A Novel Application of Magnetic Nanoparticles in Solid Phase Extraction of ^{90}Sr in Urine

By

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Abstract

Two solid phase extraction (SPE) systems based on novel magnetic nanoparticles (MNP) were tested for selective extraction of ^{90}Sr for emergency radiobioassay. Poly-2-Acrylamido-2-methylpropane sulfonic acid (p-AMPS) coated MNPs were used to extract ^{90}Sr from urine, which was eluted with 200 mM phosphate for measurement by liquid scintillation counting. The linear dynamic range was 12 Bq/L to 600 Bq/L with an overall recovery of $87\% \pm 10\%$ and minimal detectable amount of $4.9 \text{ Bq/L} \pm 0.5 \text{ Bq/L}$. A 3% error with an average relative bias of 12% was found. The extraction time is 45 minutes per batch of 9 samples. The second SPE system was 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 immobilized to the surface of p-acrylamide coated MNPs for the extraction of ^{90}Sr in urine. Trapping efficiency of ^{90}Sr was found to be $101\% \pm 5\%$. The elution efficiency using EDTA was found to be $87\% \pm 8\%$.

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List of abbreviations

MNP	magnetic nanoparticle
SPE	solid phase extraction
AMPS	2-acrylamido-2-methylpropane sulfonic acid
EDTA	ethylenediaminetetraacetic acid
ANSI	American National Standards Institute
MDA	minimum detectable amount
DVB	divinylbenzene
LSC	liquid scintillation counter
p-	poly-
FTIR	fourier transform infrared

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1. Introduction

1.1 Background on ^{90}Sr

1.1.1 What is ^{90}Sr ?

^{90}Sr (Strontium-90) is a radioactive isotope of strontium formed through the fission of uranium or plutonium¹. It is an alkaline earth metal, same as calcium and thus it has similar chemistry. Strontium has a half-life of 29.1 years and undergoes beta decay, which is the process in which a neutron becomes a proton and emits an electron and an antineutrino². ^{90}Sr undergoes two decay steps, going from ^{90}Sr to ^{90}Y and finally ^{90}Zr ³. The half-life for ^{90}Y is 64 hours while ^{90}Zr is a stable element⁴. They both are likely to lose 2 electrons to attain a full valence shell and will react with water or acids to form $\text{M}(\text{OH})_2$, where M can be either calcium or strontium. Yttrium, on the other hand, has an extra electron, in the d-orbital. Yttrium, therefore forms trivalent compounds, such as $\text{Y}(\text{OH})_3$. Figure 1.1 demonstrates the decay series of ^{90}Sr .

Figure 1.1: Decay series of ^{90}Sr

1.1.2 How ^{90}Sr enters the body

Strontium-90 can make its way into the environment, and subsequently into the human body via inhalation or through ingestion via two possible methods. Firstly, the ^{90}Sr which is already present in the environment from nuclear explosion testing and through the Chernobyl reactor meltdown can be absorbed in the body. In 2011, there

was a leak of spent nuclear fuel at the Fukushima nuclear plant in Japan which released ^{90}Sr into the ocean, among other radionuclides. The release of ^{90}Sr into the waterways could potentially contaminate water and food supplies worldwide^{5,6}. Another method of exposure to ^{90}Sr could come from a radiological dispersal device (RDD), commonly known as a dirty bomb, likely to be involved in a terrorist attack. In the event of exposure, methods for the detection of ^{90}Sr are crucial and must be rapid, effective and require the smallest volume possible⁷.

1.1.3 Health effects of ^{90}Sr

The adverse effects of ^{90}Sr contamination arise from its chemical similarity to calcium and the radioactivity of ^{90}Sr and its daughter, ^{90}Y . Its chemistry allows ^{90}Sr to bind itself to bones, categorizing it as a “bone seeker”. Once bound to the bones, the radioactivity increases the risk of bone and marrow cancers¹. However, a large portion of the ^{90}Sr will leave the body through excretion (both urinary and fecal) and therefore, radiobioassay techniques can be used for its determination in the excreta. Using an appropriate biokinetic model, the remaining body burden of ^{90}Sr , and therefrom, the potential internal radiation damage can be assessed. Radiobioassay for ^{90}Sr is usually accomplished by the analysis of urine samples¹.

