



## Development of Fluorescent Spectroscopic Techniques for Fluorescent Microsphere Enumeration

O. Pryce, J. Quinton, and L. Heathwaite

Lancaster University, Environmental Science, Lancaster, United Kingdom (o.pryce@lancaster.ac.uk)

### Abstract

The use of microspheres is becoming increasingly common for the tracing of flow pathways of colloids and solutes in the saturated and unsaturated zones. A common enumeration method for microspheres is epi-fluorescent microscopy; this method can be heavily time consuming, especially if automated counting is not available as multiple measurements have to be taken of a single sample. The use of fluorescent spectroscopy for microsphere enumeration has been successful for analysis of samples from the saturated zone, providing rapid analysis. The speed of analysis, sensitivity, and in certain cases portability of fluorescent spectroscopy make it an idea for fluorescent tracer analysis. Unfortunately the presence of sediment and dissolved organic matter (DOM) in samples limits its potential for unsaturated zone microsphere research. We report on the development of sample preparation methods designed to separate microspheres from particulates and DOM, to allow analysis via fluorescent spectroscopy.

Three different methods were examined for sample preparation. Firstly, microspheres with a diameter of 22 nm were separated from particulates by filtration (0.45  $\mu\text{m}$ ). This highlighted the ability of fluorescent spectroscopy to enumerate microspheres regardless of their size. Secondly, larger microspheres (2.2 - 0.25  $\mu\text{m}$ ) pre-filtered at a range of pore sizes (30 - 2.7  $\mu\text{m}$ ) to remove sediment, and were subsequently captured by filtration (0.2/0.45  $\mu\text{m}$ ) and dissolved. Thirdly, microsphere (2.2  $\mu\text{m}$  - 22 nm) were centrifuged, followed by either direct analysis of the supernatant or dissolution. Results were heavily dependent upon microsphere diameter and sediment concentration; however it was shown that microspheres could be successfully enumerated from samples containing sediment and DOM, and that this provides a more rapid alternative to epi-fluorescent microscopy. The increase in speed of analysis will allow improved research into the flow pathways of colloids and solutes in the vadose zone using fluorescent microspheres.