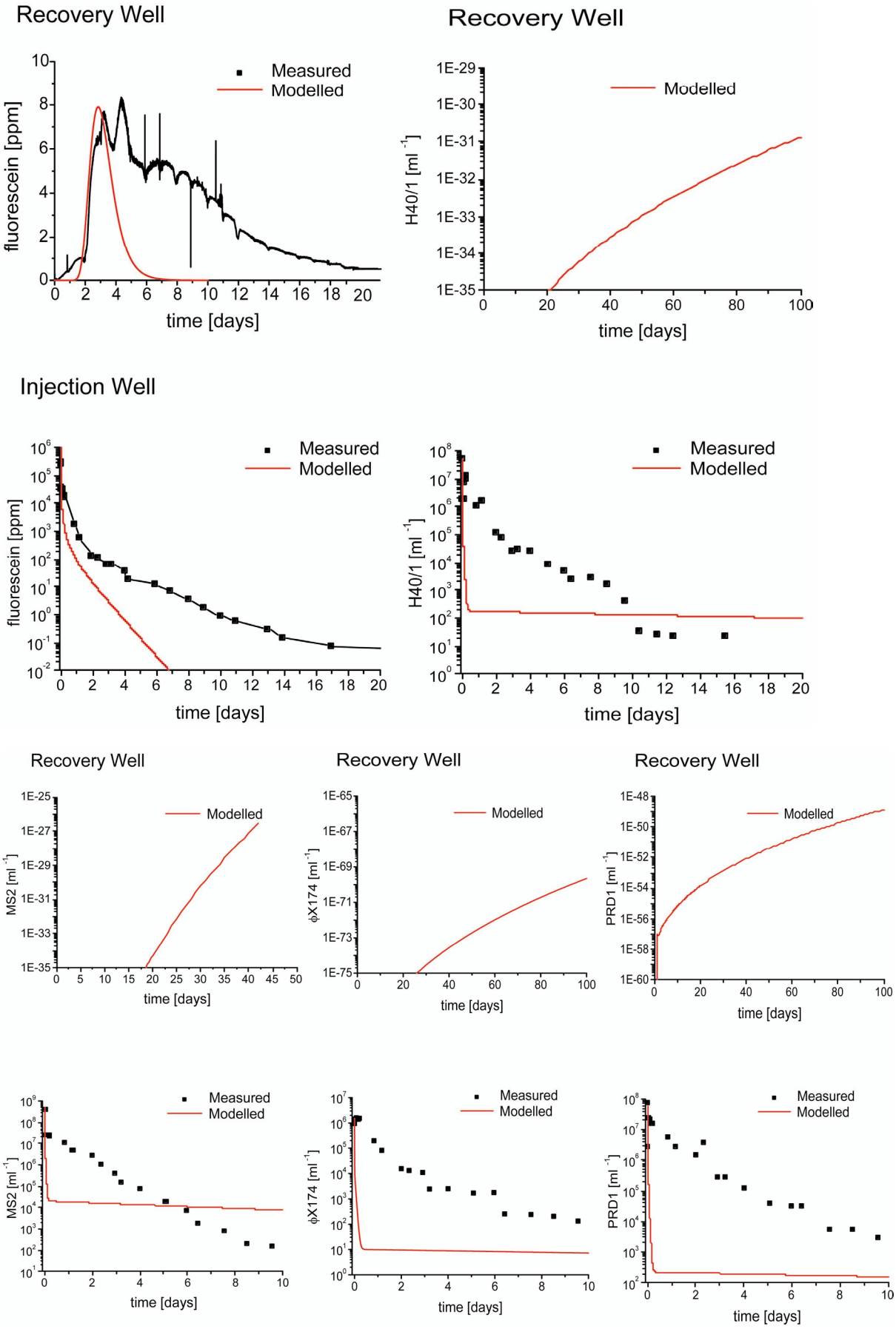


Figure 6.17 Measured and modelled results for Test 7 (phage cocktail, full borehole)

