This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This Accepted Manuscript will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
A modular library of crown ethers and monoazacrown ethers supported by CuAAC reactions onto magnetic nanoparticles and polymers has been prepared and evaluated as extracting materials for Pb$^{2+}$ from aqueous and organic solutions.
Clickable Complexing Agents: Functional Crown Ethers for Immobilisation Onto Polymers and Magnetic Nanoparticles

Carolina Mendoza,* Susanna Jansat,† Ramón Vilar‡ and Miquel A. Pericàs§*‡

1 Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Crown ethers and monoaazacrown ethers supported onto well defined cobalt and Fe₃O₄ magnetic nanoparticles have been prepared and used as magnetically recoverable extracting materials for Pb²⁺ from aqueous and organic solutions. The assembly of the functional nanoparticles involves the integration of the complexing units by means of a copper-catalysed alkyne–azide cycloaddition reaction leading to a 1,2,3-triazole linker. The same binding motifs have been also used to functionalise polystyrene (PS) and polystyrene-polyethyleneglycol (PS-PEG) resins, and the resulting materials were also employed in the removal of Pb²⁺ from solutions. The relative merits of these two approaches are compared.

Introduction

Crown ethers, for its extraordinary ability to bind cations, have revolutionised the field of coordination chemistry since its introduction in the late 60's of the last century.¹ The removal of metal ions from aqueous and organic solutions is a subject of great interest due to the high environmental impact associated with the presence of toxic heavy metal (e.g. As³⁺, Hg²⁺ and Pb²⁺) in clean and waste waters,² the recovery of highly valuable metals used in industry and jewellery (e.g. Pt, Pd or Au)³ and even the removal of metals from the body after exposure and contamination.⁴ A potentially important, yet underdeveloped approach for the removal of metal cations from solutions is represented by the use of complexing agents that could be readily separated from the solution once loaded with the target contaminants. Metal oxides, for instance, have the ability to bind metal ions in solution, by adsorption and ion exchange mechanisms.⁵ Therefore, a number of remediation techniques make use of the sorptive and extracting properties of metal and metal oxide nanomaterials to remove or stabilise inorganic, organic and radioactive contaminants.⁶

Functional magnetic nanoparticles (MNPs)⁷ stabilized with ligands that have the ability to coordinate metal cations could be applied in metal recovery and in decontamination processes. In this approach, the ligands decorating the nanoparticle’s surface would bind the cations, and the nanoparticles could then be removed from the solution by the application of an external magnetic field. Recently, some examples of this approach have appeared in the literature.⁸ MNPs encapsulated by a thiol containing polymer have been used as adsorbent for Pb²⁺, Ag⁺ and Hg²⁺.⁹ Also, thiol functionalised superparamagnetic iron oxide (Fe₃O₄) nanoparticles (5.8 nm) have been reported for the removal of heavy metal ions from aqueous solutions,¹⁰ and a fluorescent receptor for Pb²⁺ has been reported using a diaza-indacene ligand bound to magnetic nickel nanoparticles (30-40 nm).¹¹ In a recent report, an ethylenediamine tetraacetic acid-like ligand has been placed onto the surface of carbon coated nanomagnets and used in the removal of cadmium, lead and copper from contaminated water.¹²

Polymeric materials can also be used for the separation of metal ions from solutions. Setting apart the classical ion exchange resins, a more specific interaction with the metal ion can be achieved through the functionalisation of polymers with ligands containing donor atoms with affinity for the target metal. For example aminobenzo-18-crown-6 and aminomethyl-18-crown-6 ethers bonded to Merrifield resin have been used for the separation of alkaline and alkaline-earth isotopes (Li⁺, Mg²⁺)¹⁵ and aza-crown ethers immobilised onto a copolymer of ethylene glycol methacrylate and glycidyl methacrylate were used in the extraction of metal ions (Ag⁺, Pb²⁺, Cd²⁺) from aqueous solutions.¹⁶

We reasoned that, for future applications, it would be interesting to develop immobilisation strategies suitable for the incorporation of the same complexing agents onto different platforms. In this way, libraries combining sets of receptors with sets of suitably functionalized supports (organic polymers and magnetic nanoparticles) could be easily constructed and this could allow the ready optimization of the resulting modular detoxification agents for specific situations.

Probably the most general synthetic strategy available today for the predictable and fully reliable combination of molecular fragments is the copper-catalyzed alkyne-azide cycloaddition (CuAAC) reaction,¹⁷ the archetypal example of click chemistry.¹⁸ Over the last years, we and other groups¹⁷,¹⁸ have demonstrated the validity of this strategy for the immobilization of catalytic
species without negative effects on either catalytic activity or stereoselectivity, and we considered that it would be particularly suitable for the purpose of this research. As part of the ongoing work in our group on the preparation of functional ε-cobalt$^{21}$ and Fe$_2$O$_4$$^{22}$ magnetic nanoparticles (MNPs) as easily recoverable and reusable catalysts,$^{23}$ we decided to use these nanomaterials, functionalized in their surfaces with azido groups, as platforms for the grafting of cation receptors. In the same manner, azido-functionalised polystyrene (PS, Merrifield type) and polystyrene-polyethyleneglycol (PS-PEG) resins would also be used as immobilization platforms. This set of materials could provide different alternatives with respect to the nature of the solution to be detoxified (aqueous or organic) and with respect to the separation technique to be used (simple filtration or magnetic decantation).

![Figure 1](image_url) Crown ethers and azacrown ethers suitable for the preparation of metal extracting composites.