1.2 Governmental response a radiological or nuclear emergency

In an effort to better prepare Canada for radiological events, the Chemical, Biological, Radiological-Nuclear, and Explosives (CBRNE) Research and Technology

Initiative (CRTI) was launched in 2002. The CRTI aims to accomplish this goal by funding scientific and technological research which may either prevent a radiological/nuclear (R/N) event or improve Canada's response if an incident occurs⁸. The following body of work is funded by the CRTI through the rapid methods for radiobioassay (CRTI06-230RD) in an effort to improve on ⁹⁰Sr analysis in human urine samples.

1.3 Performance criteria for radiobioassay

The American National Standards Institute's (ANSI) created a standard for emergency radiobioassay, described in ANSI N13:30. These standards state that a radiobioassay method should be able to detect ⁹⁰Sr in urine with a relative accuracy of 40% and a relative bias between -25% and 50% in urine.

The individual relative bias follows the equation⁸:

$$B_{ri} = \frac{C_i - C_s}{C_s}$$

where C_i is the measured concentration and C_s is the known concentration of the sample. To obtain the relative bias for multiple samples the following equation can be used⁸:

$$B_r = \frac{\sum_i^n \frac{(C_i - C_s)^2}{n}}{C_s}$$

Since ⁹⁰Y (also a beta emitter) is a decay product of ⁹⁰Sr, a challenge arises due to the interference of ⁹⁰Y during liquid scintillation counting (LSC). Since it is difficult to determine the ⁹⁰Sr/⁹⁰Y ratio before analysis, ⁹⁰Y must be chemically separated from the

sample without affecting the measurement of ^{90}Sr at the required levels of detection, precision and accuracy⁹.

The required minimum detectable amount (MDA) for an emergency radiobioassay method for ^{90}Sr was calculated by Li and Kramer. It has been set at 10% the activity requiring immediate intervention, called the action level. The calculated amount took into account biokinetic models and that the urine sampled was excreted 3 days after an R/N emergency. The MDA required for this measurement has been found to be 19 Bq/L¹⁰. To calculate the MDA in Bq/L is determined the following formula is used⁸:

$$MDA = \frac{(4.65 \times S_b + 3)}{E \times t \times V}$$

S_b is the standard deviation of the measurement of multiple blanks. E is the percent efficiency of the procedure, t is the time of measurement in seconds and V is the volume of sample used in liters.

1.3 Current strategies for monitoring ^{90}Sr in humans

The conventional method for ^{90}Sr analysis, especially in radiobioassay, has been to use traditional solid phase extraction (SPE) resins, such as Eichrom Sr-spec resin. Different techniques have been developed. Some techniques require lengthy preparation times, long analysis times, and special equipment, while others do not meet the required detection limit^{7,9}, or present a combination of these issues.

1.3.1 Eichrom resin

Eichrom Sr spec resin is a commercially available resin for chromatographic separation of ^{90}Sr from interferants in solution. The resin consists of 3 parts: a sorptive moiety, an organic spacer and a solid support. The sorptive moiety used for the Sr spec resin is 4'4'5di-tert-butyl-cyclohexano-18-crown-6. The spacer is an organic material that holds the sorptive moiety to the solid support. Polymers with the polyacrylamide backbone have been found to work for this application. The solid support can either be inorganic (usually a metal oxide), or organic and is used to keep the sorptive moiety in the solid phase during the chromatographic separation¹¹. Eichrom Sr spec resin absorbs ^{90}Sr in the presence of 3-8M nitric acid. There is no retention of ^{90}Y by the resin. Once the column is eluted with an aqueous solution containing 0.05M HNO_3 , the ^{90}Sr is stripped from the column in its entirety. This allows for the measurement of ^{90}Sr while removing any matrix effects, such as matrix components or ^{90}Y interferences^{12,13}.

