**PROGRESS REPORT FOR AINGRA07006P**

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<th>PROJECT TITLE</th>
<th>Radiolabelling of functional polymeric microspheres</th>
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**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 ethyl 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 acids groups) at the surface.

![Polyethylene glycol methyl ether acrylate](image)

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 hexaaazabicyclo[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. $^{57}$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:** $^{57}\text{Co}$ (II) (moles) covalently bound to the microspheres. The number of active sites is related to the moles of Co(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.

PUBLICATIONS / REPORTS arising as a result of your work.


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’