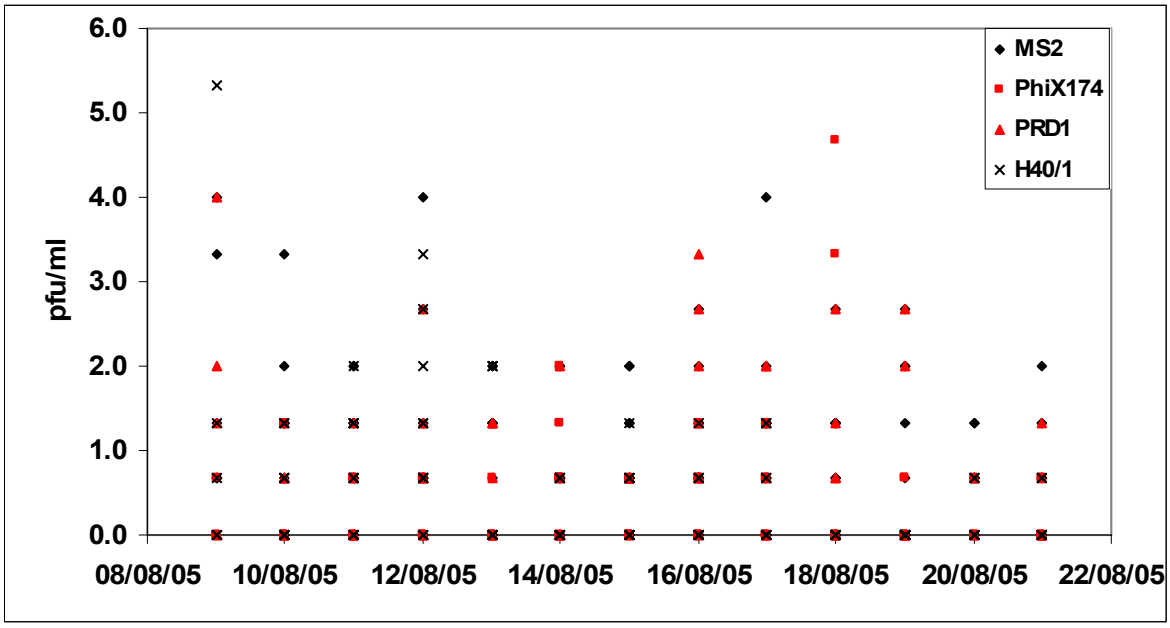


Figure 6.18 Measured results for the pumped well samples in Test 7

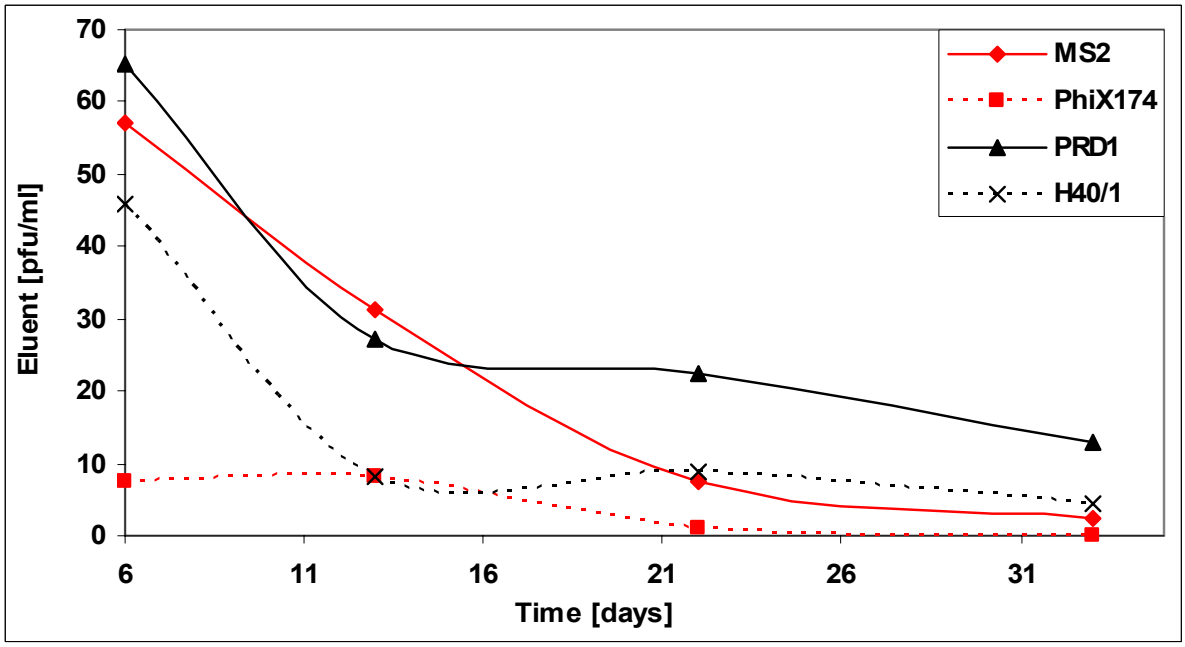


Figure 6.19 Phage concentrations from Biocap 30 filter samples, Test 7

The single medium, radial flow conditions assumed by the model are even less valid for interpreting this test than for the previous tests given:

- the broad nature of the fluorescein breakthrough curve;
- the fact that different types of pathway have been identified in this experiment;
- the presence of a great variation in lithological properties within the profile.

Accordingly, only an example model calculation was undertaken with the simulation of the first main breakthrough peak displayed by the fluorescein. The model again suggests that no phage should be detected.

Once again, the model used to interpret the field experiments assumed homogeneous flow conditions. This assumption was violated for this experiment because most of the flow would occur through fractures or other highly conductive layers, and some flow would pass through the bulk sandstone.

The fluorescein recovery curve shows multiple peaks support the assumption that this system was highly heterogeneous (Figure 6.17). Because the applied model attempted to reproduce only the first peak, it provides an explanation for only some of the processes going on in this complex experiment. Figure 6.17 shows that the employed model cannot reproduce the observations in the injection well. However, the model results provide a theoretical basis to interpret the phage data.

The correlations of the four phage species were predicted by the model to be far below the detection limit of the analytical methods. However, the observed concentrations were in a similar range for all four phages. Furthermore, all phages seemed to be transported faster than expected by the model. This observation indicates that the detected phages were not following transport through a porous medium, but are far more likely to have been carried along fast flow paths through fractures. Such fractures were not necessarily detectable with fluorescein due to the potentially large distance fractures involved, yet phage were detectable under these conditions due to the greater sensitivity of the assay method. The maximal resolution of fluorescein is of the order of 10^4 to 10^5 (in this experiment), whereas injected concentrations of phages are of the order of 10^6 to 10^8, i.e. 1–4 orders of magnitudes more sensitive.

