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Fluorescent polystyrene microspheres as tracers of colloidal and particulate materials: Examples of their use and developments in analytical technique

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ABSTRACT: Fluorescent polystyrene latex microspheres are utilised widely in biological and medical applications but they also offer potential as tracers of particle movement in the sub-surface. Microspheres, manufactured in a variety of sizes, are available dyed with any one of a number of fluorescent dyes. This allows several differently sized particles to be investigated simultaneously. Comparatively few investigations of their transport through geologic media have been reported but where they have been used they have been enumerated by epifluorescence microscopy to permit the calculation of their concentration in the aqueous phase. This procedure is both laborious and time consuming and provides no information on the disposition of the microspheres filtered or sorbed by the geologic matrix. A method has therefore been developed to allow a much more rapid and sensitive determination of the microsphere content in waters and in solid geologic matrices. It has proved useful in both laboratory column experiments and field investigations concerning migration. Two example applications of microspheres in hydrogeological investigations are described in the paper.

1. INTRODUCTION

Tracer testing has been shown in countless cases to be an extremely useful practical technique for investigating hydrogeological problems and in general suitable tracers can be selected to satisfy operational requirements. Most readily available tracers are however solute tracers and these are not ideal for colloid or particulate migration studies except for comparative purposes. The range of colloids/particles which are available for groundwater tracing is somewhat limited. Those which have been used include colloidal silica particles, radio-labelled polymeric materials, bacteria and bacteriophage. In most cases, the tracers are either not easy to prepare or are very time consuming to analyse.

Another potential suite of particulate tracers is fluorescent polystyrene latex microspheres. Although these tracers are not new, their application in medical and microbiological research has been widely reported, application in the field of hydrogeology appears to be limited to tracing groundwaters to investigate the migration of bacteria (Harvey et al, 1989 and 1993), evaluating drilling fluid contamination (Chapelle and McMahon, 1991) and measuring fracture apertures in clay (Hinsby et al, 1996). In each of these cases, analysis and quantification of the microspheres was by epifluorescence microscopy - a relatively slow and time consuming technique which results in very little advantage over the other particulate tracers.

However, the recent developments in the analytical techniques which are reported in this paper for fluorescent polystyrene latex microspheres have resulted in a considerable improvement in detecting and measuring the tracer. It has resulted in a suite of tracers which are now more cost-effective, versatile, sensitive and rapidly analysed. To demonstrate the use of the tracer, examples of laboratory and field experiments for studying specific natural processes are described.

2. FLUORESCENT MICROSPHERES

The microspheres used in the work described in this paper are manufactured by Polysciences Inc. and are available in a variety of diameter sizes ranging typically from 0.05 - 90.0 μm. Prepared by surfactant-free emulsion polymerization, the polystyrene latex beads have hydrophobic fluorescent dyes incorporated into their structures during synthesis. The fluorescent dyes are similar to those used commonly in groundwater tracing. The microspheres can be supplied as a suspension in pure de-ionised water at a concentration of 2.5% w/v of beads in aqueous suspension. The density of the microspheres is 1.055 g/ml.

Two types of bead are available - plain microspheres and carboxylated microspheres. Both types of bead have a low level of negatively charged sulphate groups associated with them but in the case

of the carboxylated microspheres, the surfaces are deliberately functionalised by negatively charged carboxyl groups as well. This offers a wider choice of tracer. Different surface charge particles can be selected to simulate different particle properties or in response to rock matrix properties depending on the nature of the experiment.

2.1 *Previous methods of analysis*

Analysis of samples for the detection and quantification of fluorescent microspheres has until now been performed by epifluorescence microscopy. This technique, which has been reported widely in the literature (Harvey et al, 1984 and Jones, 1979), involves filtration of the sample on to a black membrane filter which is then placed under a microscope. A light source of suitable wavelength (usually UV) is then selected and deflected by a dichroic mirror down through the objective on to the filter surface. The light from the fluorescing particles, which is of lower energy than that used for excitation, passes back up the objective, through the dichroic mirror to the eyepiece. Suitable filters can also be used to reduce interference and improve detection. Quantification is made by manually counting the microspheres for a known area of filter paper, determining a total number for the whole paper and a concentration by relating to the volume of sample filtered. Variability in the properties of the filter paper may however result in erroneous results because only small areas of the paper are viewed at any one time and the more areas which are viewed to improve confidence is very time consuming, tedious and impractical where large numbers of samples require analysis.

