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Abstract

Microspheres have become an important material in the biomedical and environmental sciences, particularly for use in the detection of pathogenic microorganisms and toxins as well as for use as carriers in drug delivery. In this study, their use in microbial detection, with particular emphasis on immunochromatographic assays, was investigated. Two main types of microspheres were studied: colloidal gold and polymeric. The original plan to combine the use of colloidal gold and fluorescent-dye-labelled PMMA microspheres as a signal generator, in order to enhance detection signals and improve detection limit, was abandoned when preliminary detection experiments showed that the use of colloidal gold was not so beneficial after all, taking into account the amount of light lost to absorption by the gold particles. Therefore, it was decided to use Rhodamine B-labelled PMMA microspheres. PMMA particles, both unlabelled and internally labelled with Rhodamine B, were synthesised by emulsion polymerisation and yielded monodisperse particles of around 200 nm and 300 nm, respectively. An attempt to co-polymerise MMA with HEMA to form 200 nm-sized monodisperse P(MMA-HEMA) microspheres in order to create functional –OH groups on the microsphere surface to be used in chemical covalent coupling with monoclonal antibodies resulted in aggregated microspheres and non-uniform particles, and were therefore not used for covalent coupling. Attachment of monoclonal antibodies onto the surface of Rhodamine-B labelled PMMA microspheres by passive adsorption also resulted in aggregated particles. Diffusion and detection experiments were carried out on the Rhodamine-B labelled PMMA microspheres. Diffusion of PMMA microspheres along a nitrocellulose strip was found to be 42% slower than the diffusion of colloidal gold along a nitrocellulose strip. Using a spectrophotometer, detection experiments were performed on dilutions of an original stock solution of 1% w/v PMMA in distilled water. The detection limit was found to be of the order of 10^{-3}.

This study has investigated the materials that constitute an immunochromatographic assay and has illustrated some of the complications associated with the synthesis of copolymer microspheres and immobilisation of antibodies onto their surfaces. Further work on how to improve on the methods discussed in this study have also been recommended.