

PROGRESS REPORT FOR AINGRA07006P

PROJECT TITLE	Radiolabelling of functional polymeric microspheres	
INVESTIGATOR(S)	Institution and Department	
Chief Investigator	Dr Leonie Barner	School of Chemical Sciences and Engineering, The University of New South Wales
Other Investigators		
Other Investigators	Raymond Joso	
ANSTO Investigators	Suzanne Smith	
Specialist Committee	M	

SCIENTIFIC OBJECTIVES

Functional microspheres with diameters in the range of 1 to 100 μm have found various applications in life sciences and in biotechnology – particularly surface functionalized microspheres that can be employed as colloidal support for latex diagnostic kits. Microspheres are attractive devices for biomedical applications as they are small in size and volume, have large specific surface area, feature the ability to diffuse and can form stable dispersions.

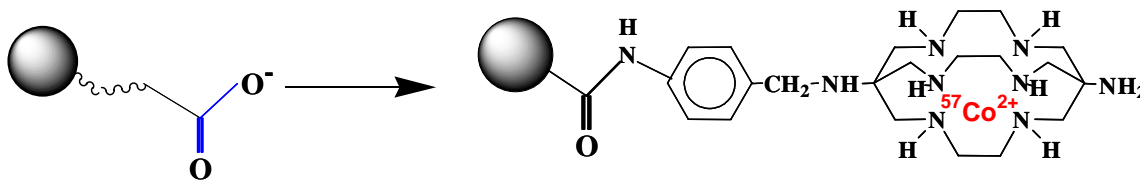
Core poly(divinylbenzene) microspheres containing functional end groups have been employed to graft poly(ethylene glycol) methyl ether acrylate (PEG-MEA) from their surface using reversible addition fragmentation chain transfer (RAFT) polymerization. The RAFT agent, 3 – benzylsulfanylthiocarbonylsulfanyl propionic acid, was used to mediate the grafting reaction which leads to grafted PEG-MEA with RAFT end functional groups (carboxylic acid groups) at the surface.



Since the grafted polymeric chains are difficult to cleave from the surface, it is difficult to obtain quantitative surface information by conventional characterization techniques (e.g. size exclusion chromatography). In this study, we introduce the application of radioisotope labeling to characterize polymer surfaces. (e.g. quantification of active groups on the surface).

PROGRESS REPORT and RESEARCH OUTCOMES

The functional carboxylic acid groups can be further functionalized to covalently conjugate a new hexaazabicyclo[6.6.6]eicosane-1,8-diamine (SarAr) ligand. The conjugation of SarAr via its amine functional group to the RAFT acid end groups on the surface of the microspheres was activated by carbodiimide 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). The conjugation reaction proceeds within 1 hour at 25°C. The ligand SarAr was designed with an additional linking strand incorporating two nitrogen atoms, to form a three dimensional 'cage' around radiotracer metal ions (e.g. $^{57}\text{Co(II)}$) resulting in stable complex cations.



SarAr rapidly (e.g. in minutes) complexes metal ions quantitatively (one metal to one ligand) in micromolar concentrations. Labeled microspheres were then recovered by centrifugation and washed several times with 2-(*N*-morpholino) ethane sulfonic acid (MES). The radioactivity from the microspheres from the washing supernatants was measured on a Gamma Counter. Note that the numbers of grafted chain are related to the numbers of $^{57}\text{Co}(\text{II})$ attached to the microspheres. From the saturation curve the number of grafted polymeric chains available on the surface can easily be quantified.

DATA

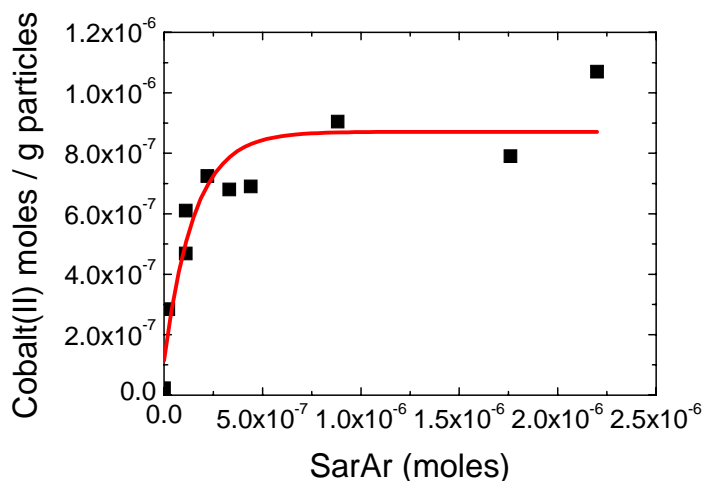


Figure 1: $^{57}\text{Cobalt}(\text{II})$ (moles) covalently bound to the microspheres. The number of active sites is related to the moles of $\text{Co}(\text{II})$ attached to microspheres. The saturation curve shows that the number of available sites is $\sim 8 \times 10^{-7}$ moles per gram of microspheres