Crown ethers and aza-crown ethers 1-4 (Figure 1) are suitable binding motifs for their ready availability and for the high selectivity depicted in front of various types of metal ions and for their adaptability to the strategy envisaged for anchoring them to the different platforms. Thus, all of them can be easily functionalized by alkylation to species containing terminal alkyne moieties. These structures are well known for their ability to act as ligands for a wide range of metal ions and cationic organic species$^{24}$ for different purposes but, to our knowledge, MNPs functionalised with crown ether have not yet been used for the removal of cations from solutions.$^{25}$

We report in the present paper the preparation of a family of immobilised functional crown ethers prepared according to the principles outlined above. Units 3 and 4, involving binding through different atoms and positions (exo- and endocyclic) were selected for this study. As a model system to test their performance we have selected the extraction of Pb$^{2+}$ from aqueous and organic solutions. The 18-crown-6 and 1-aza-crown-6 units have been shown to bind this metal cation with a significant association constant. According to literature data, association constants for the binding of the unsubstituted 18-crown-6 with Pb$^{2+}$ are in the range of log $K = 3.58$-$4.27$ in water,$^{26,27}$ log $K = 6.99$ in methanol,$^{27}$ and log $K = 6.5$ in a 70:30 v/v methanol:water mixture,$^{28}$ while the 1-aza-18-crown-6 (4), binds to Pb$^{2+}$ with an even larger association constant, log $K = 8.4$ in a methanol/water (95:5 v/v) mixture, at 25°C.$^{29}$

Results and discussion

Synthesis of functionalised cobalt nanoparticles

Monodisperse cobalt nanoparticles can be synthesised through the thermal decomposition of organometallic compounds in high-boiling organic solvents containing stabilizing surfactants.$^{30}$ Puntes et al. reported the use of cobalt octacarbonyl [Co$_2$(CO)$_8$] in the presence of a mixture of surfactants as a way to obtain ε-phase Co nanoparticles.$^{31,32}$ Surface functionalisation of these nanoparticles can be achieved by incorporating to the surfactant mixture used during their synthesis long chain carboxylic acids functionalised at the α-position with the desired groups.$^{21c}$ For this purpose we prepared bifunctional molecules that contained a carboxy group at one end (for immobilisation purposes) and a crown ether (for cation binding purposes) at the other end.

For the assembly of functional cobalt nanoparticles 6a-b it was more convenient to follow a slightly modified procedure where the functional units 5a-b were prepared in a separate step, being then used as ligands for cobalt nanoparticles generated in their presence (Scheme 1). The implementation of this procedure required the prior preparation of both 11-azidoundecanoic acid$^{33}$ and propargyl derivatives of crown ethers 3-4. These intermediates were then cycloaddend through a well established protocol for CuAAC reactions, using CuSO$_4$/sodium ascorbate in a 1:1 mixture of water and tert-butyl alcohol under microwave irradiation (see ESI). With compounds 5a-b in hand, functionalised nanoparticles 6a-b were prepared (Scheme 1). In both cases, transmission electron microscopy (TEM; see Figure 2 for the case of 6a) showed the formation of regular, spherical nanoparticles. Elemental analysis of the materials confirmed the presence of nitrogen containing species on the surface of the nanoparticles.

![Figure 2](image_url) TEM image and size distribution of cobalt nanoparticles 6a.
The nitrogen content (%N) allowed us to calculate the degree of ligand incorporation onto the nanoparticles. The functionalisation was 0.091 mmol/g for 6a and 0.113 mmol/g in the case of 6b.

5 Synthesis of functionalised iron oxide nanoparticles

Cobalt nanoparticles, although showing well-controlled shapes and allowing high levels of functionalisation, present the inconvenience of a rather cumbersome preparation: high temperatures are required to induce the decomposition of $\text{Co} (\text{CO})_\text{5}$ and the isolated yield of nanoparticles is usually low. To avoid these difficulties the use of readily available Fe$_2$O$_3$ nanoparticles as an alternative support was pursued. To modify the surface of the iron oxide nanoparticles, a ligand exchange reaction can be used starting from the as-prepared oleic acid stabilised nanoparticles. We investigated two different types of functional agents suitable for this purpose, namely alkoxysilanes with variable chain length and phosphonic acid derivatives. Fe$_2$O$_3$ nanoparticles coated with alkoxysilanes were prepared following the route shown in Scheme 2A. In the first step, oleic acid stabilised nanoparticles were produced by coprecipitation of aqueous solutions of Fe$^{\text{II}}$ and Fe$^{\text{III}}$ chlorides with ammonium hydroxide, in the presence of oleic acid following a previously reported procedure. In this manner, fairly disperse Fe$_2$O$_3$ nanoparticles of 8.4 ± 2.6 nm mean diameter (Figure 3, a) were obtained (see ESI for X-ray diffraction spectrum). The presence of oleic acid on the nanoparticle surface was confirmed by IR spectroscopy (Figure 4). $\text{O}$-$\text{Azidoalkyltrialkoxysilanes}$ 8i-iii, which would be placed in the surface of the magnetite nanoparticles for further functionalisation through CuAAC reactions were prepared by standard procedures (see ESI). Then, the functionalisation of the oleic acid stabilised nanoparticles was achieved following a ligand exchange process previously reported, (Scheme 2A, step b). In all cases spherically shaped 7–10 nm nanoparticles were obtained as shown by TEM images (Figure 3b). The incorporation of the o-azidoalkyltrialkoxysilanes in nanoparticles 9i-iii was evidenced by IR spectroscopy, through the appearance of a band at around 2097 cm$^{-1}$, corresponding to the N$_3$ asymmetric stretching and the appearance of bands between 1020-1050 cm$^{-1}$, due to Si-O-Si vibrations (Figure 4). What is more, the almost complete disappearance of the bands at 3005 cm$^{-1}$ (=C-H) and 1684 cm$^{-1}$ (C=O) evidenced the effective exchange of the oleic acid ligand by the different o-azidoalkyltrialkoxysilanes.