Overall, the detection of phage in the pumped borehole samples is consistent with the detection of enteric viruses in deep sandstone systems in Birmingham and Nottingham (Powell *et al.* 2000) previously thought to be virus-free. The CCTV imaging of the boreholes indicates many observable discontinuities. But without completing hydraulic tests with packers, it was not possible to conclude if these were real fractures.

6.5 Summary of project investigations

- Field (12ºC) and laboratory (12ºC) survival studies using all four phage (PRD1, MS2, ϕX174 and H40/1) suggested inactivation rates in the range 0.0009–0.0052 day^{-1}.
- Nine laboratory column experiments on four UK Triassic sandstone cores were carried out using single phage suspensions (PRD1, MS2, ϕX174 and H40/1) and a conservative tracer (fluorescein). The results indicated rapid attenuation of the phage by more than one type of attachment process. Over the few centimetre scale of the cores, both attachment and detachment were observed. When flushed with water at an initially higher pH, some previously attached phage were released. The results of the column experiments suggest that travel distances for the vast proportion of the phage injected are limited to less than a few metres.
- Four single phage (PRD1, MS2, ϕX174 and H40/1) forced-gradient tracer experiments were carried out in approximately half-metre packered unfractured sandstone intervals between two boreholes separated by ~7.5 metres in the Birmingham Triassic sandstone aquifer. An additional test was completed in a fractured sandstone interval. Fluorescein recoveries ranged from 47 to 81 per cent, but no phage were detected in the pumped borehole.
- Back-pumping of the fractured sandstone test interval showed slow release of viable phage from the sandstone, although this may vary if pumping took place for less than a month before reversing the pumping.
- A similar forced-gradient tracer test was completed using the full depth (35–40 metres) of the same injection and pumped boreholes used in the packer test

experiments. The test solution contained the phages PRD1, MS2, ϕX174 and H40/1 and fluorescein; 76 per cent of the injected fluorescein was recovered. Breakthrough occurred for all four phages, though in relatively low concentrations; <10 pfu/ml in the case of the auto-sampler samples and <100 pfu/ml in the case of Biocap 30 filters (changed at weekly intervals). This implies dilutions are greater than two orders of magnitude seen for fluorescein.

The implications of these findings are discussed in Chapter 7.

7 Conclusions

This project set out to investigate the mobility of viruses using field experiments, supported by laboratory work on survival times and virus/rock interactions. A specially written numerical model was used in the interpretation of the data collected.

The work was undertaken with specific reference to sandstones of the Triassic Sherwood Sandstone Group and used the bacteriophages PRD1, MS2, ϕX174 and H40/1 as surrogates for human pathogenic viruses – specifically adenovirus and rotavirus, poliovirus, norovirus, and adenovirus and rotavirus respectively.

Field and laboratory studies were undertaken to determine survival times for all four test phages. The laboratory experiments were completed at 12^{o}C, the approximate temperature of shallow UK groundwaters, and the field experiments involved suspension of dialysis tubing in wells. In both cases, only the rates in aqueous suspension were investigated; inactivation rates for phage attached to rock surfaces were not studied.

The half-lives in groundwater at 12°C of the phage (MS2, ϕX174, PRD1 and H40/1) employed in these core and field tests were several months long (12, 21, 21 and 21 months respectively). Measured rates were in the range 0.0009–0.0052 day^{-1}, suggesting typical survival times of up to around 1.75 years.

This indicates that the chosen phages are excellent microbial tracers but, more importantly, suggests that phage and hence some enteric viruses may remain viable in aquifers for considerable lengths of time. If human pathogenic viruses behave similarly, these figures indicate that travel distances are likely to be limited, with average linear velocities in the Sherwood Sandstone Group aquifers of typically of the order of tens to a few hundred metres per year.

The survival experiments show these low inactivation rates under both field and laboratory conditions. Small differences were observed between the four bacteriophage inactivation rates for both the laboratory and field studies; the differences are slightly greater for the field results indicating a more realistic environmental inactivation rates for field tracer studies.

The results from column experiments suggest that inactivation rates may change when the virus is adsorbed to a surface. This supports the findings of Yates and Jury (1995), who suggested that an important factor in virus survival is the forces created at the water or surface interface.

Overall, these studies prove phages chosen as surrogates for human enteric viruses do survive for extended periods in the environment and may be transported rapidly through the Sherwood sandstone.

Nine laboratory column experiments on four UK Triassic sandstone cores were carried out using single phage suspensions and fluorescein as a conservative tracer. Simultaneous application of fluorescein and phage to laboratory columns of sandstone was used to develop models of the overall transport behaviour of phage in aquifers.

The results indicated rapid attenuation of the phage by more than one type of attachment process over the few centimetre length of the cores. It appears that both attachment and detachment occur, but much more work is necessary to identify the mechanisms involved. Nevertheless, the breakthrough curves were fitted using a

numerical model which included kinetic and equilibrium attachment and inactivation. Assuming these processes to be appropriate, parameters were also determined.