1.3.2 Description of previous analytical methods using traditional SPE resins

Alvarez et al. described a technique which is used for routine radiobioassay of workers potentially exposed to ^{90}Sr . A 24-hour urine sample is collected from each worker and 60 mL of HNO_3 is added to it. The solution is stirred and heated to a temperature of 70-80°C before 1 mL of H_3PO_4 , 2 mL of $\text{Ca}(\text{NO}_3)_2$, tracers, $\text{Sr}(\text{II})$ and NH_3 are added. This mixture is stirred for 1 hour before allowing the precipitate to settle overnight. The sample is then wet ashed by adding 10 mL of concentrated HNO_3 and evaporating the sample to dryness. This step is repeated several times until all the

organics are removed and the sample is then dissolved in 75 mL of 8 M HNO₃. A 10 mL portion of this solution is then passed through an Eichrom resin that has been preconditioned with 8 M HNO₃. The column is washed with 8 M HNO₃ and ⁹⁰Sr is finally eluted using 5 mL of 0.05 M HNO₃. This aliquot is measured by LSC. It requires approximately 24 hours for sample preparation¹⁴. Another method, by Antonio et al., describes an equally time consuming technique as Alvarez et al published in 2009¹⁵.

Li et al.¹⁶ described a field deployable rapid bioassay method for ⁹⁰Sr where urine is stabilized with 1% HCl and passed through an Eichrom prefilter cartridge to decolourize. The urine is then mixed with 0.5 mL of 0.5 M Na₂HPO₄ and 0.45 mL of 5 M NaOH to mask the ⁹⁰Y through the formation of an yttrium phosphate complex. After stabilizing for 10 minutes, the sample is passed through an anion exchange resin preconditioned with 100 mL of 10 mM triethanolamine. A 5ml aliquot is measured by LSC. Although the method meet the required performance criteria for accuracy and precision as defined by ANSI N.13:30, the detection limit was 121 Bq/L, which is higher than required limit of detection for an emergency radiobioassay method for ⁹⁰Sr set at 19 Bq/L as described by Li and Kramer⁹. Sadi et al. describes another technique with similar sample throughput, using in lab techniques. They achieved a MDA of 40 Bq/L¹⁷.

A high performance extraction chromatography analysis was developed by Plionis et al. using the Eichrom Sr-spec resin. In this method, 4 mL of sample was run through the column at a flow rate of 1 mL/min. The analyte was stripped from the column for 24 minutes and then the column was reconditioned for 2 minutes. The detection limit was 47 decays per minute using 2 mL of urine mixed with 2ml of HNO₃.

This amounts to a detection limit of 391 Bq/L, which is not low enough for emergency radiobioassay¹⁰. This method also required an analysis time of 30 minutes per sample¹⁸. The detection limit could have been decreased with larger loading volumes, however, this would likely increase the time of analysis.

1.3.3 Analysis of ⁹⁰Sr without traditional SPE resins

A faster method for analyzing ⁹⁰Sr was developed by Hrdina *et al.* where ⁹⁰Y was precipitated through the formation of a hydroxide at pH 7.5. The precipitate was removed via centrifugation. Magnetic nanoparticles were coated with an AMPS polymer cross-linked with Divinylbenzene (DVB). 4'4'5di-tert-butyl-cyclohexano-18-crown-6 was immobilized to the surface. These MNP's were used to extract ⁹⁰Sr from the urine sample at pH 9. The result was an 83% recovery of ⁹⁰Sr whose detection limit was dictated by the pre-concentration factor and a removal of 89% ⁹⁰Y. However, this method required sample preconditioning through the adjustment of the pH and centrifugation for the co-precipitation of ⁹⁰Y as Y(OH)₃ at pH 7.5. These steps lead to a 2 hour analysis time¹⁹.

1.4 Theoretical background

This work aims exploit the application of magnetic nanoparticles (MNPs) in selective solid phase extraction of a radionuclide. The MNPs in suspension can be coated by an organic polymer by synthesizing the polymer in presence of the MNPs. The selectivity toward radionuclide extraction can be achieved by either incorporating a

suitable functional group to the polymer backbone or by impregnating the polymer coating by a suitable complexing agent. Due to the smaller size of the polymer coated MNPs, they can be easily dispersed in a sample solution in order to achieve more efficient phase transfer of the analyte from the sample solution phase to the surface modified MNPs. After the extraction and preconcentration of the radionuclide, the surface modified MNPs can be collected at the bottom of the sample container by the application of an external magnetic field. This allows a simple and convenient way of preconcentration of a radionuclide from a large volume of sample solution to a very small quantity of surface modified MNPs. After the preconcentration and matrix removal, the radionuclide can be eluted to a small volume of a stripping solution ready to be measured by a suitable detection technique.