Nitrogen elemental analysis allowed the calculation of the degree of ligand incorporation onto the nanoparticles ($f = 0.238 \%\text{N}$). The determined functionalisation were 0.480 mmol/g for 9i, 0.297 mmol/g for 9ii, and 0.233 mmol/g for 9iii. Finally, the incorporation of the cation binding units 10a and 10b onto the MNP was carried out using the CuAAC reaction in dimethylformamide (DMF), with CuI as catalyst in the presence of $\text{N}_3\text{N}$-diisopropylethlyamine (DIPEA). Following this procedure six new types of silane-coated iron oxide nanoparticles (11al-iii and 11bi-iii) were prepared, incorporating two different macrocycles on their surfaces and three different spacers (with 3, 5 or 11 carbon atoms) between the 1,2,3-triazole unit and the surface of the nanoparticle. The incorporation of the ion-binding units onto the nanoparticles could be easily monitored by IR spectroscopy. This showed the disappearance of the azide band at 2097 cm$^{-1}$ while the presence of the macrocycles was shown by the appearance of a band at ca. 1105 cm$^{-1}$, as can be seen in Figure 4 for the case of 11bi-iii. The functional nanoparticles 11 were obtained as a mixture of small aggregates and discrete particles of 9 to 11 nm (TEM images Figure 3, c and d), no change in the appearance or size of the nanoparticles being observed over the CuAAC reactions. Additionally, functional nanoparticles 12 obtained by CuAAC reaction of phenylacetylene with azide nanoparticles 9i were prepared (see ESI), to evaluate any possible effect of the triazole ring on the extraction of metal ions. In this case, the tris(triazolyl)methanol copper(I) chloride complex, [CuCl(TTM)], was used as the catalyst to promote the CuAAC reaction. Elemental analysis (%N) indicated a functionalisation of 0.32 mmol/g for the resulting MNPs.

As a preliminary step for the preparation of nanoparticles 17, where binding with the functional unit takes place through interaction with a phosphonic acid group (Scheme 2B), the preparation of 11-bromoundecylphosphonic acid (14) was first required. This was achieved through a five-step sequence starting from 11-bromoundecan (see ESI for full details).

| Scheme 2 | A) Preparation MNPs of Fe$_2$O$_3$ functionalised with cation binding groups. Conditions: a) Oleic acid, 30% NH$_3$, H$_2$O, 80 °C, 30 min; b) Acetic acid, toluene, rt, 5 days or 100 °C, 24 h; c) CuI, DIPEA, DMF, 40 °C. B) Preparation of functional iron oxide nanoparticles coated with |
phosphonic acid derivatives. Conditions: a) Oleic acid, oleylamine, 1,2-hexadecanediol, benzyl ether, 200 °C, 2 h, then 300 °C, 1 h; b) 14, CHCl₃, rt, 24 h; c) NaN₃, DMF, 65 °C, 12 h; d) 10a or 10b, CuI, DIPEA, DMF, 40 °C.

Compound 14 was then grafted onto the surface of 6.2±2.0 nm oleic acid/oleylamine stabilised iron oxide nanoparticles 13 prepared by the thermal decomposition method reported by Sun. The functionalisation reaction was performed in chloroform and the incorporation of 14 was evidenced in the IR spectrum of MNPs 15 (Figure 5) by the appearance of very intense bands in the region between 1260 and 850 cm⁻¹ that have been assigned to P-O and P-O-Fe stretchings. The obtained nanoparticles were spherically shaped (6.4±1.0 nm) and well dispersed (see ESI for TEM images). The degree of ligand incorporation onto the nanoparticles was determined to be 2.36 mmol/g by quantitative phosphorus determination.

Figure 3 TEM images of a) MNPs 7, b) azide functionalised MNPs 9i, c) and d) MNPs after the functionalisation with crown ethers derivatives (11ai and 11bi), e) and f) MNPs 17a and 17b.

Magnetic nanoparticles 15 were next transformed into the ω-azidoalkylphosphonyl functionalised nanoparticles 16 (5.6±1.0 nm) by reaction with an excess of sodium azide in DMF at 65 °C (Scheme 2B, step c), a transformation evidenced by the appearance of the strong N₃ band in the IR spectrum (Figure 5) at 2097 cm⁻¹. The presence of nitrogen in the surface of the nanoparticles was confirmed by elemental analysis and the degree of functionalisation of MNPs 16 was found to be 2.2 mmol/g. For the incorporation of the metal-binding units, ω-azidoalkylphosphonyl functionalised MNPs 16 were reacted with alkynes 10a and 10b with CuI catalysis. The presence of the macrocycles in the resulting MNPs 17a-b was evidenced by the appearance of bands at around 1100 cm⁻¹ and 1353 cm⁻¹ in the IR spectrum (Figure 5).

Figure 4 IR spectrum of MNPs 7, azide functionalised MNPs 9iii and MNPs after the incorporation of the azacrown ether residue (11biii).

Figure 5 IR spectrum of MNPs 13, bromo functionalised MNPs 15, azide functionalised MNPs 16 and MNPs after the incorporation of the crown ether residue (17a). For comparison the spectrum of 10a was included.

The loading of the binding units in the ready-to-act MNPs was determined by elemental analysis (%N), being 1.0 mmol/g for 17a and 1.27 mmol/g for 17b. TEM images (Figure 3e and 3f) indicated that the macrocycle-functionalised nanoparticles had a narrow size distribution, with average diameters of about 6.0 nm. Moreover there was no change neither in size nor in the spherical shape of the nanoparticles during the functionalisation reactions.
Preparation of functionalised polystyrene and polystyrene-polyethylene glycol resins.

(Azidomethyl)polystyrene (18) and (2-azidoethoxy)polystyrene-polyethylene glycol (19) were prepared by reaction of the corresponding halogenated commercial resins with sodium azide following previously reported procedures. Alkynes 10a and 10b were reacted with the azide-functionalised polymers using CuI/DIPEA as catalyst and a 1:1 DMF/THF mixture as solvent (Scheme 3). The completeness of the reaction was established by IR spectroscopy, in a similar fashion as with the iron oxide nanoparticles (see above). The obtained resins 20a-b and 21a-b have extractant loadings of around 0.2 mmol/g for the PS-PEG polymers and around 0.6 mmol/g for the PS resins (determined by nitrogen elemental analysis). These functionalisations accurately reflect those of the starting commercial resins (see ESI). To evaluate any possible contribution of the triazole unit to the extraction of metal ions, functional PS-PEG resin 22 was also prepared by CuAAC reaction with phenylacetylene.