The column experiments demonstrated significant attachment of phage to the sandstone matrix. Fluorescein recovery was approximately 100 per cent, but the phage recoveries were substantially lower [PRD1 (22.9, 10.3 and 2.9 per cent), MS2 (3.1 and 6.4 per cent), ϕX174 (0.99, 1.8 and 5.1 per cent) and H40/1 (6.47 per cent)]. The results suggested a component of slow detachment and indicated that attached phage may have greater inactivation rates than were seen in the survival experiments in the aqueous phase. The results of the column experiments suggest that travel distances through the sandstone matrix for the vast proportion of the phage injected would be limited to less than a few metres.

The model used highlighted significant uncertainty. This originates in:

the measurements;
the fact that the commonly used advection–dispersion approach to model virus transport did not always reproduce accurately the phage recovery observed curve, though it worked well for a conservative tracer such as fluorescein.

PRD1 and MS2 column experiments showed a considerable shoulder (slower decrease compared with the model) on the descending limb of the curve. ϕX174, on the other hand, revealed a much sharper recovery curve (steeper increase and faster drop) than predicted by the model. The first observation could be due to non-ideal flow behaviour in the cores.

Four single phage forced-gradient tracer experiments were carried out in approximately half-metre packered unfractured sandstone intervals between two boreholes separated by approximately 7.5 metres in the Birmingham Triassic sandstone aquifer. An additional test was completed in a fractured sandstone interval.

Fluorescein recoveries ranged from 47–81 per cent, but no phages were detected in the pumped borehole for any of the tests. This does not mean that viruses cannot be transported over distances of seven metres, but it does show the strong protective effect on pathogen transport on inter-granular groundwater transport. Thus, these field tests appear to support the findings of the laboratory column experiments, with both studies suggesting very limited mobility for the phage and therefore, by inference, human pathogenic viruses.

Back-pumping of the fractured sandstone test interval showed slow release of viable phage suggesting that, at least, some viruses are not irreversibly attached and that they remain viable.

A further forced-gradient tracer test was completed using the full depth (35–40 metres) of the same injection and pumped boreholes used in the packer test experiments. The test solution contained a cocktail of all four phages and fluorescein; 76 per cent of the injected fluorescein was recovered.

Unlike the previous experiments, phage breakthrough occurred in this experiment. All four phages were detected at the pumping borehole, though at concentrations several orders of magnitude lower than would be expected given an unreacting solute. The earliest breakthrough times were very short, suggesting residence times of <1 per cent of the average unreacting solute, although breakthrough continued to occur until close to the end of the experiment at approximately 45 days when the fluorescein concentrations were close to background level.

When a large enough volume of sandstone is tested, it would appear that pathways can be found which allow the phage to travel much greater distances than they would normally be able to do. The possibility that the pathways are associated with fractures is reinforced by the rapid breakthrough of some of the viruses. This would allow reconciliation of the data with the results of the laboratory column experiments; the findings of the field tests are consistent with the discovery of human pathogenic viruses in small concentrations in the deep sandstone aquifers of Birmingham and Nottingham (Powell *et al.* 2003). However, other major and contributory processes cannot yet be discounted including:

- the presence of phage with atypical properties that allow them to be rather more mobile;
- the presence of sandstone units with properties atypical of those used in the laboratory experiments.

The understanding of virus transport in the packered homogenous sandstone interval and packered fractured sandstone interval is insufficient to predict virus movement in these environments. Although it was known the phage could move through the formation, it was not possible to identify the pathway by which this occurred at the local scale. The chosen bacteriophage all had fairly similar acidic pI values (3.5, 6.6–6.8, 3–4 and >4.5 respectively); if the experiments were repeated using phage with an alkali pI value, the results may be very different.

The field tests demonstrated that the bulk sandstone matrix at the Birmingham site can be very effective at removing bacteriophage. Despite this, the open borehole test showed that transport pathways do exist in the sandstone aquifers which are not easy to predict or to identify. It seems likely that the bacteriophages were transported through fast flowing fractures, but the attempts to identify and isolate such fractures were in vain.

It is tentatively concluded that:

- phage survival is limited to around two years at most, severely limiting the distances that viruses could travel (especially considering the limited evidence from this project that attachment may increase the rate of inactivation);
- phages are attenuated by attachment during passage through the matrix of most sandstones of the Sherwood Sandstone Group;
- travel distances are likely to be less than a few metres in such sandstones, although relatively rare pathways – possibly associated with fractures – exist that allow a small proportion of phage to travel much greater distances;
- although no direct evidence has been obtained, the results of the work on phage are consistent with monitoring data on human pathogenic viruses and hence there is no evidence that viruses of apparently similar physicochemical properties behave differently from one another, even if they have different host organisms.

These findings suggest that quantitative prediction of virus mobility is likely to be very difficult. To a first and very close approximation (>99 per cent) which would be satisfactory in almost all other cases of groundwater pollutant transport, the laboratory data show that viruses are immobile in the sandstones. However, this approximation is not adequate because the small proportion of viruses that are mobile over significant distances are still abundant enough to cause concern. In place of the usual approaches for predicting pollutant behaviour based on averaged properties ±n standard deviations, virus assessment may represent an instance where extreme property values (virus charge, fracture connectivity) need to be the main target for consideration despite being of low likelihood.

At the field scale, the model worked well but reached its limits when incorporating high attachment and detachment rates. Further development of the model is necessary to account for the heterogeneity of the sandstone at the field scale.

