The Development of Magnetic Molecules for the Seletive Removal of Comtaminents

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ABSTRACT

"Magnetic molecules" are a new type of decontaminant for removing dilute dissolved contaminants from solution. Magnetic molecules have a specific ion exchange function to selectively react with a particular type of ionic contamination in a liquid solution. The magnetic molecules also have a very small magnetic ferritin core (ferritin is an iron-III mammalian storage protein having about 10nm diameter), which enables the magnetic molecule to be removed from solution by magnetic filtration. The ion exchange function is attached to the magnetic ferritin core by organic reaction sequences. The ion exchange function selectively bonds to a specific type of contaminant ion. For example, ion exchange functions can selectively target radioactive contaminant ions such as cobalt, cesium and plutonium. The procedure for decontamination is that the appropriate magnetic molecule (which targets the contaminant which it is desired to remove) is added to the solution and the solution is then passed through a magnetic filter. The contaminant binds to the magnetic molecule and is then removed by the magnetic filter. The magnetic molecule/contaminant can then be recovered from the magnetic filter by backwashing.

Work has been undertaken towards the development of magnetic molecules for use as radioactive decontaminants for radioactive waste management purposes. Previously we have reported on the functionalisation of ferritin with the chelating agent DTPA and have shown that this can be used to bind Ca(II) in solution and separate it from Na(I) ions by the process of equilibrium dialysis. Approximately 100 DTPA molecules could be bound to the surface of the ferritin molecule. Synthetic conditions have been optimised, and which will be reported here, ferritin has been functionalised with approximately 1200 DTPA molecules per mole of ferritin and used successfully to achieve a quantitative separation of Co(II) from Cs(I) ions by

equilibrium dialysis. This separation has been carried out over a range of pH values from 5 to 9. Further selective groups are now being added to the basic magnetoferritin structure and separations achieved.

INTRODUCTION

There are many known techniques for removing dissolved impurities from water. Existing water purification techniques include evaporation, ion exchange and reverse osmosis. Although these techniques produce pure water, they are not capable of selectively removing certain target impurities while leaving all other impurity constituents dissolved in the solution. The selective removal of contaminant ions from an aqueous solution is a very common requirement in radioactive decontamination applications in nuclear power plants and other nuclear facilities. In reactor liquid cooling systems and effluents radioactive species may exist in very low molar concentration (typically about 10^{-15} to 10^{-12} moles per litre) while other harmless dissolved species are present in much greater concentrations. In nuclear applications, it is desirable to selectively remove only the radioactive species while leaving the harmless dissolved species in the water. The separated radioactive waste requires careful containment and disposal. The volume of radioactive waste must be rigorously minimized for safety and economic reasons. If harmless dissolved species are removed and handled together with the radioactive contaminants, the resulting waste volume will be excessively large creating disposal problems.

Methods for selectively separating the radioactive ions from the contaminated solution have been developed which are based upon the substantial difference in chemical properties of the radioactive ions and the harmless dissolved species. The most typical way of removing contaminants from solution is to transfer the contaminants to a different phase, normally from liquid to solid. Solid particles are added to the contaminated solution which selectively bind to the radioactive ions but do not bind to other harmless ions. The solid particle and attached radioactive ions are then removed from the solution using solid liquid separation techniques. This technique for removing radioactive ions has been applied on an industrial scale. Many nuclear facilities use the solid absorber clinoptilolite, for example, to selectively remove cesium and strontium ions from the plant's effluents.

There are, however, problems to be overcome in designing a selective removal process as described above. In order to have adequate capacity to hold the contaminants, the particles which bind to the radioactive ions must either be large and porous or very small. Large porous particles evenly absorb and distribute the contaminants throughout the volume of the particle. Robust porous particles, such as clinoptilolite, are difficult to create and usually have limited selectivity to absorb only the desired radioactive ions. Although clinoptilolite absorbs cesium and strontium ions, many other types of harmless ions will also be absorbed.

Smaller particles and large porous particles are substantially different. The smaller particles are not porous and target contaminants can only bind to their outer surfaces. If the particles are sufficiently small they will have an adequate absorption capacity, but they then become more difficult to separate from the solution using solid liquid separation techniques. Small particles do have the advantage of being more easily created to selectively absorb target contaminants while being inert to non-target ions.