Metal extraction experiments

Once the different types of extracting materials were prepared and fully characterised, their ability to extract metal ions from aqueous and organic solutions was evaluated. The main goal of these studies was the identification of optimal combinations of support and complexing agent for specific situations (solvent nature, pH of aqueous solutions), and also determining the influence of additional factors (immobilization strategy, complexing unit loading) on the performance of these combinations. To perform these studies, the extraction of Pb²⁺ from aqueous and organic solutions was selected as a representative and yet not fully solved case.

Extraction with MNP's

The metal extracting capability of the nanoparticles was investigated by placing the extracting material in contact with either aqueous or acetonitrile solutions of Pb(ClO₄)₂ of known concentration (([Pb]₀). The samples were shaken in a closed flask for the specified period of time, and then the nanoparticles were separated with the help of an external magnet. The resulting clear solution was analysed to determine the final concentration of Pb²⁺ ([Pb]₉). Both concentrations of Pb²⁺ were determined by flame atomic absorption spectroscopy (FAAS). The amount of lead that was extracted from the solution by the nanoparticles was calculated according to equation 1.

% Pb extracted = 100 x ([Pb]₀ - [Pb]₉)/[Pb]₀ (1)

Table 1 shows the results for the removal of Pb²⁺ from acetonitrile solutions, using functionalised cobalt and iron oxide nanoparticles. Extractant 6a, functionalised with a 18-crown-6 ether was first tested. Interestingly, up to 79% of the initially present cation was removed from solution (entry 1). In a similar manner, a significant amount of Pb²⁺ was also removed form the solution using extractant 6b (entry 2).

Functionalised iron oxide nanoparticles were also employed in the removal of Pb²⁺ from acetonitrile solutions. Also in this case, the nanoparticles are able to bind and remove Pb²⁺ from the solution (entries 3 and 4, Table 1). However the extracted percentages are much smaller than in the case of cobalt nanoparticles. In particular, for the case of 11bi, just 10% of the Pb²⁺ was removed because the nanoparticles were dissolved in the solution, and could not be separated by the external magnetic field.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Extractant</th>
<th>Extractant to Pb²⁺ ratio</th>
<th>Contact time /h</th>
<th>% Pb extracted⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6a</td>
<td>1:1</td>
<td>21</td>
<td>79(1)</td>
</tr>
<tr>
<td>2</td>
<td>6b</td>
<td>1:1</td>
<td>21</td>
<td>68(1)</td>
</tr>
<tr>
<td>3</td>
<td>11ai</td>
<td>1:1</td>
<td>21</td>
<td>47(1)</td>
</tr>
<tr>
<td>4</td>
<td>11bi⁶</td>
<td>1.5:1</td>
<td>21</td>
<td>10(1)</td>
</tr>
</tbody>
</table>

⁴Mean values of two independent determinations. Observed result variability is given in parentheses. ⁶Nanoparticles of 8.1 nm diameter and f = 0.604 mmol/g were used.

In view of the promising results obtained in the removal of lead cation from acetonitrile solutions, we decided to test the behaviour of our systems in aqueous media. Table 2 shows data of the extracting experiments using functional iron oxide MNPs, in the removal of Pb²⁺ from aqueous solutions.
Using an equimolar amount of extracting groups and metal cations in solution, between 21% to 62% of the Pb can be removed from the aqueous solution in a single operation. The nanoparticles containing the 18-crown-6 moiety in combination with the shorter, more readily available spacers (n = 1 and 3) allow higher extraction percentages than those bearing monoaaza-18-crown-6 units. In general, as the chain length increases a slightly positive effect on the extraction values is noted independently of the nature of the extracting unit. In order to secure that the extracting power of these materials arises from the binding units present in their structures, control experiments were performed with MNPs 9i (<2%, entry 8) and 12 (14%, entry 9). This last result seems to indicate that the triazole group can contribute to some extent to the extraction of lead cations from the aqueous solution. As we will see later, this impression is not confirmed by the results obtained with the same complexing agents clicked on polymers. Functional nanoparticles 17a and 17b, where the functional unit is bonded to the magnetic core through a phosphonate unit, were also used in the extraction experiments from aqueous solution. The experiments were performed in a similar manner as with MNPs 11, and the results are also shown in Table 2 (entries 10-13). Again, 18-crown-6 functionalised nanoparticles seem to be more effective than monoaaza-18-crown-6 in removing lead from the solutions achieving nearly a 49% of removal with 17a. Interestingly, when a 2.8:1 ratio of extractant was used, 92% of the initially present metal loading was removed from the solution in a single extraction (entry 12).

Table 2 Extraction of Pb²⁺ form aqueous solutions using functionalised Fe₃O₄ nanoparticles.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Extractant</th>
<th>Contact time /h</th>
<th>Extractant to Pb²⁺ ratio</th>
<th>% Pb²⁺ extracted</th>
<th>% Pb²⁺ extracted&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11ai</td>
<td>41</td>
<td>1:1</td>
<td>29(1)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11bi</td>
<td>41</td>
<td>1:1</td>
<td>21(1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11aii</td>
<td>41</td>
<td>1:1</td>
<td>45(4)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11bi</td>
<td>41</td>
<td>1:1</td>
<td>30(6)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11aii</td>
<td>41</td>
<td>2:1</td>
<td>66(3)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>11aiii</td>
<td>41</td>
<td>1:1</td>
<td>53(5)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>11iib</td>
<td>41</td>
<td>1:1</td>
<td>62(1)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>9i</td>
<td>41</td>
<td>1.5:1</td>
<td>2(1)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>41</td>
<td>2:1</td>
<td>14(1)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>17a</td>
<td>21</td>
<td>1:1</td>
<td>49(4)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>17b</td>
<td>21</td>
<td>1:1</td>
<td>38(2)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>17a</td>
<td>21</td>
<td>2.8:1</td>
<td>92(2)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>17a</td>
<td>41</td>
<td>2.6:1</td>
<td>86(4)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean values of two independent determinations. Observed result variability is given in parentheses.