In the natural environment, viruses are usually injected continuously (e.g. through leaking sewage systems or sewage application) whereas the tests performed during this project were performed as a peak and not a continuous injection. It can be shown that even for continuous injections of 10^4 viruses per litre (typical for sewage), transport distances to reach concentrations below one virus per litre (in bulk sandstone) are of the order of a few meters to a few tens of meters only (Table 7.1). Interestingly, these transport distances qualitatively correlate quite well with the height of the energy barriers (Figure 6.10). Using the concept of bulk transport of viruses in an ideal porous medium (as it is assumed in most regional groundwater models to determine protection zones) and using transport parameters as determined with the core and the survival experiments, the determined protection zones may underestimate the influence of transport through fractures (Table 7.1).

Table 7.1 Typical transport distances (in meters from injection point) of different phages with input concentrations of 10^4 per litre to reach concentrations <1 per litre under steady state conditions

Flow velocity (m/day)	PRD1	H40/1	ϕX174	MS2
1	0.3	0.25	0.15	0.2
10	1.7	1.3	0.8	1
100	14	11	6	8

Even with continuous injections, phage/viruses will over a long period of time eventually coat all the available binding sites, thus allowing the latest phage/viruses to travel further and further into the aquifer or fracture. In addition, there will be a large reservoir of phage/viruses ready to be desorbed when there is a low pH input such as rainfall.

The low number of phage detected during the open borehole test confirms transport through fractures is occurring. However, high numbers of phage were not recovered making it necessary to allow consider attachment to the matrix (fracture walls and bulk rock). The results from the six field tracer tests demonstrate that testing only one packered interval at the Birmingham well field site is not representative of transport properties in the entire sandstone aquifer.

Biocap 30 filters proved effective in concentrating bacteriophage in field tracer tests. They provide an enclosed filter system which prevents the risk of sample contamination while concentrating the numbers of bacteriophage present to a detectable or assayable level. The filters worked well in the laboratory where approximately 30–90 per cent of injected phage were eluted.

This project illustrates the use in field tracer tests of four bacteriophage (MS2, PRD1, ϕX174 and H40/1) to predict the fate and transport of human enteric viruses in the Sherwood sandstone aquifer. The purpose of the four phage was to act as surrogates for poliovirus (MS2), adenovirus (PRD1 and H40/1), norovirus (ϕX174) and rotavirus (PRD1 and H40/1). Another possibility is to use R17, f2 or fr, which are also considered surrogates for poliovirus and norovirus in relation to size, shape, genetic make-up and pI.

8 Recommendations for further work

8.1 State of present knowledge of virus mobility in UK Permo-Triassic sandstone aquifers

When combined with previous results available from allied research investigations and the limited number of other studies undertaken in the UK, the work undertaken in this project suggests that:

- viruses are mobile within the groundwaters of Permo-Triassic sandstone aquifers;
- only a very small proportion of the viruses released into Permo-Triassic sandstone aquifers are mobile over distances of significance to water resource assessment;
- the mechanisms of attachment and degradation are largely unknown;
- the survival times for viruses in groundwaters are probably less than two years;
- within this time, viruses can travel much greater distances than might be predicted by estimates based on average linear velocities.

Two approaches (process-oriented and empirical) are possible to improve the accuracy of prediction of virus movement in sandstone groundwaters. These are discussed in the following sections.

8.2 Process-oriented approaches

This approach is the better in the long-term because it should lead to flexible and secure prediction methods in which there would be considerable confidence. But to achieve this, much basic research would be required (Table 8.1).

Table 8.1 Research needed for process-orientated approach to improving the accuracy of prediction of virus movement in sandstone groundwaters

Topic	Aspects
Surface chemistry of the aquifer	• Charge pH relationships, and their dependence on ionic strength
Surface chemistry of the viruses	• Charge pH relationships, and their dependence on ionic strength • Aggregation kinetics • Differences between properties of any sub-populations present
Heterogeneity of the aquifer system	• Hydraulic heterogeneity and therefore time for reactions • Pore-size heterogeneity • Chemical heterogeneity • Fracturing
Interactions between viruses and the rock surfaces	• Building on the knowledge of surface chemistry developed in the studies listed above: – rates – capacities – dependence on environmental conditions.
Interactions between viruses and other particles in the groundwater	• Importance of inorganic colloids • Importance of organic colloids • Importance of other biocolloids (including predation)
Interactions of viruses and other debris introduced at the virus source	• Virus aggregation at source • Humics and other cell debris interactions
Interactions between viruses and water/air interfaces in the unsaturated zone	
Further establishment of the validity of using phage as surrogates for human pathogen viruses	

Most viruses are effectively immobile (Chapter 7) and it is only where viruses and rocks with extreme properties coincide that virus movement becomes of concern. Because the movement of even extremely small proportions of viruses is a potential issue, it becomes important to consider these extreme situations. If this is correct, a philosophical shift may also be necessary, moving conceptualisation towards the consideration of unlikely properties rather than median property values.

To achieve the understanding necessary, much work needs to be undertaken using:

- laboratory experiments;
- field experiments (both borehole-to-borehole and push–pull testing);
- geochemical modelling;

- reactive transport modelling;
- detailed characterisation.

