Using high-throughput techniques to identify complexants for ^{137}Cs, ^{60}Co and ^{90}Sr†

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High-throughput synthetic and screening methods have been used to prepare and evaluate caesium, cobalt and strontium selective sequestering agents derived from aza-crown ethers. Storage phosphor autoradiography was the screening method used to determine potential lead compounds. The most effective complexants for Cs$^+$ have a very high selectivity over potassium ions and will bind picomolar quantities of Cs$^+$ in the presence of mM K$^+$. The binding and selectivity of these compounds with ^{60}Co and ^{90}Sr have also been investigated and selectivity was also determined with tap water and Ca^{2+}, respectively. Experiments with tap water spiked with ^{137}Cs and ^{60}Co were carried out to confirm the decontamination capabilities of some of our compounds and a good correlation between autoradiography and these results were found.

Introduction

Radioactive contamination of surfaces and aqueous solutions can present substantial health and environmental hazards, and the development of materials that can be used to scavenge such contaminants is an essential part of any strategy for the remediation of contaminated materials. The technical obstacles to such remediation are, however, severe since the radioelement of interest may be present in nanomolar or lower concentrations and still present a hazard. These difficulties are exacerbated in cases where the contaminant radioisotope is present at low concentrations and stable elements with similar chemical properties are present in large excess. This is particularly true when developing selective scavengers for ^{134,137}Cs$^+$ in the presence of K$^+$ and for ^{89,90}Sr^{2+} in the presence of Ca^{2+}. In such circumstances, an effective decontaminant must display both a high affinity for the element(s) of interest and a high selectivity against potential interferences.

Traditionally, radionuclide complexants have been developed iteratively in a time-consuming process. In other fields, high-throughput techniques have been used to great effect, notably in the field of drug discovery and pharmacokinetics;[1,2] such techniques offer rapid screening of targeted libraries of compounds, and provide a useful shortcut in the discovery process. To our knowledge, such an approach has not been applied to the design of selective complexants, despite the ease with which radioisotopes lend themselves to high-throughput screening. We now report the synthesis and high-throughput (HT) screening of a library of potential ^{137}Cs, ^{60}Co and ^{90}Sr complexants.

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Results and discussion

Library definition

The main focus of this paper is concerned with Cs$^+$ complexation, but our investigation also extended to Co$^+$ and Sr^{2+}, and the results for these radionuclides are presented in the ESI.† The aza-crown ether structure has previously been shown to bind Cs$^+$ efficiently and has been used in the separation of ^{137}Cs from liquid nuclear waste. Vibhute et al.[3] used a dibenzo-24-crown-8 derivative to remove ^{137}Cs selectively from solutions containing s- and p-block elements. Amide-type open-chain crown ethers have also been found to exhibit a high affinity for Cs as well as other alkali earth ions through formation of pseudomacrocycles, and have been used to extract Cs from bulk NaCl.[4,5]

Our initial library of compounds was based around the aza-crown structure containing one nitrogen atom in the ring. The rings were attached to functionalised polystyrene or silica solid supports either directly or via different intermediate chains. We used six different solid supports, five intermediate chains and three ring sizes (based on 12-, 15- and 18-membered rings containing four, five and six donor atoms respectively) in combinations that gave a library of 26 compounds. Varying in surface coverage, from 1.3 to 5 mmol g^{-1} of active component, the supports also range in particle size from 100 to 1100 μm in radius. The synthesis and characterisation of the library is presented in the ESI† with key compounds summarised in Fig. 1. Whilst the nature of the bound reagents has not been fully analysed at this stage what is important is the reproducibility of the synthesis and the principle and execution of the idea. Screening this library for affinity and selectivity towards ^{137}Cs, ^{60}Co and ^{90}Sr permits us to examine effects of varying crown sizes, solid support and speciation to maximise selectivity over K$^+$, tap water and Ca^{2+}, respectively.