MAGNETIC MOLECULES DESIGN PARAMETERS

The operational parameters required for the new separation technology were listed as follows:

1. High selectivity for the target contaminant

2. Fast reaction kinetics

3. Satisfactory absorption capacity for the contaminant

4. Easily separated from the treated liquid

5. Waste volume reduction potential

6. Reliable technology and application

Highly selective reactions between chemicals species are present in both plant and animal biological systems. This is most often achieved by the three dimensional arrangement of a specific receptor molecule having the correct size, shape and charge distribution to allow a very close 'fit' between it and the target molecule. This approach is able to be reproduced in organic chemical synthesis to design very specific chelating molecules. A considerable body of work has been developed in this field over the last fifty years and the data now available forms the basis for the selection of specific chelating groups that can be used to achieve the selectivity needed for point 1 of the design parameters.

A fast reaction time, in this case for chelation of the contaminant, is dependent on a number of factors, some of which can be affected by the size of the reacting entities. The fastest mixing and hence reaction conditions exist when there is an intimate mix of the reactants in solution. It is not possible to use the chelating molecules alone as these could not subsequently be removed from solution any easier than the original contaminant. The chelating molecule must be attached to a discreet particle for its removal from solution. Use of a small particle has several process advantages; it gives a much higher surface area per volume of material, e.g. a factor of 1,000 for 1 µm diameter particles compared with 1mm diameter particles. The concept of chelating groups attached to the surface of small particles offers the most direct reaction opportunity for the chelating group with the contaminants in solution (good reaction kinetics) and also gives the greatest opportunity for the quantity of chelating groups attached to the available surfaces (capacity). This approach satisfies parameters 2, 3 and 5 on the design list.

Small particles can be difficult to remove from solution by standard barrier filtration due to 'blinding' of the filter membrane and the necessary use of high pressures (and hence energy) to overcome the resistance to flow presented by a fine filter membrane (ultra filtration techniques typically require slow flow rates at 50 to 100 psi) . An alternative to barrier filtration is magnetic filtration. In this instance the liquid is passed through an open steel mesh while subjected to a strong magnetic field gradient. Any magnetically susceptible particles follow the magnetic gradients present in the filter volume and ultimately are trapped on the steel mesh surface. This is a well proven industrial technology which is used, amongst other applications, to remove para and ferro magnetic iron oxide impurities from 25% clay slurries producing high quality white clay used in paper manufacture. By using a small magnetic particle on which to attach the selective chelating group items 4 and 6 of the design parameters can be satisfied.

Assessment of the design parameters in conjunction with available scientific knowledge and processing technology suggested that the development of a new separation technology was a plausible research undertaking.

FEASIBILITY STUDY

From a selection of candidate magnetic moieties it was decided that the properties required for the magnetic molecule were best satisfied by a naturally occurring protein called ferritin. Ferritin is an iron storage protein used by all mammalian species and some plants, fungi and bacteria to store and access iron as their metabolisms require. Its unique property for iron storage has been exploited commercially in the electronic recording industry [1]. The ferritin molecule can be thought of as supramolecular cage [2], which consists of a spherical protein shell enclosing a core of iron oxide of approximately 4,500 atoms. Naturally occurring ferritin is not magnetic as the iron is stored in the form of an antiferromagnetic oxide similar to mineral ferrihydrite. This non magnetic ferritin is named apoferritin.