This is a long-term and ambitious goal, and is unlikely to yield results for many years. But there would undoubtedly be important by-products of the research, the main one being the development of a quantitative model of the aquifer surface chemistry which would also be of direct interest in the attenuation and remediation of a wide range of other pollutants, both by sorption and by redox processes.

The apparently extremely rapid movement of viruses in the sandstone, as indicated by previous work and by the full borehole field experiment reported here, suggests that there may well be fundamental issues in solute movement through aquifers which have yet to be fully understood.

8.3 Empirical approaches

The simplest approach to the development of a method for estimating the risk of virus contamination of a groundwater is to undertake detailed monitoring. This monitoring should involve:

- both piezometers and pumped wells;
- the development of a database including all relevant factors including information about:
  - water chemistry;
  - the presence of other microorganisms;
  - well depths;
  - pumping rates and times;
  - local geology;
  - unsaturated zone depth;
  - antecedent weather conditions;
  - land use/cover.

Seasonal variation should also be considered as limited evidence from monitoring at piezometers suggests that there may be a seasonal variation in virus populations related to seasonal variations within the human population.

Early work may be able to establish that certain land uses (e.g. some types of agricultural use) may not be at risk. Eventually this database would provide the means for estimating risk, possibly using correlations with the other environmental factors to refine risk estimates.

The limited survival times of viruses in groundwater mean that there should be little chance of a time-bomb effect such as seen with nitrate and, as such, the collected survey data are likely to be interpretable without the complications of forecasting breakthrough times. Groundwaters at risk from viruses are likely to be only the most recently recharged and some work on monitoring or mapping out age indicators – including chlorofluorocarbons (CFCs) and $^{3}H/^{3}He$ – might also be worth considering. Investigation of the virus occurrence in potential source fluids would support any correlations between virus occurrence and land use data indicated by the monitoring data.

8.4 Other aquifers

Limited evidence of virus movement has been obtained from other UK aquifers, including chalk (Slade 1985), and from evidence collected elsewhere in the world but on aquifer types similar to those present in the UK (e.g. sand and gravel).

In some of these aquifers, the likelihood of mobility of viruses is greater than in the Permo-Triassic sandstones simply because of the dominance of fracturing. In all cases, the two main approaches outlined above for the sandstones are also valid.

8.5 Additional information for follow-on tests required from this project

Completion of this project left a number of questions unanswered. The following sections outline further laboratory and field tests in the Triassic sandstone aquifer required to provide conclusive evidence of bacteriophage adsorption and migration.

8.6 Laboratory tests

Three types of laboratory tests are required:

- laboratory batch and core experiments
- laboratory and field inactivation studies
- box tracer experiments.

8.6.1 Laboratory batch and core experiments

Laboratory batch and core experiments are needed to determine the attachment of a range of enteric viruses/bacteriophage to different aquifer materials.

Batch experiment would incorporate different aquifer materials in conjunction with colloids to determine their influence on attachment and detachment. The aim would be to identify potential human enteric viruses (and their relevant bacteriophage surrogates) and assess the influence of physical and chemical aquifer properties on transport of microbial contaminants within a range of UK aquifers. This would involve design and construction of a number of cores from different aquifer types (chalk, limestone, sandstone, sands and gravels and top soil) through which a number of surrogate bacteriophage or enteric viruses are run, highlighting specific characteristics for transport and attenuation.

8.6.2 Laboratory and field inactivation studies

Long-term laboratory and field inactivation studies would use a range of acidic and alkali bacteriophage/human enteric viruses in combination with different groundwaters, different aquifer materials and colloids. These studies would also include polymerase chain reaction (PCR) analysis of the bacteriophage or viruses to determine if a number of different subpopulations are present with varying inactivation or survival rates.

8.6.3 Box tracer experiments

Box tracer experiments would investigate virus fate and transport on an intermediate scale between the laboratory and field. The use of traditional laboratory column and batch experiments to predict field transport conditions or coefficients has the following limitations:

- Virus transport is known to not be linear with distance, restricting the scaling up of results from laboratory to field.
- Traditional short column experiments address one-dimensional transport and do not address the heterogeneity experienced in the field.
- Microorganism subpopulations appear with differing transport and survival characteristics.

This project would involve design and construction of a two box set-up providing a two-dimensional flow field to determine transport coefficients with distance and heterogeneity (by packing the box with materials of varying permeability or surface charge). The larger box would allow transport investigations in homogeneous or heterogeneous repacked soils over several metres. The smaller box would aid comparisons of scale and design while being packed with undisturbed soil or sand.

Both box set-ups would be equipped with sampling ports and online monitoring equipment throughout their depth and length to provide detailed information on flow paths. Fluorescently labelled colloids and microorganisms would be used to provide continuous results. The box set-up would be suitable for experiments in both unsaturated and saturated conditions, and for testing a range of contaminants.

This initial design and construction phase would include verification experiments using a conservative tracer and bacteriophage. Additional experiments could include viruses, bacteria, protozoa and colloids. This set-up could also be used to test:

- the transport of nanoparticles and determine their decontamination efficiency;
- whether any adverse environmental effects such as harmful by-products remain within the matrix and groundwater.

8.7 Field tests

These would involve:

- virus monitoring programme
- additional field tracer tests at the University of Birmingham site
- injection-recovery test
- risk assessment of virus transport in groundwater sources.