With the goal of determining conditions for the extraction of >90% of the initially present Pb²⁺ in a single operation using the more readily available MNPs 11ai, a set of experiments was performed using increasing amounts of this extractant (Table 3). As it can be seen, a 91% of the initially present lead was removed from the solution in a single treatment when a 10:1 extractant to metal ratio was used.

**Extraction with functional polymers**

Functional polymers 20a and 20b were also used for the selective removal of lead from organic solvents. THF was chosen as solvent, because it was adequate for the swelling of the polymers. Results of the extraction experiments are shown in Table 4.

Table 4 Extraction of Pb²⁺ from THF solutions, using functional polystyrene resins.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Extractant</th>
<th>Extractant to Pb²⁺ ratio</th>
<th>Contact time /h</th>
<th>% Pb²⁺ extracted</th>
<th>% Pb²⁺ extracted&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20a</td>
<td>1:1</td>
<td>24</td>
<td>91(2)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20b</td>
<td>1:1</td>
<td>24</td>
<td>86(3)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>2:1</td>
<td>24</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean values of two independent determinations. Observed result variability is given in parentheses.

As it can be seen, high extraction percentages, of around 90% were achieved with polystyrene-immobilised complexing agents 20a-b. To show that there was no unspecific binding to the resin, the extraction experiment was also carried out using polymer 18, and, no decrease in the amount of Pb in the solution being observed (entry 3).

Resins 20b and 21a-b were also tested for extraction from water (Table 5). Polystyrene resin 20b was inefficient under these conditions (entry 1), likely because of poor swelling. Amphiphilic resins 21a and 21b performed much better with respect to swelling in water, and its extracting potential was studied at different pH values. Using resin 21b a 43% of extraction was achieved in water under neutral conditions, while in acidic media (pH 1 and pH 3) the extraction capacity was highly reduced due to the protonation of the tertiary amine in the monoaaza-18-crown-6 moiety. Resin 21a works poorly in water at pH = 7 (14%), but its extraction ability was improved at pH 1 (45%). Again, control experiments were performed using azido resin 19 (entry 13) and phenyltriazole functionalised resin 22 (entry 14). No decrease in the amount of Pb was observed in any of the cases. The result in entry 14 indicates that, at least in this case, the presence of the triazole group does not contribute to any extent to the binding of cations, in contrast with what we observed for the case of magentite nanoparticles (see above). As observed for the extraction with functional MNPs, increasing the amount functional resin used as extractant allows increasing the removal of lead up to 72% in a single operation (entry 11).
Typical procedure for the preparation of cobalt nanoparticles functionalised with crown ether derivatives: Preparation of functional nanoparticles 6a

These nanoparticles were prepared by slight modifications of a previously reported procedure.21c,31 Crown ether derivative 5a (0.29 g, 0.529 mmol) and triethylphosphine oxide (TOPO) >97% (0.042 g, 0.0363 mmol) were weighed in a 50 mL two-necked round-bottomed flask and dissolved under argon atmosphere in 6 mL of anhydrous and degassed o-dichlorobenzene. To this suspension, 85.5 µL of oleic acid 98% (0.0762 g, 0.2644 mmol) were injected and the mixture was heated in a silicone oil bath at 160 ºC, with mechanical stirring using a shaker. When the bath had reached the desired temperature, a solution of 0.201 g (0.529 mmol) of [Co₆(CO)₁₈] (≥ 90%) in 5 mL of anhydrous, degassed o-dichlorobenzene was rapidly transferred into the reaction media by injection. The reaction mixture was mechanically stirred at 160 ºC for 1 hour. After cooling to room temperature, 24 mL of anhydrous and degassed methanol were added. The resulting suspension was stored in the freezer under argon until the precipitation of the particles (3 hours). The particles were separated by magnetic decantation and washed once with anhydrous, degassed methanol (20 mL) and with anhydrous, degassed dichloromethane (40 mL). The solvents were removed in vacuo and the residual solid was dried under a nitrogen stream. In this manner, 35.3 mg of cobalt nanoparticles 6a were obtained with particle size of 18 nm (s = 3.3) and f = 0.091 mmol/g. Elemental analysis: C, 5.81; H, 0.84; N, 0.38. FTIR νmax/cm⁻¹: 3443, 2958, 2922, 2853, 1620, 1460, 1413, 1225, 1101, 801, 524, 457.

Preparation and functionalisation of magnetite nanoparticles

Typical procedure for the preparation of magnetite nanoparticles coated with oleic acid (7)

These nanoparticles were prepared by slight modifications of a previously reported procedure.34 Iron(II) chloride tetrahydrate (99%) (3.01 g, 15 mmol) and iron(III) chloride hexahydrate (97%) (8.36 g, 30.0 mmol) were dissolved in degassed ultra pure water (140 mL) in a 500 mL round-bottomed flask and heated to 80 ºC, shaking vigorously with a shaker. Oleic acid (98%) (2.1 mL, 0.60 g, 0.68 mL) dissolved in degassed acetone (15 mL) was added to the reaction mixture, followed by 30% aqueous NH₃ solution (18.2 mL). After addition, further amounts of oleic acid were added (5 × 1.0 mL) over a 5 to 10 minutes period. The black reaction mixture was held for 30 minutes at 80 ºC and then slowly cooled to room temperature. A 1:1 v/v mixture of methanol and acetone (100 mL) was then added to support the precipitation. After allowing the nanoparticles to settle overnight with the help of a magnet, the supernatant was separated using a cannula and the particles were washed with a 1:1 v/v mixture of methanol and acetone (5 × 100 mL). The nanoparticles were dried, first with an argon stream and then under vacuum. In this manner, 3.8 g of nanoparticles were isolated as a dark brown solid with a particle size of 8.40 nm (s = 2.56). FTIR νmax/cm⁻¹: 3453, 3005, 2956, 2923, 2852, 1632, 1426, 1409, 1261, 1229, 801, 524, 457.
Typical procedure for the preparation of magnetite nanoparticles coated with oleic acid (13)