The core in apoferritin can be reconstituted through well recorded synthesis to become a ferromagnetic form such as magnetite [3]. This product is named magnetoferritin and is the base unit for the magnetic molecule technology. The magnetoferritin molecule has an outer diameter of approximately 12 nm (0.012µm) and an inner core of 8 nm diameter. The advantages of this molecule are three fold, the magnetic susceptibility is uniform and sufficient to respond to magnetic filtration, the size is uniform and extremely small (83,300 diameters fit between the 1mm marks of a rule) and the protein shell provides an organic matrix which can be functionalized with selective chelating groups. Figure 1 illustrates the outer protein shell of the ferritin molecule. The illustration highlights the fact that the molecule does not have a smooth geometric surface but rather an open structure with the folded protein sub units forming a cage around a central core. This structure affords a much greater opportunity for attaching the selective functional groups and therefore increases the absorption capacity of the functionalized molecule.
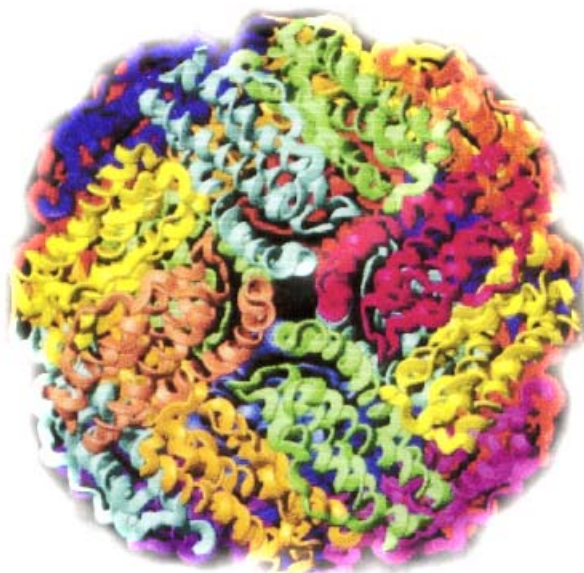

Fig. 1. Illustration of the Ferritin protein

BROAD SPECTRUM CHELATOR

It should be noted that while DTPA is a broad spectrum chelating agent that lacks the specificity to effect efficient separations of many cations in solution it is a very useful molecule with which to investigate binding of a complexant to the surface of ferritin. This is because DTPA is readily available in pure form, is economic and because the preferred route of binding molecules to the surface of proteins is *via* the formation of amide bonds. Two potential reaction schemes were proposed and tested for coupling the chelating agent DTPA (Figure 2) to peptide residues (amine groups) present on the surface of the ferritin molecule (Figure 1) *via* the formation of amide bonds. The successful scheme was chosen for its relative simplicity, the milder reaction conditions it required (an important consideration when working with biological molecules) and the lower cost of the chemical reagents.
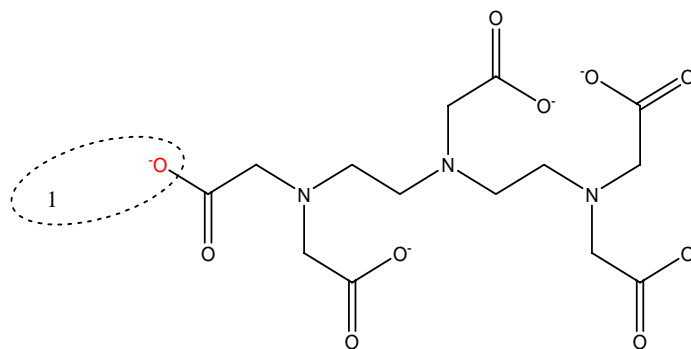

Fig. 2. The Diethylenetriaminepentaacetate (DTPA) anion. The molecule is functionalised at one of its carboxylate residues, *e.g. via* position 1 as shown, so that it can be attached to the ferritin protein surface; in the process one coordination site is lost.

When successfully attached to a ferritin molecule the bound DTPA has a similar chelating behavior to EDTA, a popular and useful chelation agent. Although not a specific chelation agent it can be used to separate single positive valent (charged) ions from multivalent positive ions. This can exploit the difference between cesium (1+) and cobalt (2+). In proving tests ferritin-DPTA was used to separate these two ions. In these tests the ferritin-DPTA was held inside a semipermeable membrane sac and contacted with a mixture of cesium and cobalt ions. While the metal ions were able to diffuse in and out of the sac the ferritin-DPTA was restricted within the sac due to its larger size. The result of this mixing is that the cobalt ions are captured by the DPTA and held within the sac but the cesium ions remain unaffected. Analysis of the ferritin-DPTA reveals how much cobalt was captured. In Table I a comparison of the capture capacity of DPTA attached to native and magnetoferritin is given.