2.2 *Development of new method for analysis*

In order to improve efficiency and allow greater numbers of samples to be analysed rapidly, we have developed a new technique for identifying and quantifying fluorescent microspheres. The technique can be used for analysing both water samples and solid samples (to investigate sorption or straining) (Harrison, 1996).

The nature of the dyes used in the manufacture of the microspheres is similar to the fluorescent dyes commonly used in groundwater tracing. It therefore became clear that similar techniques, as those used for fluorescent dye tracers, should be applicable for the microspheres. However, whilst truly colloidal particles which are kept in suspension by Brownian motion should be analysable without any sample preparation, those particles which were larger (> 1 μm) and had a tendency to settle may present problems when instrument calibration and subsequent sample analyses is performed.

To overcome this problem, a known volume of sample was filtered to trap the microspheres (filter size was selected on the basis of microsphere size). The beads were then preferentially eluted by passing a known volume of Acetone AR through the filter. Due to the nature of the microspheres, the Acetone effectively dissolved the polystyrene matrix of the microspheres releasing the fluorescent dye into solution. Both nylon 66 and inorganic membranes were found to retain and elute the beads quantitatively and reproducibly.

This methods was found to have a number of clear advantages. Firstly, any extraneous insoluble particulate material which may have had an interfering effect could be filtered out. Secondly, the microspheres are manufactured with the dye incorporated within the whole bead. Dissolution resulted in a release of all of the dye which in solution was then able to fluoresce. Whilst the bead remained intact, only the dye at or close to the surface of the microsphere was available. Analysis of the dissolved microspheres resulted in a significant increase in sensitivity. The difference for two differently dyed beads is shown in Figure 1. Analysis of samples can be performed using any fluorescence spectrophotometer which is suitable for groundwater dye tracing, however care must be taken to avoid evaporation of the acetone. In the experiments reported here, an Hitachi F2000 instrument was used with 5 ml quartz glass cuvettes. The measured detection limits of the microspheres in water and acetone with this instrument (without pre-concentration) were better than 10 μg/l for blue and green microspheres in water and 1 μg/l in acetone.

The third advantage of the analytical method outlined above is the ability to concentrate samples in order to improve detection. Large volumes of water sample may be filtered and then eluted with acetone using a relatively small volume of solvent. The amount of sample concentration is effectively restricted only by the amount of 'raw' sample available.

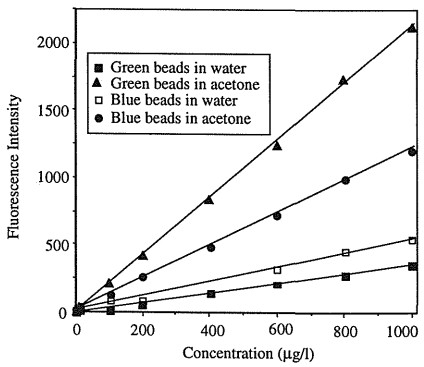

Figure 1. Calibration curves for two microsphere types in water and acetone.

2.3 *Analysis of solid samples*

Where water-rock interaction processes are of interest tracer tests can play an important part in developing an understanding. Breakthrough curves can yield important information but do not enable the distribution of tracer within the water-rock system to be physically investigated. In order to do this samples of the matrix are required to be examined. This can prove extremely difficult in many cases but where fluorescent microspheres are used detailed analysis is possible. Cores of rock which have been exposed to the tracer, fracture faces and adjacent matrix material can all be examined by mixing the solid material with acetone to dissolve and release the dye from the sorbed or trapped microspheres. Fluorimetric analysis can then yield valuable information on water-colloid/particle-rock interaction and quantitative results.

3 EXAMPLES OF MICROSPHERE USE

Two examples of the use of fluorescent microspheres as tracers to investigate particulate movement in aquifer material are presented below. In the first case, the test reported was part of an investigation to study the rôle of colloids in radio-nuclide transport (Harrison, 1996). In the second case, the microspheres were used to investigate the potential for pathogen migration from the surface, through the unsaturated zone, to the water table and a major water supply aquifer.

3.1 *Migration through glacial sand*

This laboratory scale experiment used 6.5 cm long glass columns packed with saturated glacial sand. To simulate groundwater flow, native filtered groundwater was pumped through the columns until steady-state conditions were obtained, i.e. a constant flow rate. Groundwater was pumped through the columns for at least 48 hours prior to any tracer testing.

