Functionalized paramagnetic nanoparticles for waste water treatment†

Ilona Urban, a Norman M. Ratcliffe, a John R. Duffield, a George R. Elder b and David Patton a

Received 10th December 2009, Accepted 4th May 2010
First published as an Advance Article on the web 25th May 2010
DOI: 10.1039/b925933c

An approach to the design, development and implementation of a new separation technology for use in the decontamination of radioactive waste streams is reported here. Calixarene-crown-6 derivatives with terminal carboxyl groups were synthesised and attached to nano-sized magnetoferritin molecules and their ability to sequester radioactive caesium(II) ions from aqueous solution was demonstrated.

Although traditional techniques for removing radioactive impurities produce pure water, they are either not selective and/or they produce large volumes of secondary waste. This is especially the case when impurities are present in very low molar concentrations compared to other harmless species. Hence, there is an urgent need for economic and environmental reasons to develop new separation technologies to selectively remove only the radioactive ions while leaving the ‘harmless’ elements in water. The operational parameters required for the new separation systems are high specificity and high complexation capacity for the trace elements. In addition, they should be easily separated from the treated liquid, and should produce minimal amounts of secondary waste. Recently, we have researched the potential of a new separation technology based on the attachment of ion selective chelating molecules to paramagnetic nano-sized particles so that the resultant conjugates can be used to target and extract trace amounts of ions selectively from aqueous waste streams.

Ion selective bifunctional chelating agents can be synthesised for a wide range of nuclides. The attachment of these chelating agents to discrete nano-sized paramagnetic particles has several advantages: nano-sized particles possess high surface area enabling the attachment of a large number of chelating agents to their surface, hence the removal of more contaminants per volume of material. They are also very small compared to the micron-sized particles used in traditional clean-up methods, therefore they have the potential to significantly reduce the volume of secondary waste. Moreover, paramagnetic nanoparticles can be removed easily from aqueous solution by magnetic filtration which is a well proven separation technology used in the steel and mineral processing industries.

Magnetoferritin, a magnetic variety of the naturally occurring iron storage protein ferritin, was chosen and tested as a possible paramagnetic nanoparticle because it is easily dispersed in aqueous systems, its size is of the right order of magnitude (ca. 12 nm diameter) and its magnetic susceptibility is uniform and sufficient to respond to magnetic filtration. In addition, magnetoferritin possesses surface functional groups which make it possible to couple other molecules to its surface.

Our approach was to synthesise and attach a caesium specific chelating agent to magnetoferritin because 137Cs is a major radiation source in radioactive waste water and is strictly controlled regarding its release to the environment. We chose calixarene derivatives as putative chelating agents because their exceptional affinity for caesium is well documented. Moreover, their cavity size and hence selectivity can be modified which could be useful in future applications: if the synthesis, attachment and caesium separation of the chosen derivative are successful it opens up the possibility to build a family of chelating agents based on calixarenes to target other problematic ions in waste water treatment.

While magnetoferritin is relatively easy to produce (Nanomagnetics, University Gate East, Park Row, Bristol, UK BS1 5UB, following the procedure by Wong et al. 12), there have been only a few reports on its applications. Similarly, literature on the attachment of calixarene derivatives to macromolecules is also very scarce. Although 1,3-calix[4]arene-biscrown-6 derivatives have excellent affinity towards caesium, they have no available functional groups through which the attachment to magnetoferritin could be achieved. However, previously it has been shown that when one of the crowns has been replaced by two hydrocarbon chains there are insignificant changes in selectivity. According to Arnaud-Neu et al. for example, the Cs/Na selectivity of dioctyl calix[4]arene-crown-6 was excellent, in the region of 33 000. Therefore it was decided to synthesise 25,27-bis[(3-carboxypropyl)oxy]calix[4]arene-crown-6 having two hydrocarbon chains with terminal carboxyl groups as shown in Scheme 1. Purification by flash chromatography was undertaken at each step, the overall yield to the final product was about 20%. High resolution MS, 1H NMR, 13C NMR and IR spectra are consistent with the product structure and intermediates.

Before attachment of the calixarene to magnetoferritin its capability to sequester caesium(II) ions from solutions was assessed. 25,27-Bis[(3-carboxypropyl)oxy]calix[4]arene-crown-6 (2.4 mg, 2.87 × 10⁻³ mmol) was dissolved in deuterated chloroform (2 ml) while solid caesium picate (17.8 mg, 4.93 × 10⁻² mmol) was dissolved in deuterated water (20 ml). Solvent extraction was performed by mixing the calixarene solution (0.8 ml, 1.15 × 10⁻³ mmol) with the caesium picate (0.8 ml, 0.2 × 10⁻³ mmol) solution for three minutes. 1H NMR spectroscopy was used as a rapid initial methodology for...
caesium chloride (92.5 Bq/ml) and caesium picrate (2.49 Bq/ml) was then shaken with a mixture of the diluted radioactive caesium mixture. The resultant emulsions were left to separate overnight. The caesium activity in the organic (1 ml) and aqueous phases (1 ml) was determined using a LKB-Wallac 1282 CompuGamma microcomputer controlled universal gamma counter.