Screening of ^{137}Cs, ^{60}Co and ^{90}Sr sequestration

In this study, we used storage phosphor technology[6] to quantify the affinity and selectivity for ^{137}Cs, ^{60}Co and ^{90}Sr of the

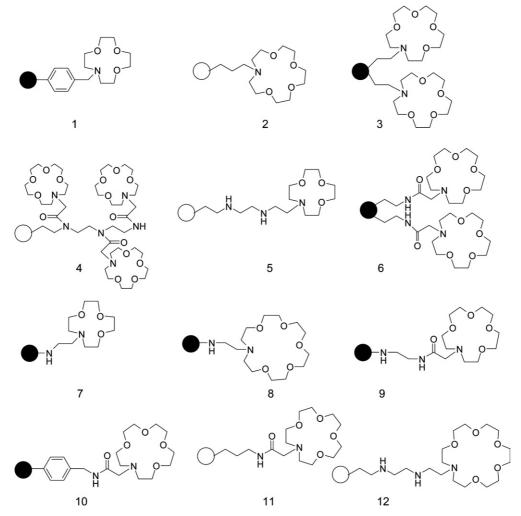**Fig. 1** Selected key compounds: ● = polystyrene solid support; ○ = silica solid support.

compounds in the library. In our system, the imaging plate is coated with fine photostimulable phosphors, in this case BaFBr:Eu^{2+}. On exposure to ionising radiation, the Eu^{2+} is formally oxidised and the electron thus produced is trapped. On excitation by a laser, the intensity of the emission associated with de-excitation is proportional to that of the incident ionising radiation. Johnston *et al.*[7] were the first to employ storage phosphor technology for autoradiography to study samples labelled with ^{32}P, ^{14}C and ^{32}S. This linear response and the very high sensitivity associated with this technique contrasts with conventional autoradiography using X-ray film, which shows a relative lack of sensitivity and a non-linear dynamic range. This has opened up a range of applications, including detection of extremely low level radioactivity on floor surfaces and lab shoes[8] and identification of radioactive particles in soil samples.[9]

Storage phosphor autoradiography calibration

Calibration measurements were used to show that storage phosphor autoradiography can be used routinely for sample activities between 100 Bq (0.23 pmoles, 31 pg) and 1 kBq (2.3 pmoles, 310 pg) of ^{137}Cs. To prepare the 96 well-plates, seven different activities of ^{137}Cs were pipetted into wells and then the

storage phosphor screen was exposed to the well plate by placing it above the open plate for 20 min and 1 hour. The screen was then "read" using a HeNe laser ($\lambda = 633$ nm) in a Typhoon™ phosphor imager (GE Healthcare, Amersham). The degree of darkening associated with each well is quantitatively related to the activity bound, and the average pixel intensities were determined using ImageQuant™ software. These values were then plotted against activity and a linear response was obtained down to sub-picomolar concentration for both exposure times, as shown in Fig. 2.

High-throughput screening

In order to screen a relatively large number of compounds simultaneously, 96 well-plates with filters on the bottom of each well were used. The material to be screened was weighed into the wells and for ^{137}Cs, as required, was pre-equilibrated with a 10 mM aqueous solution of K$^+$ then contacted with 250 Bq (0.575 pmoles) carrier-free ^{137}Cs (Cerca LEA, France); contacted directly with ^{137}Cs; or contacted with a 10 mM K$^+$–^{137}Cs solution. The plate was filtered under vacuum as soon as the last well had been spiked with ^{137}Cs. The same experimental procedure was followed for the ^{60}Co and ^{90}Sr measurements (Cerca LEA,
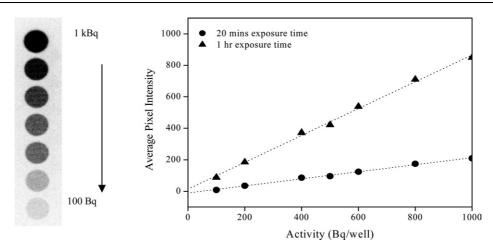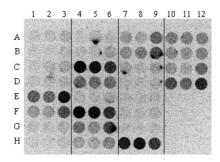
Fig. 2 Autoradiograph of varying activities of ^{137}Cs and graph illustrating the linear relationship between average pixel intensities and activity per well after exposure times of 20 minutes and 1 hour.