The results are presented as molar ratios at different pHs and reactant concentrations. As one DPTA molecule chelates one cobalt ion it provides an estimate for how many chelation groups are attached to a ferritin molecule. The response to pH is a function of the properties of the DPTA and the lower overall capacity of the magnetoferritin a reflection of the reduced DPTA reaction (attachment) sites available due to the presence of the iron core.

Table I. Cobalt Absorption Results

Ferritin Type	Native	Native	Native	Mag-ferritin	Mag-ferritin	Mag-ferritin

pH	5	5	9	5	9	7
Dialysis sac contents and system	DTPA:Ferritin (molar ratio)					
Ferritin-DTPA dialysed against 10^{-5}M CoCl$_2$	248	152	66	363	247	331
Ferritin-DTPA dialysed against 10^{-4}M CoCl$_2$	1288	1266	1364	467	319	737

Laboratory tests using a magnetic filter in conjunction with the magnetic molecules successfully demonstrated the feasibility of the method to separate the two radionuclides cesium and strontium. A patent was applied for on the basis of these results[4].

The next phase of development required the identification and addition of specific chelation groups to achieve the necessary separations desired. It was possible that this could involve many and various types of molecules and a disproportionate work effort to design suitable reaction conditions for attaching the different molecules to the ferritin.

MODULAR APPROACH

A previous literature study indicated that the use of a modified calixarene molecule for the selective chelation of cesium had been demonstrated by a number of research groups. The calix[4]arenes are most often used due to ease of preparation and functionalization [5].

For cesium chelation the calix[4]arene is modified by the addition of a crown ether bridge as shown in figure 3. The form of the crown is an important contributor to the selectivity of the calixarene molecule
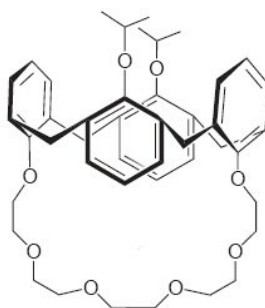

Fig. 3. 1,3-Calix[4]arene crown-6

For example the 1,3-Calix[4]arene crown-**5** is selective for potassium over cesium whereas the crown-**6** provides high selectivity for cesium over potassium[6].

For the purpose of demonstrating selection between cesium and strontium the 1,3 Calix[4]bis crown-6 structure was chosen as the best candidate. Although not the most selective calixarene former for Cs its ease of synthesis made it the best choice at this stage.

The choice of the calixarene molecule, as a base for specific chelation, has an additional benefit. A great deal of work has and is going on, to use the calixarene molecule as a basic unit from which to build selective chelation forms. The work covers transition metals, lead, mercury and cadmium, lanthanides, actinides alkali and alkaline metals, [7]. By establishing a single method for attachment of the calixarene to the ferritin then the ion selective options can be increased by substituting in the appropriate modified calixarene.

Papers on the use of calixarenes as applied to nuclear wastes and fuel cycle nuclide separation have been published by groups in nearly all of the US DOE laboratories. These are already identifying waste problems and calixarene molecules suitable for specific target molecules, [8,9 & 10].

PROCESS DESIGN

The technology is designed to remove dissolved ionic species from aqueous solutions in a manner that targets a specific contaminant or contaminants. The basic concept of the technology application is shown in Figure 4.