The activity in the organic phase containing the chelating agent increased significantly after solvent extraction with a concomitant decrease in the aqueous phase indicating that about 42% of the radioactive caesium was extracted. Meanwhile control experiments showed that insignificant caesium extraction (only 1%) occurred in the absence of calixarene (Table 1).

To enable the use of the synthesized compound in the proposed new magnetic separation technology it has to be attached to magnetoferritin in such a way that its ability to separate caesium(i) ions is retained. Amine groups of macromolecules are the most often targeted functional groups for the attachment of specific chelating agents through the formation of amide bonds by activated carboxylic acids. Many activation methods were assessed to maximize the number of calixarene molecules bound to the surface of magnetoferritin in a consistent and reproducible manner. This task proved to be very difficult since the calixarene derivative was only soluble in organic solvents in which the magnetoferritin became very unstable. However, formation of acyl halides was found to be the most effective for the attachment of 25,27-bis[(3-carboxypropyl)oxy]calix[4]arene-crown-6 to magnetoferritin (Scheme 2). Acyl fluoride was particularly useful, due most likely to its much lower rate of hydrolysis compared to other acyl halides. High resolution MS, 1H NMR, 13C NMR and IR spectra were consistent with the acyl fluoride, which was used for the attachment to magnetoferritin without further purification. Buffered (HEPES, 0.05 mol dm⁻³, pH 8.6) magnetoferritin solution (5.5 ml containing 11 mg, 1.2 × 10⁻⁵ mmol magnetoferritin) was added to sodium bicarbonate solution (14.5 ml, 0.1 mol dm⁻³) while 25,27-bis[(3-carboxypropyl)oxy]calix[4]arene-crown-6 fluoride (34.1 mg, 4.1 × 10⁻⁵ mol) was dissolved in DMSO (1 ml). The dissolved fluoride (0.5 ml, 2.05 × 10⁻⁵ mol) was mixed with the prepared magnetoferritin solution (20 ml, 1.2 × 10⁻⁵ mmol magnetoferritin) and left at room temperature overnight. A control solution was also prepared by mixing the magnetoferritin solution (20 ml, 1.2 × 10⁻⁵ mmol magnetoferritin) with DMSO (0.5 ml). The following day the sample and control solutions were cleaned by dialysis and used to sequester radioactive caesium from solution.

Radioactive caesium (2 ml, 370 Bq/ml) was added to three solutions (1 ml each): one containing the functionalized magnetoferritin, another the magnetoferritin without chelating agent and the third deionized water. The solutions were left to equilibrate for about 1 h and loaded into a separation column with the magnetic field on (Fig. 1). The column was washed with water and the effluent was collected in fractions (3 × 10 ml). After the third fraction the magnetic field was switched off and three further fractions were collected. The radioactivity in each fraction was measured and the activities were calculated. Table 2 lists these values.

The activity of the deionised water was only tested to obtain information on the time needed for the radioactive caesium to leave the column. Results showed that 90% of the radioactivity left the system with the magnetic field still on. A similar trend was observed when the control magnetoferritin was

---

**Table 1** Data to show the ability of 25,27-bis[(3-carboxypropyl)oxy]calix[4]arene-crown-6 to complex radioactive caesium

<table>
<thead>
<tr>
<th></th>
<th>Activity before solvent extraction (Bq ml⁻¹)</th>
<th>Activity after solvent extraction (Bq ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>Control</td>
</tr>
<tr>
<td>Organic phase</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aqueous phase</td>
<td>92.3</td>
<td>95.8</td>
</tr>
</tbody>
</table>

---

**Scheme 2** Formation of acyl fluoride and its attachment to ferritin.
Fig. 1 Schematic of a magnetic separation set up for purifying waste water.

Table 2 Extraction of radioactive caesium from aqueous solutions using functionalized magnetoferritin nanoparticles

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Blank water</th>
<th>Blank magnetoferritin</th>
<th>Functionalized magnetoferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic field on</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.80</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>48</td>
<td>47.9</td>
</tr>
<tr>
<td>Magnetic field off</td>
<td>4</td>
<td>5.8</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

filtered and can be further concentrated by evaporation. These nanoparticles are up to five orders of magnitude smaller than the existing ion exchange beads and they are ion specific due to their functionalisation. Using these particles can reduce the volume of secondary waste significantly, which is desirable for both economic and environmental reasons.

Further work to scale-up syntheses and to produce small scale separation equipment is under way.

We acknowledge the financial support of Bradtec and EPRI for funding a PhD studentship for Ilona Urban.

Notes and references