France) but in these cases tap water was used for ^{60}Co selectivity and a Ca^{2+} solution was used for ^{90}Sr. In the case of ^{137}Cs, the effect of leaving the compounds in contact with the solutions for longer was also examined. Instead of filtering immediately, the K^+ and Cs^+ solutions were left for an hour before filtering (image not shown). No significant increase in uptake was observed, showing that metal binding and equilibration occurs rapidly.

An example of radiometric screening data is given in Fig. 3 after 22 hours. The radiometric screening data for ^{60}Co and ^{90}Sr is given in the ESI.† In the plate shown, 28 compounds were screened with each compound tested three times. In the first column of each group (columns 1, 4, 7 and 10) only ^{137}Cs (250 Bq) was added; in the second columns (2, 5, 8 and 11), the wells were pre-treated with 10 mM K^+ before adding ^{137}Cs. In the third group (3, 6, 9 and 12), ^{137}Cs and 10 mM K^+ were added simultaneously. All solutions used were adjusted to pH 7 before addition to the wells.

It can be seen immediately that there are a number of compounds (e.g. C4–C6 (**6**)) that exhibit significantly stronger binding of ^{137}Cs. There is also evidence to suggest that some of these compounds actually bind the Cs selectively over K^+, since the presence of vast excesses of K^+ does not prevent Cs^+ binding (e.g. C4–6 (**6**), D4–6 (**3**)). Furthermore, in some cases Cs^+ binding can be improved by the presence of K^+ (E1–E3 (**5**), D10–D12 (**4**)), implying that some sites in the structure may bind to potassium and "pre-organise" the structure for Cs^+ binding. Those compounds that are poor at binding ^{137}Cs are identified by the wells where the pixel intensity is very low. Examples of these are in wells A1–A3 (**7**), G1–G3 (**9**), H1–H3 (**10**) and F7–F9 (**8**). The compounds that are good at binding generally contain more rings and have larger ring sizes (15-crown-5 and 18-crown-6).

The diameters of the Cs^+ ion and K^+ ions are 3.34 and 2.66 Å, respectively, so, in principle, the Cs^+ ion should not be able to fit inside the cavity of either the 15-crown-5 or the 18-crown-6, which have cavity sizes of 1.7–2.2 and 2.6–3.2 Å, respectively. It has been shown previously[10] that with compounds like these it is possible for alkali and alkaline earth metals to form 'sandwich' structures of the form ML_2. It is notable that the most effective systems are those in which macrocycles are appended from a branched chain, and in which such ML_2 binding does not rely on the presence of a second chain bound nearby on the support surface. Effectively these branched chains favour chelation by two macrocycles at once, and minimise the loss in entropy on coordination of a second macrocycle to the metal centre. By contrast, K^+ ions will fit into a single cavity and favour ML complex formation over ML_2 complex formation. In the absence of this additional chelate effect, the binding affinity would be expected to be much reduced, as indeed it is.

Correlation with water-decontamination experiments

In order to confirm the autoradiography results we carried out decontamination experiments by running tap water spiked with ^{137}Cs through solid-phase extraction columns containing the appropriate compound (the decontamination of ^{60}Co spiked tap water has also been investigated and the results are given in the ESI†). After collecting the filtrate, a gamma spectrum was

	1	2	3	4	5	6	7	8	9	10	11	12
A		7			16			2			15	
B		1			19			4			12	
C		13			6			18			25	
D		14			3			17			23	
E		5			9			20				
F		26			6			8				
G		9			24			21				
H		10			11			22				