8.7.1 Virus monitoring programme

A UK enteric virus monitoring programme is needed to determine the risks to UK groundwater resources from human enteric viruses.

This aim of this project would be to determine if there is a seasonal variation in the contamination of UK aquifers. Sampling would be completed four times per year for two years at locations throughout the UK and testing a range of different aquifers (chalk, limestone, sandstone, sands and gravels). Samples would be analysed for both

chemical and microbial contamination; bacterial contamination by standard plate counts and contamination by enteric viruses through PCR analysis.

8.7.2 Additional field tracer tests

Additional field tracer tests would be completed on the University of Birmingham field test site. These tests would incorporate a range of bacteriophage (acidic and alkali) in conjunction with conservative tracers (fluorescein) to test different packered fractures with and without overlying clay layers.

Tests would also include continuous injections of lower concentrations (10^4 viruses/litre) of bacteriophage over prolonged periods to determine whether the adsorption capacity of the sandstone aquifer is influenced by both the concentration and volume of contaminates.

8.7.3 Injection-recovery test

An injection-recovery (push–pull) test would provide a quantitative assessment of bacteriophage adsorption to the sandstone matrix along with transport characteristics in bulk sandstone on an intermediate field scale. It would also provide information on bacteriophage die-off over time.

The following assumptions can be made prior to undertaking an injection-recovery test:

- Groundwater transport characteristics will vary depending on the sandstone matrix or the presence of fractures (Figure 8.1).
- Virus transport and attachment will also vary between these heterogeneities. Hence it is planned to carry out the test on non-fractured rock.
- Inactivation rates are known from previous and ongoing studies.
- Filtration during the injection is assumed to be reversible during recovery. Physical filtration does not seem to be constraining flow and can be quantified based on the recovery curve.
- Attachment and detachment will occur. Based on equilibrium theory, the phage should be released from the sandstone as clean groundwater is pumped back to the bore. Therefore, if the test is continued until phages are below detection, it will provide information on the average values of retardation and removal under natural groundwater conditions and for the rock volume affected by phage.
- The recovery curve (see Figure 8.2) is expected to be dominated initially by filtered phage, followed by phage in solution, and then a gradual release of detached phage. This is similar to that found after the back-pumping test described in this report, undertaken one month after conclusion of the phage cocktail test. Note that the latter test was performed on a fracture.

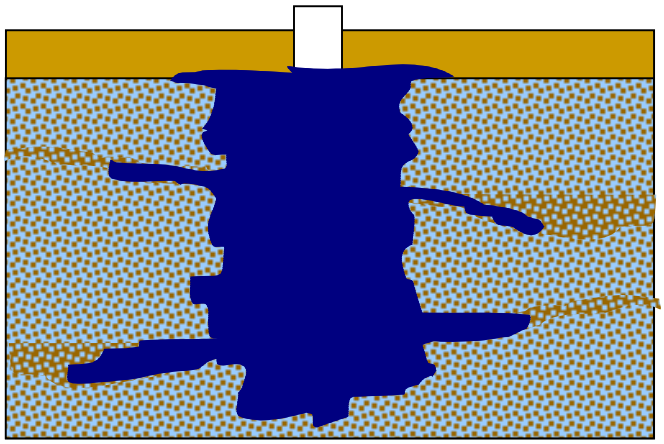


Figure 8.1 Virus and fluorescein plume transport in injection-recovery test

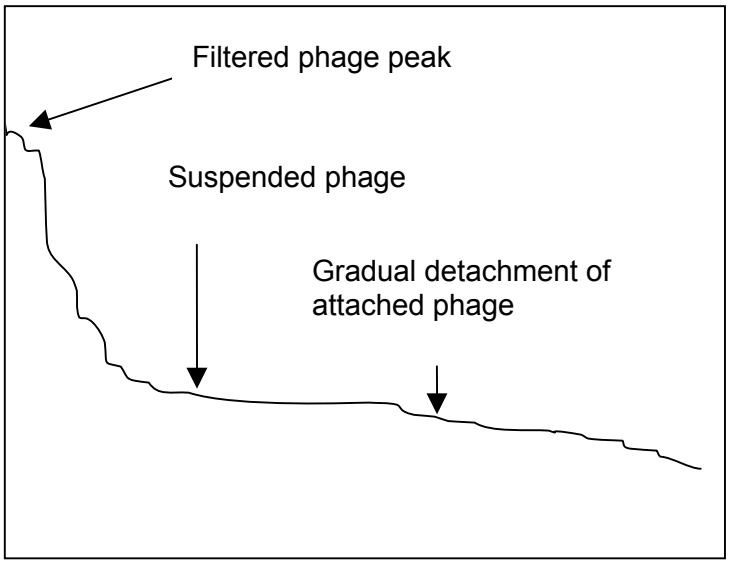


Figure 8.2 Predicted phage recoveries for injection-recovery test

8.7.4 Risk assessment of virus transport in groundwater sources

Recent work undertaken by University of Surrey and the University of Birmingham quantified virus fate and transport in sandstone aquifers. The implications of this work are difficult to quantify due to limitations in direct extrapolations with distance and scenario. Virus removal with distance is not a linear function and will be further distorted by heterogeneities. Virus behaviour can be expected to vary in different soil or stone matrices. Organic colloids present in sewage may interact differently with viruses and soil or stone from silica colloids.