These nanoparticles were prepared by slight modifications of a previously reported procedure.42 Iron(III) acetylacetonate 98% (0.706 g, 1.96 mmol), oleic acid (1.9 mL, 5.99 mmol) oleylamine (1.60 g, 1.93 mL, 5.99 mmol) and 1,2-hexadecanediol 90% (2.58 g, 8.98 mmol) were mixed in 20 mL of benzyl ether 99% under nitrogen. The mixture was heated at 200 °C for 2 h and then at 300 °C for one more h. The mixture was then allowed to cool down to room temperature and 40 mL of ethanol were added. Centrifugation allowed the separation of a dark-brown solid that was dissolved in hexane (5 mL) and reprecipitated by addition of 40 mL of ethanol. The nanoparticles were obtained as a black solid (80 mg) with a particle size of 6.18 nm (s = 1.98). FTIR ν_max/cm⁻¹ 3440, 2955, 2920, 2850, 1630, 1594, 1524, 1401, 1380, 1248, 1103, 385, 584.

Typical procedure for the preparation of azido-magnetite nanoparticles: Synthesis of functional nanoparticles 9i

These nanoparticles were prepared by slight modifications of a previously reported procedure.36 Nanoparticles 7 (0.96 g) were dispersed by sonication (20 minutes) in about 480 mL of degassed toluene and then 3-azidopropyltrimethoxysilane 8i (1.92 g, 9.33 mmol) was added, followed by glacial acetic acid 99.5%, d = 1.05 g/mL (0.192 mL, 3.36 mmol) and ultrapure water (0.269 mL, 14.93 mmol). The reaction mixture was stirred at room temperature for 3.5 days. The black particles were allowed to settle overnight with the help of an external magnet. The supernatant was separated using a cannula, and the nanoparticles were washed with toluene (3 × 42 mL) and methanol (3 × 26 mL) and dried under vacuum. In this manner, 0.78 g of nanoparticles were recovered as a brown powder with a particle size of 8.83 nm (s = 3.01) and f = 0.35 mmol/g. Elemental analysis: C, 36.1; H, 0.80; N, 1.48. FTIR ν_max/cm⁻¹ 3442, 2955, 2924, 2852, 2098 (N₃), 1637, 1420, 1239, 1182, 1103, 1022, 585. TGA (30-1000 °C, 10 °C/min, N₂) %weight loss (onset [°C]): 9.79 (244.01), 4.58 (633.10).

Typical preparation of MNPs functionalised with crown ether: Synthesis of functional nanoparticles 11ai

Magnetite nanoparticles 9i (90 mg, f = 0.480 mmol of ligand/g) were dispersed in 5 mL of anhydrous DMF in a Schlenk flask under argon, using ultrasonication for 15 min to ensure dispersion. The alkane 10a (43 mg, 0.13 mmol), dissolved in 1 mL anhydrous DMF was then added to the reaction media, followed by DIPEA 99.5% (0.136 mL, 0.78 mmol), and finally Cul (3.3 mg, 0.017 mmol) as a solid. The reaction mixture was stirred at 40 °C under argon and the reaction progress was followed by IR spectroscopy. Once the IR bands associated to N₃ had disappeared (2-10 days) the reaction was cooled to room temperature, methanol was added (c.a. 50 mL), and the mixture was left overnight in the freezer. The precipitating particles were separated by magnetic decantation. The supernatant was discarded and the particles were washed with methanol (3 × 10 mL) using centrifugation to separate the solvent. The solid material was dried with an argon stream and then in vacuo. In this manner 68.1 mg of functional magnetite nanoparticles were obtained as a brown solid, with a particle size of 10.88 nm (s = 3.97) and f = 0.343 mmol/g. Elemental analysis: C, 8.88; N, 1.47; H, 1.44. FTIR ν_max/cm⁻¹ 3405, 2919, 1726, 1629, 1454, 1350, 1248, 1103, 835, 584.

Magnetite nanoparticles stabilised by 11-bromoundecylphosphonic acid (15)

Compound 14 (0.235 g, 0.75 mmol) and 0.111 g of magnetite nanoparticles 13 were shaken in 15 mL of CHCl₃ at room temperature for 24 h. Then the nanoparticles were collected using an external magnet and the organic solution was removed. The nanoparticles were washed 3 times with ethane sonicating each time to ensure total abstraction of ligands in excess. The product was obtained as a brown solid (0.40 g), with a particle size of 6.37 nm (s = 0.97). According to the %P determined by ICP-AES, a functionalisation f = 2.36 mmol/g was calculated. Elemental analysis: C, 31.97; H, 5.66; N, 0.10; P, 7.32. FTIR ν_max/cm⁻¹ 2917, 2850, 1466, 1082, 940, 559.

Magnetite nanoparticles stabilised by 11-azidoundecylphosphonic acid (16)

To a dispersion of 15 (0.13 g, 0.31 mmol, f = 2.36 mmol/g) in 4 mL of DMF, NaN₃ 95.5% (0.15 g, 2.3 mmol) was added and the mixture was heated at 65 °C for 12 h. The mixture was allowed to reach room temperature, the nanoparticles were separated and washed with water (3 × 5 mL) and methanol (3 × 5 mL). The product was obtained as a brown solid (0.122 g), with a particle size of 5.56 nm (s = 1.04). According to the %N determined by elemental analysis a functionalisation of the nanoparticles of f = 2.25 mmol/g was determined. According to the %P determined by ICP-AES a functionalisation f = 2.28 mmol/g was calculated. Elemental analysis: C, 33.15; H, 5.82; N, 9.11; P, 7.07. FTIR ν_max/cm⁻¹ 2917, 2851, 2097, 1079, 560.