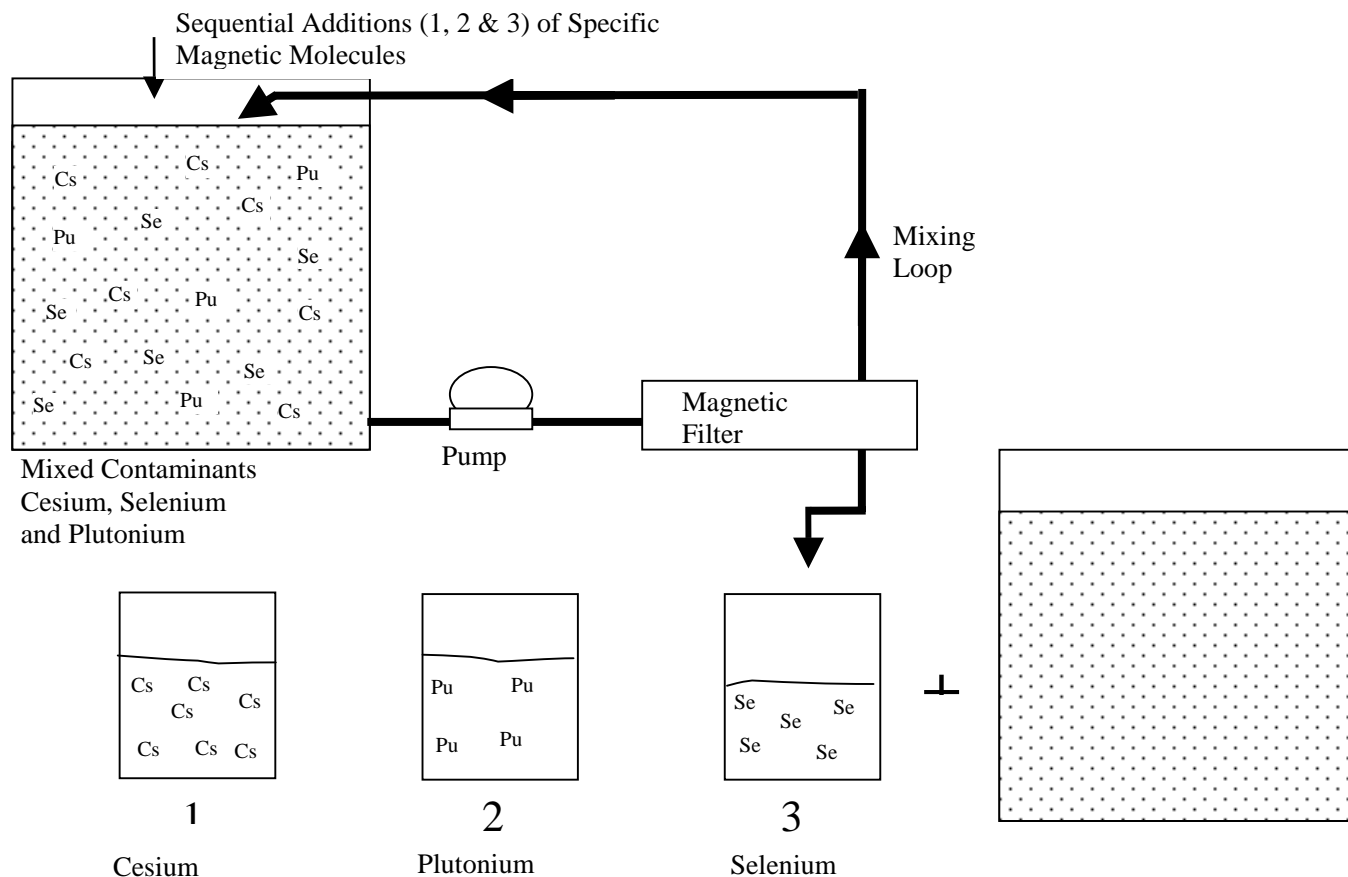Sequential Additions (1, 2 & 3) of Specific
Magnetic Molecules

Fig. 4. Separator for ionic species

The separation system illustrated in Figure 4 is referring to the sequential use of magnetic molecules with different selective groups attached.

The first batch of magnetic molecules with selectivity for cesium (Cs) contamination is injected into the tank and thoroughly mixed by pumping around the loop with the magnets deactivated. The solution is then run through the filter with the magnets activated and the magnetic molecules are held in the filter.

Regenerating the filter flushes the magnetic molecules and the entrapped contamination into a separate holding vessel, number 1 figure 5.

This action is repeated for the other contaminants (Pu & Se numbers 2 and 3 of figure 5) using the appropriate selective magnetic molecule. This approach allows complete separation of the chosen contaminants and enables the bulk waste to be treated in an appropriate cost affective manner. Alternatively, by using different magnetic molecules in combination, or a cocktail of three or more types, it would be possible for example to remove all actinides or toxic heavy metals in one application. The use of a range of different selective molecules with a common removal method allows great flexibility in the approach taken with waste mixtures that vary in composition.