Once steady-state conditions had been established, a conservative tracer, 0.2 ml of $Na^{36}Cl$ (ß-emitter) was injected on to the columns at the inlet end by means of a sample loop (Figure 2). A fraction collector was used to sample the effluent from the columns for subsequent analysis. Scintillation counting was then performed on the samples to determine the breakthrough curve of the conservative tracer for each of the sand columns used.

After completion of the tests using a conservative tracer, a tracer test using the fluorescent microspheres was performed. Bead suspensions containing 2.5% w/v (as supplied by the manufacturer) were diluted ten times using groundwater. Again 0.2 ml of the diluted tracer was injected via sample loop on to the column and the

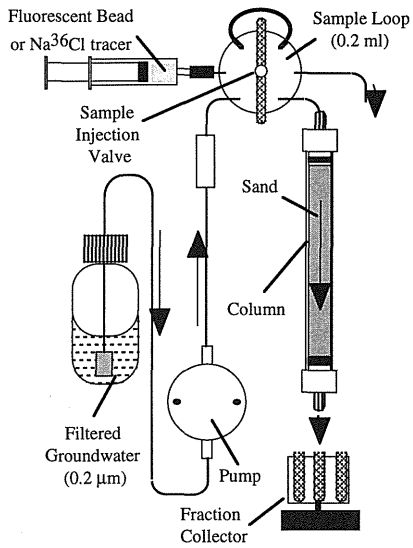

Figure 2. Column experiment apparatus.

effluent sampled by fraction collector.

The 5 ml of the collected fractions were then filtered and the filters eluted with 5 ml of acetone, as described above, prior to analysis. Where analysis revealed low concentrations of beads, preparation of samples was repeated using a concentration step to improve detection and quantification.

Once a complete breakthrough curve had been obtained, the flow through the column was stopped and the column material (core) extruded. As the core emerged from the glass column it was sectioned, with each section sample placed in a glass bottle of known weight. A measurement of the length of core extruded in each section/sample was also recorded. Samples were then dried for 24 hours (at 100° C) before being weighed. The samples were then broken up by hand and 5 ml of acetone AR added to each bottle before sealing. The bottles were shaken for 2 hours to allow complete dissolution of the microspheres before a small volume was filtered and analysed using the fluorescence spectrophotometer.

Figure 3 shows the comparison between conservative and microsphere tracer breakthrough curves for a column experiment and the distribution of the 'trapped' colloids unaccounted for by the breakthrough curve within the column. Results of these column tests were subsequently used to design field scale tracer test and enabled a study of the potential rôle that colloids can play in contaminant (radionuclide) migration.

3.2 *Migration through unsaturated fissured chalk.*

This field experiment was aimed at investigating the

controlling mechanisms of pathogen migration. An area of unconfined chalk was selected with an unsaturated zone approximately 20 m deep. The top soil of this area measuring 7 m x 7 m was removed to expose the surface of the chalk. At the centre of this area, a borehole (cased to 20 m) was drilled to 24 m below ground level and a sampling pump installed with an intake at 23 m below ground level.

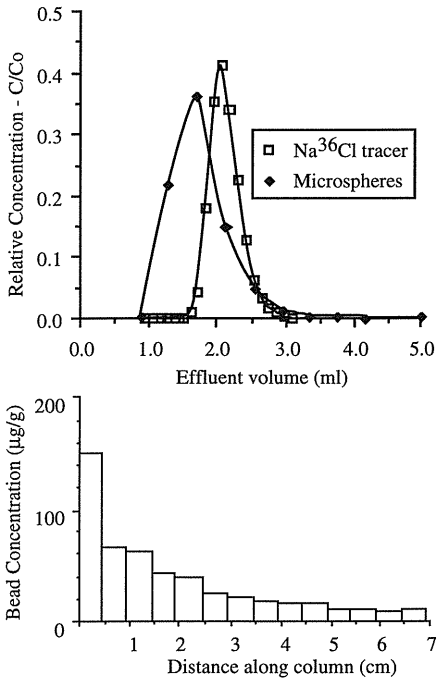

Figure 3. Column breakthrough curves and distribution of trapped beads.