1.4.1 Magnetic particle synthesis

The synthesis of magnetic nanoparticles uses a technique described by Kang et al. The synthesis requires the oxidation of FeCl_2 and FeCl_3 in a 1:2 molar ratio under basic conditions (pH 11-12). These magnetite (Fe_3O_4) nanoparticles have an average diameter of 8.5 ± 1.3 nm. More importantly, the methods used do not require any surfactants, as required in other methods. This removes a purification step which was once required in the formation of iron oxide nanoparticles²⁰. The reaction is as follows (Figure 1.2):

Figure 1.2: Reaction of iron (II) chloride and iron (III) chloride to form magnetic nanoparticles

1.4.2 Radical chain polymerization

In a radical chain polymerization reaction, an initiator is used to form a radical from of desired molecules (the monomer) to initiate a reaction between monomer molecules to form a polymer. There are 4 steps to a radical chain polymerization: production, radicalizing the monomer, propagation and termination.

The production stage consists of the use of an initiator to produce free radicals. This is generally formed by the homolytic cleaving of the initiator (I) to form a pair of radicals ($R^{\bullet}$). These radicals are termed primary radicals. The radicalization of the monomer is a reaction between the monomer and the radical species. In the case of this study, the π bond of the acrylamide species double bond is cleaved, creating a single bond and turning the monomer into a radical. These are the chain initiating species. The chain initiating species react with other monomers at the double bonds, cleaving the pi bond and creating a longer radical. The termination occurs when two radicals react at their radical sites, creating a single bond and pairing the two electrons preventing further reaction with the monomer. The steps of radical chain polymerization are described by figure 1.3²¹:

Figure 1.3: Steps of radical polymerization²⁰

Cross linking of polymers is an act of polymerizing monomers with multiple polymerization sites. This can be done by itself or in conjunction with co-monomers. The polymerization of monomers with multiple polymerization sites leads to non-linear polymer chains. An example is the polymerization of styrene cross-linked with divinylbenzene as is shown in figure 1.4²².

Figure 1.4: Polymerization of styrene cross-linked with DVB²¹

1.4.3 Solid phase extraction (SPE)

SPE is an analytical technique designed to pre-concentrate analytes by passing large volumes of sample through a solid sorbent material that will trap the desired compound (the analyte). The analyte is then eluted from the solid phase using a smaller volume of extracting solvent. SPE uses various interactions to trap the analyte. The two that are of interest in this work are ion exchange and crown ether complexation induced by ion pairing.

The disadvantage to traditional SPE systems is that they are column, cartridge, or disk based and require a preconditioning step and constant monitoring²³. If the SPE system is in the form of magnetic particles suspended in solution, these steps are not necessary.

The first type of SPE technique that is used in this study is ion exchange SPE. Ion exchange systems use a suitable functional group incorporated within the polymeric backbone to extract oppositely charged analytes. The disadvantage of this method is that it does not differentiate between analytes and interfering compounds of the same charge type. However, in the case of cation exchange systems, if the number of charges on the analyte and its interferent(s) is different, it can be eluted separately. A species with a higher charge will have a stronger interaction with the sorbent than a species with a lower charge. If the target has a lower charge, it can be eluted with a low to moderate strength eluent and analyzed without eluting the interferent(s). If the charge is higher, a washing step could be employed to remove the interferent(s) using a low to moderate strength eluent followed by a stronger eluent to remove the target from the SPE system²².