Typical example of CuAAC reaction between functional magnetite nanoparticles 16 and alkynes: Synthesis of MNPs 17a

Magnetite nanoparticles 16 (70 mg, f = 2.25 mmol/g) were dispersed in 5 mL dry DMF in a Schlenk flask under an argon atmosphere. The alkene 10a (100 mg, 0.302 mmol), dissolved in 1 mL dry DMF was added to the suspension of the nanoparticles, followed by DIPEA 99.5% (0.474 mL, 352.0 mg, 2.72 mmol), and finally Cul (11.5 mg, 0.0605 mmol) as a solid. The reaction mixture was magnetically stirred at 40 °C under argon. The progress of the reaction was monitored by IR spectroscopy, following the decrease of intensity of the azide band. If required, additional amounts of Cul and alkene were added. Once the azide band had dissappeared (between 4 to 7 days), the reaction mixture was cooled to room temperature, and the DMF was evaporated in the vacuum line to about one half of its volume. Then, 40 mL of methanol (HPLC grade) were added and the particles left to
precipitate overnight in the freezer, with the aid of an external magnet. The supernatant was separated via cannula, the particles were washed twice with 5 mL methanol (HPLC grade) and two times with distilled water (5 mL each) using centrifugation to separate the particles. The particles were again washed with methanol (2 × 5 mL) and were dried with an argon stream. 66.6 mg of magnetite nanoparticles were isolated, with a particle size of 6.37 nm (s = 1.08) and $f = 1.00 \text{ mmol/g}$. Elemental analysis: C, 36.65; H, 5.78; N, 4.24. FTIR $\nu_{\text{max}}/\text{cm}^{-1}$ 3445, 2920, 2851, 1638, 2919, 2865, 1638, 1571, 1506, 1493, 1453, 1438, 1348, 1296, 1249, 1093, 946, 845, 761, 701; $\delta$H NMR (500 MHz, CD$_2$Cl$_2$) $\delta_{\text{H}}$ 7.46 (H, triazole), 7.11 (s, br, polymer backbone), 6.64 (s, br, polymer backbone), 5.37, 4.63 (2 H, s br) 3.74 (2 H, m, polymer backbone), 1.52 (s, polymer backbone) ppm. $\delta$C NMR (126 MHz, CD$_2$Cl$_2$) $\delta_{\text{C}}$ 145.73, 128.58, 128.2, 126.2, 123.1 (CH triazole), 111.30, 78.67, 71.09, 70.97, 70.06, 68.31, 65.28, 40.92 ppm.

Typical functionalisation of azido-polystyrene-polyethylene glycol with crown ethers: Preparation of polystyrene-polyethylene glycol resin 21a

Alkyne 10a (0.14 g, 0.421 mmol), DIPEA 99.5% (0.384 mL, 2.202 mmol) and copper(I) iodide (0.0043 g, 0.02 mmol) were added to a suspension of 0.22 g azidomethylpolystyrene 18 ($f = 0.77 \text{ mmol/g}$) in 1:1 DMF/THF (2 mL) and the mixture was stirred at 40 °C for 5 days. The reaction mixture was monitored and once the IR-signal of the azido group had completely disappeared, the resin was collected by filtration and sequentially washed with water (60 mL), THF (60 mL), MeOH (120 mL) and 21a THF (60 mL). The solid was dried in vacuo for 24 hours at 40 °C to afford 0.185 g of 10a with a functionalisation $f = 0.597 \text{ mmol/g}$. Elemental analysis: C, 79.96; H, 7.65; N, 2.51. FTIR $\nu_{\text{max}}/\text{cm}^{-1}$ 3059, 3025, 2919, 2850, 1945, 1719, 1601, 1493, 1451, 1350, 1151, 1068, 903, 754, 693; $\delta$H NMR (500 MHz, CD$_2$Cl$_2$) $\delta_{\text{H}}$ 7.46 (H, triazole), 7.11 (s, br, polymer backbone), 6.64 (s, br, polymer backbone), 5.37, 4.63 (2 H, s br) 3.74 (2 H, m, CH$_2$-CH$_2$N$\equiv$), 3.65 (s, -(O-CH$_2$-CH$_2$O)-), 1.86 (s, polymer backbone), 1.52 (s, polymer backbone) ppm. $\delta$C NMR (126 MHz, CD$_2$Cl$_2$) $\delta_{\text{C}}$ 145.73, 128.58, 128.2, 126.2, 123.1 (CH triazole), 111.30, 78.67, 71.09, 70.97, 70.06, 68.31, 65.28, 40.92 ppm.

Typical functionalisation of azidopolystyrene-polystyrene-polymethylene glycol with crown ethers: Preparation of polystyrene-polymethylene glycol resin 21a

Alkyne 10a (0.037 g, 0.113 mmol) dissolved in 1:1 DMF/THF (3 mL), DIPEA 99.5% (0.17 mL, 0.975 mmol) and copper(I) iodide (0.0015 g, 0.0075 mmol) were added to a suspension of 0.3 g of azido-terminated polystyrene-polyethylene glycol 19 ($f = 0.25 \text{ mmol/g}$) in 1:1 DMF/THF (3 mL) at 40 °C and the mixture was shaken for 24 hours. The reaction mixture was monitored and once the IR signal of the azido group had completely disappeared, the resin was collected by filtration and sequentially washed with water (180 mL), MeOH (160 mL) and THF (160 mL). The solid was dried in vacuo for 24 hours at 40 °C to afford 0.203 g of 21a with $f = 0.252 \text{ mmol/g}$. Elemental analysis: C, 62.61; H, 8.74; N, 1.06; FTIR $\nu_{\text{max}}/\text{cm}^{-1}$ 2865, 1638, 1602, 1492, 1453, 1348, 1296, 1249, 1093, 946, 845, 761, 701; $\delta$H NMR (500 MHz, CD$_2$Cl$_2$) $\delta_{\text{H}}$ 7.79 (1 H, s, triazole), 7.04 (s, br, polymer backbone), 6.57 (s, br, polymer backbone), 4.60 (2 H, s), 4.52 (2 H, s), 3.86, 3.72, 3.59, 3.45, 2.09, 1.73 (s, br, polymer backbone), 1.52 (s, polymer backbone) ppm. $\delta$C NMR (126 MHz, CD$_2$Cl$_2$) $\delta_{\text{C}}$ 145.3 128.5, 110.2, 85.3, 71.9, 70.8, 70.5, 69.7, 50.5, 40.9.