The development of a risk-based model of virus transport in groundwater is proposed to address the likely residence times or distances required to achieve seven log10 reductions before abstraction. The development of such a model would include:

- screening analysis of different risk pathways (e.g. based on slope, soil type and application of a groundwater model to identify groundwater flows);

- review of virus transport and survival coefficients in UK groundwater and internationally, integrated with virus transport studies at the Robens Centre for Public Health (RCPEH) at the University of Surrey;
- detailed analysis of key risk pathways focusing on modelling of virus transport in groundwater based on experimental results and compared with literature reports.

9 Glossary

Adsorption	The process of attachment of a particle from solution onto a collector surface.
Back-scattered electron microscopy (BSEM)	Employs a focussed beam of electrons that is scattered across the surface of the sample. A solid-state detector is used that is sensitive to the electrons that are backscattered from the sample.
Bacteriophage	Viruses that infect certain bacteria.
Cathodoluminescence (CL)	The light emitted by specimens as a result of electron bombardment in a certain wavelength range.
Comminuted	Crushed or fragmentised material.
Coliforms	Coliform bacteria are a commonly-used bacterial indicator of sanitary quality of foods and water
Desorption	Describes the opposite process to adsorption, i.e. from the attached to the aqueous state.
Detrital sediment	Pertaining to particles of rock derived from the mechanical breakdown of pre-existing rocks by weathering and erosion.
DVLO (Derjaguin–Landau–Verwey–Overbeek)	Theory of colloid stability, which is governed by the equilibrium between the repulsive double-layer interactions and attractive van der Waals forces.
Energy dispersive X-ray analysis (EDXA)	Micro-analytical technique based on the characteristic X-ray peaks that are generated when the high-energy beam of the electron microscope interacts with the specimen.
Faecal streptococci (FS)	Group of gram-positive cocci; they are non-sporulating and non-motile bacteria belonging to the genera Enterococcus and Streptococcus. They are useful faecal indicators in that they rarely multiply in temperate climate groundwaters, are more resistant to environmental stress and chlorination than coliforms, and can persist for longer than coliforms in the environment.
Granulation seam	Individual quasi-tabular bands of crushed rock, commonly less than 1 cm in thickness, which are characterised by intense grain size reduction, grain rotation and compaction.
Particle size analysis (PSA)	Textural and grain distribution analysis of materials.
pI	Isoelectric point or charge of the virus or molecule;, the pH at which a molecule in a solution will no longer move in an electric field because it no longer has a net electric charge.
Lyophilised	Dried sample of bacteriophage or bacterial host.
Mini-permeameter	Injects gas into the rock sample through a small compressible tip. The gas flows into the rock and then back out through the surface. The gas flow rate is

	measured with three electronic mass flow meters, allowing calculation of permeability.
Optical televiewer	Generates a continuous oriented 360° image of a borehole wall using an optical imaging system in order to provide detailed, oriented, structural information.
Secondary electron microscopy (SEM)	Performed by scanning a focussed probe across the surface of the sample to be studied.
Sulphite–reducing clostridia	Anaerobic, spore-forming, non-motile gram-positive rods of exclusively faecal origin. The spore nature of this organism means it can survive much longer in water than either coliforms or streptococci (possibly even for years).
Thermotolerant coliform bacteria (TTC)	Members of the total coliform bacteria group that grow at 44°C. They tend to be more closely related to faecal or sewage pollution, and do not generally replicate in groundwater systems. This group of bacteria are variously known as faecal coliforms, presumptive *E. coli* and faecal coliforms (pres.) because, in many cases, the majority of faecal coliforms found in contaminated groundwaters are *E. coli* (though this can vary over quite a range).
Total viable counts (TVCs)	This test aims to grow all heterotrophic culturable bacteria at temperatures that reflect environmental conditions (22°C) and human body temperature (37°C). This can give an indication of the level of culturable indigenous microorganisms in the water.
X-ray diffraction (XRD)	Describes the analysis of X-ray photon scattering.
Zeta potential	Electrostatic potential of a molecule or particle such as a cell (virus/bacteriophage) measured at the plane of hydrodynamic slippage outside the surface of the molecule or cell. Usually measured by electrophoretic mobility. Related to the surface potential and a measure of the electrostatic forces of repulsion the particle or molecule is likely to meet when encountering another of the same sign of charge.

List of abbreviations

BGS	British Geological Survey
BH	borehole
BSEM	back-scattered electron microscopy
CCTV	closed circuit television
CEC	cation exchange capacity
CFU	colony forming units
CL	cathodoluminescence
DLVO	Derjaguin–Landau–Verwey–Overbeek [theory]
EDXA	energy dispersive X-ray analysis
HDPE	high density polyethylene
mbgl	metres below ground level
ML/d	million litres per day
MPN	most probable number
NFU	Nephelometric turbidity units
PCR	polymerase chain reaction
PFU	plaque forming units
pfu/ml	plaque forming unit per millilitre
pl	isoelectric point
ppb	parts per billion
ppm	parts per million
PSA	particle size analysis
PVC	polyvinyl chloride
PVDF	polyvinylidene fluoride
SEM	scanning electron microscopy
SRC	sulphite-reducing clostridia
Sst	sandstone
TC	total coliforms
TVC	total viable count
XRD	X-ray diffraction

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