Selective affinity of Sr^{2+} with in the cavity of 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 was utilized as second mode of interaction for SPE of ^{90}Sr on the surface modified MNPs. However, in order to promote the ^{90}Sr -crown ether complexation in a neutral polymeric phase, $^{90}\text{Sr}^{2+}$ present in the aqueous phase needs to be ion-paired with a counter anion that will neutralize the charge on ^{90}Sr and increase its affinity to be partitioned into surface modified MNPs. The advantage of this technique is that it is

useful for selective targeting of analytes. The ion pair used in this work is strontium and nitrate as described by the equation shown in figure 1.5.

Figure 1.5: Ion pairing of Sr^{2+} and NO_3^{1-}

1.4.4 Liquid scintillation counting

LSC is a method used to measure the number of radioactive decays within a sample over a given period of time. The sample is mixed with a LSC cocktail. The cocktail is made up of two main components, a solvent and a phosphor. The solvent absorbs the energy from the radiation particles and transfers it to the phosphor allowing the phosphor to emit light. This light is detected and is proportional to the number of radiation particles produced, as well as the concentration of the radioactive species.

The solvent is generally made up of aromatic organic solutions. The π clouds on aromatic species are effective in capturing the energy of radiation particles. Once this energy is captured, it is transferred to the phosphor since aromatic species do not tend to release energy once absorbed by either emitting light or through the destruction of the molecule²⁴.

The phosphor consists of a primary and secondary scintillator, as shown in Fig. 1.6. The primary scintillator is a molecule which can be excited by the solvent to the point of fluorescence and can form a dipole moment in its solvent shell. The dipole moment will allow the primary scintillator to absorb energy from solvent molecules that

are not in direct contact with the scintillator, improving the efficiency of the transfer. Naphthalene is an example of a scintillator. The secondary scintillator is a molecule that can absorb the light emitted by the primary scintillator and then emit a photon of higher wavelength than the one absorbed. This secondary emission has been found to improve the efficiency of the scintillation counting methods²³.

The energy of the beta particles can be measured by the LSC and a spectrum can be generated. The energies of beta particles from different elements vary, allowing for some identification of the analytes in a sample. If there is enough energy separation, it is possible to quantify different radionuclides without chemical separation. Figure 1.6 below is an example of a spectrum containing ^3H , ^{14}C and ^{32}P .²³

Figure 1.6: LSC spectra for ^3H , ^{14}C and ^{32}P . Pulse energy is the energy of the light being captured by the detector²³.

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The analytical signal from a LSC counter can be quenched by two separate mechanisms: chemical quenching and colour quenching. Chemical quenching is the result of beta particle emissions being absorbed before they reach the solvent or

phosphor²⁵. Nitric acid is an example of a chemical quencher²⁶. Colour quenching is the result of the solution absorbing the light of the phosphor before it can reach the detector. Figure 1.7 demonstrates the mechanism for Beta counting along with color and chemical quenching. Figure 1.8 uses three superimposed spectra to demonstrate that chemical quenching will decrease the signal evenly at all energy levels whereas colour quenching will decrease lower energy signals, leaving higher energy ones unaffected.

Figure 1.7: Mechanism for LSC counting. Excited molecules are shown as purple. A beta particle collides with and excites the solvent. The solvent transports the energy to one of two molecules: the phosphor (right) or a chemical quencher (left). The chemical quencher prevents the energy from reaching the phosphor. The phosphor then emits the light, which is captured by a colour quencher and is not detected (left) or the photodetector (right)²³.

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Figure 1.8: LSC spectrum showing the difference between a chemically quenched system and a colour quenched system.²⁷

1.5 Objective of this study

1.5.1 Magnetic separation for SPE

Magnet nanoparticles can be dispersed to fill the entire volume of a sample to allow the SPE sorbent to extract the analyte into the solid phase and can be compressed into a small volume and collected at the bottom of a container using an external magnetic field. Once collected, the analytes can be stripped from the magnetic particles using a small volume of an appropriate eluent and be ready for measurement.

1.5.2 Ion exchange solid phase extraction and selective elution

2-Acrylamido-2-methylpropane sulfonic acid (AMPS) is an acrylamide described by the structure shown in Figure 1.8. Acrylamides undergo radical chain polymerization as described in Section 1.3.1 forming p-AMPS. The sulfonic acidic group (SO_3H) of AMPS

has a pK_a of -2²⁸. Using the Henderson-Hasselbach equation (shown below), the ratio of conjugate base (A^-) to acid (HA) in solution can be found.