The use of the magnetic molecules process can be scaled relatively easily from laboratory through to industrial application. It is dependant on efficient mixing and the size of the magnetic filter, of which currently there are commercially available models treating up to 50 gpm (190 litres min^{-1}). The application could be by a fixed unit used on the liquid waste in hold-up tanks before release, in a similar fashion to that of filtration and ion exchange. Alternatively, the unit could be mobile for various liquid waste treatment scenarios on different sites or different locations on a large site.

It is useful to have some discussion on the parameters which come into consideration when planning an application of the new technology. In a previous section the very small size of the molecules was given; a diameter of approximately 12 nm. This can be given some proportionality if it is considered that the volume in one grain of sand 1 millimeter in diameter (1/25th of an inch) is the same as is contained in 10^{15} (1 million trillion) of these entities. With the correct selectivity this can produce a very definite benefit when considering waste management volumes. At full capacity, the material used in the proof of principle tests (10mg) could absorb 0.63 curies of Co-60. This translates to 1 gram of magnetic molecules absorbing 63 curies of cobalt 60 or 13 curies of strontium 90.

The magnetic material regenerated from the filter will be small in volume even allowing for a many fold excess of magnetic molecules to contaminant. The waste will however be the equivalent of a chelated waste and can be stabilized in several different ways:

- If heated the protein shell and chelate group can be combusted at relatively low temperature to be lost as gaseous products such as carbon dioxide. The residue will be a melt or sinter of the iron cores and the contaminant. It will contain the activity in an inorganic matrix suitable for cementing, or disposal in a HIC.

- Should the contaminant wish to be recovered then the protein shell can be denatured at 80^0 C in caustic conditions or destroyed using conditions developed for low temperature organic resin oxidation. The contaminant now concentrated in a small volume of solution can be extracted by normal chemical means.

Further work will need to be undertaken to determine the optimal disposition options for the small volume of waste concentrates produced by the processed, based upon waste classification and optimal decay time.

An alternative approach to mixing the molecules then extracting onto a filter is to hold unused magnetic molecules on the surfaces of filter media and allow the liquid waste to pass over them. This is a similar approach to pre-coating filter surfaces. A very small amount of molecules could treat a large volume of waste because only the selected contaminant would react with the molecules. The contact time and surface area could be chosen for best advantage. Once finished the molecules would be regenerated from the filter in the normal fashion. Similar methods employing Cobalt and Cesium specific filter media are now being tested at U.S. plants.

AREAS OF APPLICATION

The most immediate area for application of this technology is the radioactive waste effluents in the commercial nuclear power plants. Hold-up and batch release tank applications as previously described could be a routine application. Other problems which occur when stations have to

perform non routine tasks for maintenance or retrieval, e.g. the production of mixed wastes, could also be addressed.

The US DOE is currently involved in a large decommissioning program to treat and dispose of legacy wastes. These wastes are of a unique nature in many cases and require innovative approaches to be developed. Magnetic molecules could provide a very precise tool in targeting specific nuclides in wastes. It could also be used in conjunction with EPRI decontamination technologies to release large plant items or specialist plant.

Magnetic molecules could also be considered for use in ground water clean up. Ground water can be channeled from large areas through a restricted channel which allows treatment of the water flow. Ground water has many naturally occurring and beneficial salts and organic matter which it is desirable to maintain, this can be achieved and the contaminants removed (nuclear and toxic metals) by applying magnetic molecule technology

A supply of appropriate selective magnetic molecules can be used as an emergency clean up method for accidental spillages or for emergency clean up of contaminated water.

CONCLUSIONS

A new selective separation technology has been proposed based on the use of selective chelating agents, for reaction with a target contaminant, bonded to the surface of magnetic molecule for subsequent removal by magnetic filtration.

Magnetoferritin has been identified as a suitable magnetic moiety for the separation technology.

A chelating group DPTA has been successfully attached to the magnetoferritin.

The selective magnetic molecule synthesised for the separation technology was successfully used in a 'Proof of Principle Test' to separate cesium and strontium.

A patent for the Intellectual Property covering the technology was applied for in the USA on the 7th May 2003

A research program has developed a reliable chemical synthesis for attaching chelating molecules to the magnetoferritin molecule.

A method for the attachment of a calixarene molecule to a ferritin core has been developed and tested.

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