Functionalized paramagnetic nanoparticles for waste water treatment†

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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(I) 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.[1] 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.[2,3] 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.[4,5]

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)[6,7] 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 ^{137}Cs is a major radiation source in radioactive waste water and is strictly controlled regarding its release to the environment.[8] We chose calixarene derivatives as putative chelating agents because their exceptional affinity for caesium is well documented.[9–11] 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.[13–16] Similarly, literature on the attachment of calixarene derivatives to macromolecules is also very scarce.[17–19] 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.[20–22] 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.[23] Therefore it was decided to synthesize 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, ^{1}H NMR, ^{13}C NMR and IR spectra are consistent with the product structure and intermediates.

Before attachment of the calixarene to magnetoferritin its capability to sequester caesium(I) 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 picrate (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 picrate (0.8 ml, 0.2×10^{-3} mmol) solution for three minutes. ^{1}H NMR spectroscopy was used as a rapid initial methodology for

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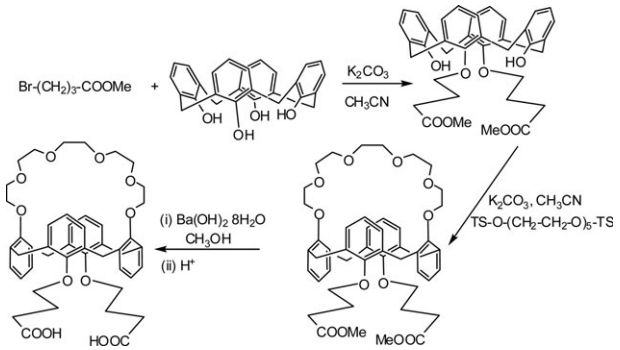


Scheme 1 Synthetic route for preparing 25,27-bis[(3-carboxypropyl)-oxy]calix[4]arene-crown-6.

assessing sequestration. The chemical shifts of the aromatic (between 6.8 and 7.3 δ) and the polyether protons (between 3.3 and 4 δ) were shifted downfield indicating that the caesium(I) ion has been complexed in the crown structure and that it has also strongly interacted with the aromatic rings. Furthermore, inspection of the spectrum showed there were no unshifted peaks, therefore complete complexation by all the calixarene molecules can be inferred.

Solvent extraction was also performed between aqueous solutions of caesium picrate containing radioactive caesium chloride and ethyl acetate with the synthesised calixarene. Three solutions were prepared: 25,27-bis[(3-carboxypropyl)-oxy]calix[4]arene-crown-6 (83 mg, 9.99×10^{-2} mmol) in distilled ethyl acetate (80 ml), caesium picrate (18 mg, 4.99×10^{-2} mmol) in deionised water (20 ml) and a secondary radioactive stock solution of caesium chloride (25 ml, 370 Bq/ml) further diluted with deionised water to a total volume of 100 ml. Calixarene in ethyl acetate (25 ml, 3.12×10^{-2} mmol) was then shaken with a mixture of the diluted radioactive caesium chloride (92.5 Bq/ml) and caesium picrate (2.49×10^{-2} mmol) solution (50 ml) for 1 h. As a control experiment, ethyl acetate without the calixarene was also shaken with the caesium mixture. The resultant emulsions were left to separate overnight. The caesium activity in the organic (1 ml) and the 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).

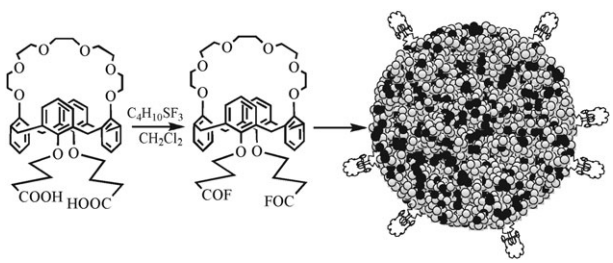
To enable the use of the synthesized compound in the proposed new magnetic separation technology it has to be attached to magnetoferritin in such a way that its ability to separate caesium(I) ions is retained. Amine groups of macromolecules are the most often targeted functional groups for the attachment of specific chelating agents through the formation of amide bonds by activated carboxylic acids.[24,25] 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, ^{1}H NMR, ^{13}C 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

	Activity before solvent extraction (Bq ml^{-1})		Activity after solvent extraction (Bq ml^{-1})	
	Sample	Control	Sample	Control
Organic phase	0	0	80.7	2
Aqueous phase	92.3	95.8	55.7	92



Scheme 2 Formation of acyl fluoride and its attachment to ferritin.

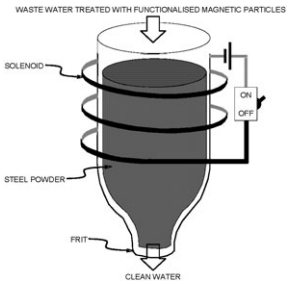


Fig. 1 Schematic of a magnetic separation set up for purifying waste water.

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

	Activity (Bq ml^{-1})			
	Fraction	Blank water	Blank magnetoferritin	Functionalized magnetoferritin
Magnetic field on	1	0	0	0
	2	6.80	1	0
	3	48	47.9	6.2
Magnetic field off	4	5.8	10.6	51.3
	5	0	2	5.5
	6	0	0	0

tested. Although the effluents collected with the magnetic field on were colorless, indicating the absence of magnetoferritin, the gamma emission of the third fraction increased significantly and 80% of radioactivity left the system with the magnetic field still on. This suggested that only a fraction of the radioactive caesium may have been associated with the control magnetoferritin. When the functionalized magnetoferritin was tested the effluents collected with the magnetic field on were also colourless and only a small increase of gamma emission was detected in the third fraction. However, a few seconds after the magnetic field was switched off a dark brown solution appeared and the gamma emission increased significantly. These results showed that 90% of radioactivity was associated with the magnetic molecule, indicating that the calixarene had been attached to the magnetoferritin successfully without losing its ability to separate caesium(I) ions from solutions.

In conclusion, with this pilot study it has been shown that magnetoferritin can be functionalised with a calix[4]arene-crown-6 derivative and subsequently used for caesium chelation. The functionalised paramagnetic particles with the radioactive caesium can be easily removed from solution by magnetic filtration 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.

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