The design of the tracer test was to inject a series of tracers evenly over the exposed area of chalk and monitor their arrival at the water table. Subsequent drilling within the area would then allow core to be recovered and pathways of tracer migration within the unsaturated zone to be identified. A range of tracers were chosen which included LiBr (conservative solute tracer), bacteriophage (0.1 µm) and three different sized and dyed microspheres (1, 6 and 10 µm).

Prior to injection, the site was irrigated with water to saturate and flush through the unsaturated zone. Tracer injection was then completed and further light irrigation of the site continued to simulate recharge. After injection the site was covered to reduce evaporative losses and to shade the site to reduce photochemical decay of the fluorescent dyes used in the manufacture of the microspheres.

Groundwater sampled from below the water table was analysed for each of the tracers. For the microspheres, a volume (1 litre) of groundwater was filtered through a 0.45 µm nylon filter and the presence of beads determined by both epifluoresence microscopy and fluorescence spectrophotometry. Presence of naturally fluorescent solid particles in the groundwater which were trapped on the filter paper made analysis by microscopy difficult. However the acetone elution significantly improved analysis by separating insoluble material and allowing more controlled fluorescence analysis.

Interpretation of the microsphere results and comparison with the other tracers showed that particles of 6 µm diameter and below could migrate rapidly through the unsaturated zone to the water table. The calculated percentage recovery of each of the tracers at the water was 0.01% for LiBr, 0.00001% for the bacteriophage, 0.01% for the 1.0 µm microspheres and <0.001% for the 6.0 µm microspheres. The approximate travel times for the tracers from the surface to the water table are shown in Table 1.

Table 1. Travel times of tracers through unsaturated zone to water table.

Tracer	Travel time (h)
Li^+ (as LiBr)	72.0
Bacteriophage 0.1 µm	18.5
Microspheres 1.0 µm	15.0
Microspheres 6.0 µm	18.5

Vertical distance between injection point and water table = 20 m.

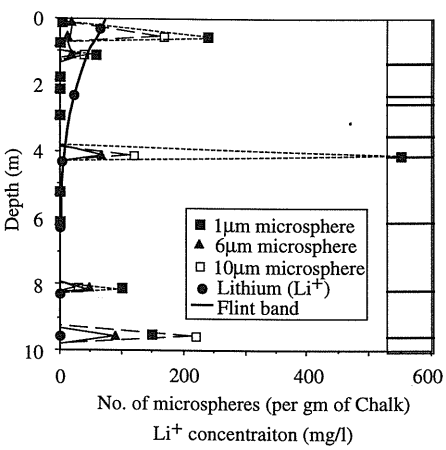

Figure 4. Distribution of microspheres, Li^+ and flint bands in the unsaturated zone.

After sufficient time had elapsed and no further tracer was measurable at the water table, two additional boreholes were drilled in the test area to a depth of 10 m. Core was recovered for each of these to investigate vertical distribution of tracers within the

unsaturated zone. Selected samples were examined for LiBr and microspheres. For LiBr (Li^+), pore water samples were extracted by centrifugation. For the microspheres, samples of chalk were prepared and analysed using the method described earlier for the solid material analysis in the column experiments. For this experiment, larger samples ranging from 0.6 - 1.6 kg were used. Comparison of the results for the microspheres showed that the vertical distribution was not uniform. Specific horizons, which appeared to coincide with flint bands, revealed the presence of microspheres but in between no beads were detected. The distribution of microspheres in one of the cores is shown in Figure 4. In the case of the LiBr tracer an exponentially decreasing concentration with depth was observed.

Interpretation showed that particle migration can occur via discrete vertical pathways within the unsaturated zone which intersect horizontal bedding plane features associated with flint bands. Migration of the particles into the matrix of the chalk is precluded because of the small pore throat apertures (<1 μm) whereas the LiBr, as a solute, can move into the matrix. This explains the observed Li^+ concentration distribution.

4. CONCLUSIONS

Developments in analytical techniques for the quantitative determination of fluorescent microspheres in both aqueous and solid samples have improved efficiency, confidence and versatility in the use of these materials as groundwater tracers The microspheres are available in a wide range of sizes, with different surface charge properties and dyed with different coloured fluorescent dyes enabling their use in a wide-range of situations alone or in combination.

Two examples of microsphere use for investigating highly relevant, but different, environmental problems have been described. Their use in both cases was successful clearly demonstrating their relevance and suitability as an investigative tool for examining groundwater and contaminant transport and behaviour.

5. ACKNOWLEDGMENTS

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