Metal extraction experiments using functionalised MNPs and resins

General procedure for the Pb$^{2+}$ extraction from acetonitrile solutions with nanoparticles

An exactly measured aliquot (5 mL) of a solution of Pb(ClO$_4$)$_2$ (of around 83 ppm) was added to a closed vial containing an equimolar amount of functional MNPs. The samples were sonicated for at least 15 min to achieve the dispersion of the nanoparticles and the vials were shaken on a horizontal shaker for specified periods of time. The nanoparticles were then magnetically decanted with the help of an external magnet. The supernatant was transferred via cannula to a Schlenk flask, the solvent was evaporated under vacuum and the residue was dissolved in an exactly measured volume of 1% v/v HNO$_3$. The concentration of lead in the supernatant was determined by FAAS.

General procedure for the Pb$^{2+}$ extraction from aqueous solutions with nanoparticles

An aliquot (5 mL) of a solution of Pb(ClO$_4$)$_2$ (of around 83 ppm) was added to a closed vial containing the required amount of magnetite nanoparticles. The samples were sonicated for at least 15 min to achieve the dispersion of the nanoparticles and the vials were shaken on a horizontal shaker for specified periods of time. The nanoparticles were then magnetically decanted with the help of an external magnet. The supernatant was transferred to a syringe, filtrated through a Nylon filter (0.45 µm) and acidified with concentrated HNO$_3$ (65%, puriss. p.a. grade) to achieve a final acid concentration of 1% v/v. The concentration of lead in the supernatant was determined by FAAS.

General procedure for the Pb$^{2+}$ extraction from aqueous solutions with PS resins

An aliquot (5 mL) of a solution of Pb(ClO$_4$)$_2$ was added to a closed vial containing the required amount of polymer. The samples were orbitally shaken on a horizontal shaker for specified periods of time. The samples were allowed to decant and a 2 mL aliquot of the supernatant was transferred to another vial, evaporated to dryness in an argon stream and the residue was dissolved in 5 mL aqueous 1% v/v HNO$_3$. The concentration of lead in the supernatant was determined by FAAS.

General procedure for the Pb$^{2+}$ extraction from aqueous solutions with PS-PEG resins

An aliquot (5 mL) of a solution of Pb(ClO$_4$)$_2$ (of around 83 ppm) was added to a closed vial containing the required amount of polymer. The samples were shaken on a horizontal shaker for specified periods of time. The samples were allowed to decant and the lead in the supernatant was separated with a syringe, filtered through a Nylon filter (0.45 µm) and acidified with concentrated HNO$_3$ (65%, puriss. p.a. grade) to achieve a final acid concentration of 1% v/v. The concentration of lead in the supernatant was determined by FAAS.
Conclusions

In summary, we have shown that CuAAC reactions (the popular and widely used click chemistry) can be used as a most practical ligation methods for the preparation of immobilized complexing agents involving a clickable crown ether or azacrown ether complexing unit and a support allowing easy separation (a magnetic nanoparticle or a resin). The prepared library of clicked extractants covers different types of magnetic nanoparticles (e-Co and Fe₃O₄) and resins (PS and PS-PEG), different approaches to the binding of the azido group to the nanoparticles (two-point carboxylic acid binding, three-point phosphonic acid binding and covalent silicate binding through α-azidoalkyl(trialkoxy)silanes), and different spacer lengths separating the functional unit from the support. Some of these complex extracting materials, such as 11ai-bi, 20a-b, and 21a-b, are remarkably easy to prepare being only a few steps away from commercial precursors.

To illustrate the potential of this approach, we have studied the application of the clicked complexing agents to the removal of Pb²⁺ from aqueous and organic solutions. Remarkably, conditions have been developed for removing >90% of the initial Pb²⁺ in a single operation from water and from organic solvents (acetonitrile and tetrahydrofuran).

When the performance of both types of materials (MNP-based and resin-based) in the planned complexation of Pb²⁺ from its solutions is considered, the merits of functional polymers and functional nanoparticles depends on the nature of the media from which Pb²⁺ needs to be extracted. To remove lead contamination from organic solvents, functional polymers such as 20a-b probably represent the best alternative. Thus, very high extraction levels (> 90%) can be achieved at stoichiometric extractant to Pb²⁺ ratios. On the other hand, small and readily dispersable, functional Fe₃O₄ nanoparticles offer the advantage of being equally suitable for organic (11ai) and aqueous (17a) solutions, while functional polymers provides only mediocre results in this last situation. Taking into account the experimental simplicity of the complexation of the Pb²⁺ cations in combination with the ready separation of the corresponding complexes by magnetic decantation, the use of complexing agents clicked onto MNPs offers substantial potential for future applications in the detoxification area.

Acknowledgement

This work was funded by MINECO (grant CTQ2012-38594-C02-01), DEC (grant 2014SGR827), EU-ITN network MagNet(ieFun (PITN-GA-2012-290248), and ICIQ Foundation. We also thank MINECO for support through Severo Ochoa Excellence Accreditation 2014-2018 (SEV-2013-0319). C.M. thanks the ICIQ foundation and Agencia de Gestió d’Ajuts Universitaris i de Recerca (AGAUR) for a predoctoral fellowship.

Notes and references

For a comprehensive review on immobilization of catalysts through poly(ethyleneimine) units for the removal of mercury(II) from aqueous solutions, see: S. Fernandes, C. M. Eichenseer, P. Kreitmeyer, J. Rewitzer, V. Zlateski, R. N. Grass, W. J. Stark and O. Réiser, RSC Advances 2015, 5, 46430.

This journal is © The Royal Society of Chemistry [year].