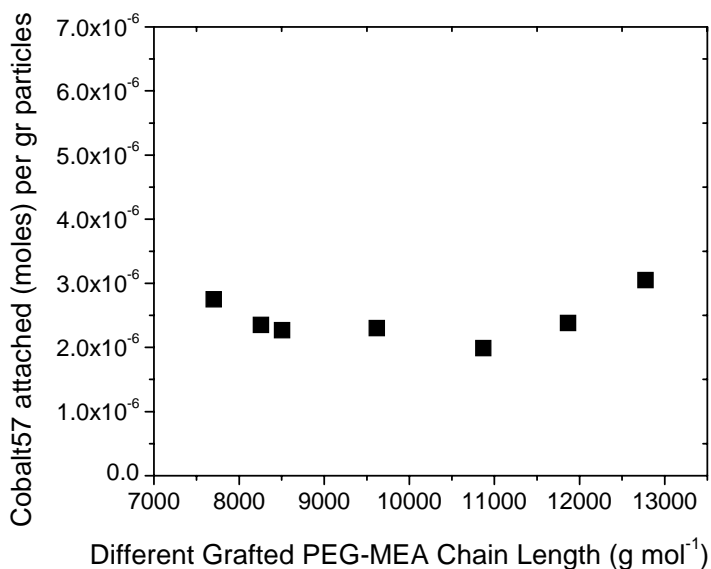


Figure 2: 7 different grafted chain lengths were synthesized (Molecular Weight: 7000 – 12500 g/mol). It was found that there is no influence of the grafted chain length on the number of available sites on the microspheres.

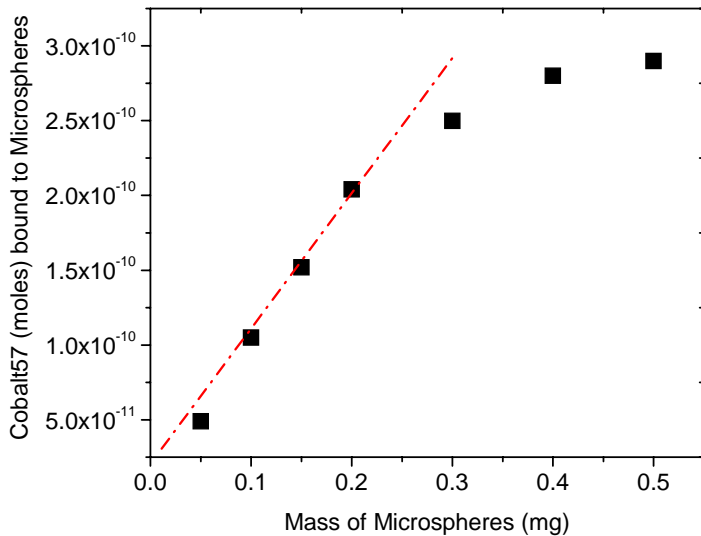


Figure 3. The result shows that there is a linear increase in the amount of Cobalt57 bound to microspheres when more microspheres were added. This also validates our experimental procedure.

Signature of Investigator preparing the report for After signing this report please fax this page with your signature for our files	Proj: AINGRA07006P Date:

PUBLICATIONS / REPORTS arising as a result of your work.

1: Functional Hydrophilic Microspheres for Biotechnology Application. Joso, R., Huang, S., Bulmus, V., Barner-Kowollik, C., Stenzel, H. M., Davis, T. P., Smith, V. S., Barner, L. 29th Australasian Polymer Symposium, Hobart Tasmania. 11-15 February 2007. (Poster Presentation), (s, c)

2: Surface Characterization of Microspheres via a Radiolabelling Technique. Joso, R., Huang, S., Smith, S. V., Barner, L. IUPAC International Symposium on Ionic Polymerization 2007, Kloster Banz – Germany. 2-7 September 2007. (Poster Presentation) (s, c)

In addition, three manuscripts are in preparation.

PhD STUDENTS

Raymond Joso: The anticipated date of conferment of his PhD award is August 2008. The tentative title of his thesis is: 'Synthesis of Functional Core-Shell Microspheres via controlled/living polymerization techniques'