Fig. 3 Autoradiograph of 96 well plate containing crown-modified solid supports with ^{137}Cs, 22 h exposure and table to show locations of compounds in the wells.
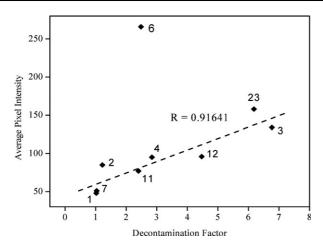
Fig. 4 Graph to show average pixel intensities *versus* decontamination factor (DF).

obtained and the counts of the γ peak at 654.22 keV were compared to a spectrum of a standard solution of ^{137}Cs containing the same activity. From these values it was then possible to determine the decontamination factor (DF) of each of the compounds tested (DF = counts of γ peak in standard solution/counts of γ peak in decontaminated sample). The DFs were compared to the average pixel intensities from the autoradiography image (Fig. 4).

For all the compounds tested, with the exception of compound **6**, there is good correlation between decontamination factor and average pixel intensity. Compound **6** does not compare as well as the autoradiography image would predict, and this may be a function of the experimental factors including flow rate, quantity of material and contact time. The DF values that are determined here are a result of adding the spiked tap water to the column, which was then pushed straight through, hence a short contact time. The filtrate was then collected and counted on the gamma spectrometer. No attempt was made to investigate flow rate or contact time.

Summary and conclusions

We have demonstrated that storage phosphor autoradiography can be used to screen compounds for their affinity and selectivity towards our target radioisotopes, ^{137}Cs, ^{60}Co and ^{90}Sr using a high-throughput approach. This has allowed us to identify potential lead compounds quickly, and allows rapid optimisation of molecular design. We have also tested some of our compounds for their capabilities to decontaminate tap water that has been spiked with either ^{137}Cs or ^{60}Co and we have found good comparisons between these results and those from autoradiography experiments. Further studies are currently in progress to exploit these leads and to establish the mode of binding.

Experimental

Instruments

Storage phosphor autoradiography was carried out on a Typhoon 9410 Variable Mode Imager (GE Healthcare) using storage phosphor screens (Amersham Biosciences). Data was analysed using ImageQuant™ software.

Gamma Spectroscopy was carried out on an EG&G lo-ax HPGE semiconductor gamma ray detector coupled to an EG&G 919 ADC/MCA.

The vacuum manifold and 96 well filter plates were purchased from Porvair Sciences. Decontamination experiments were carried out using solid-phase extraction (SPE) columns (Chromabond®, Fisher Scientific).

Materials

^{137}Cs, ^{60}Co and ^{90}Sr (all in 0.1 M HCl) were purchased from Cerca LEA, France. Potassium carbonate (Merck, VWR), caesium carbonate, cobalt chloride hexahydrate, calcium hydroxide and strontium hydroxide (Aldrich) were used for autoradiography experiments and for pH adjustments.

Decontamination experiments

Decontamination experiments were carried out with ^{137}Cs/^{60}Co spiked tap water. Metal ion content of the tap water was determined as Mg = 280 ppm, Na = 589 ppm, K = 766 ppm, and Ca = 317 ppm. Selected solid supports were put into solid-phase extraction columns. The compounds were chosen based on autoradiography results demonstrating both good and bad ^{137}Cs–^{60}Co binding. Equal amounts of each compound (0.12 g) were weighed out into 3ml solid-phase extraction columns. A standard solution containing 1 kBq of radionuclide in tap water was counted on the gamma spectrometer. The procedure for all experiments was: firstly 2 ml of tap water were run through the column followed by 5 ml of spiked tap water and finally 2 ml of tap water were run through again to remove any residual radioisotope left in the column. The decontamination factors (DF), were determined from the counts of the ^{137}Cs gamma peak at 654.22 keV and for ^{60}Co the gamma peak at 1159.99 keV (counts of standard solution/counts of filtrate = DF).

Acknowledgements

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