Figure 1.8: Structure of 2-acrylamido-2-methylpropane sulfonic acid

$$pH = pK_a + \log\left(\frac{[A^-]}{[AH]}\right)$$

When this equation is rearranged, it forms

$$pH - pK_a = \log\left(\frac{[A^-]}{[AH]}\right)$$

$$10^{pH-pK_a} = \frac{[A^-]}{[AH]}$$

This demonstrates that the higher the pH and the lower the pK_a , the higher the dissociation of the acid. With a pK_a of -2, AMPS is almost fully dissociated, and thus it would make a good candidate for an ion exchange resin at pHs above 0²⁹. Hrdina et *al.* found that at pH 9, p-AMPS coated magnetic particles trapped ^{90}Sr almost quantitatively. However, it was also found that ^{90}Y also binds to the particles. This binding could not be prevented with the use of selective chelating agents blocking the ion exchange sites. The use of co-precipitation was explored; however this removed a portion of the target analyte, decreasing the effectiveness of the method¹⁹. The first part of the present study will be to demonstrate the use of p-AMPS as an ion exchange SPE system to trap both ^{90}Sr and ^{90}Y for preconcentration followed by selective elution of ^{90}Sr into an aqueous phase which used phosphate as a counter ion, thus eliminating the need for a crown ether. This research direction has the advantage of using less

expensive magnetic particles which require a shorter production time and offer a faster analysis time.

1.5.3 SPE of ^{90}Sr using 4'4'5-di-tert-butyl-cyclohexano-18-crown-6 and HNO_3

It has been demonstrated that 4'4'5-di-tert-butyl-cyclohexano-18-crown-6 (structure shown in Figure 1.9) can be immobilized to polymeric compounds attached to solid supports, such as p-acrylamide, commercially known as Sr spec resin by Eichrom Technologies LLC. The structure of acrylamide is shown in Figure 1.10.

Figure 1.9: 4'4'5-Di-tert-butyl-cyclohexano-18-crown-6³⁰

Figure 1.10: Structure of acrylamide³¹

The capture of $^{90}\text{Sr}^{2+}$ by the crown ether immobilized to the polymeric support requires an acidic counter anion. It was also shown by Hrdina et al. that polymers with an acrylamide backbone which are cross-linked with divinylbenzene (DVB) could be used for the surface immobilization of 4'4'5-di-tert-butyl-cyclohexano-18-crown-6¹⁸. Hrdina used the sulfonic acid groups from the AMPS as the counter ions. However, the use of

an anion exchange polymer caused the $^{90}\text{Y}^{3+}$ to bind to the magnetic particles through cation exchange. The second part of this work aims to modify the anionic polymer Hrdina et al's with a neutral polymer, p-acrylamide. With the removal of the anionic functional group, the affinity for ^{90}Sr on the neutral polymer coated MNPs will be determined by the selective crown ether complexation induced by ion-pairing. The advantages to this modification include the ability to selectively extract a radionuclide without the need to carry out a traditional solid phase extraction with a column or cartridge that requires packing, preconditioning, loading, and stripping of the analyte from a sample solution using a vacuum box.

1.5.4 Biosprint 15 and automated magnetic separation

The Biosprint 15 is a device that allows for the automated manipulation of magnetic material using robotic arms to displace magnetic material from one well into another. Section 2.4 has a description of the device. Increasing the ease of an analysis using the magnetic nanoparticles by using a Biosprint 15 was tested. Both the ion exchange MNP's described in Section 1.4.1 and the ion pairing SPE particles described in Section 1.4.2 were tested with the Biosprint 15.

2. Material and methods

The materials and methods section is separated into 4 sections. The first involves the synthesis of the magnetic nanoparticles. The second section covers the method development of p-AMPS particles for radiobioassay of ^{90}Sr in urine. The third chapter describes the proof of concept of using p-acrylamide as a backbone for surface immobilization of 4'4'5di-tert-butyl-cyclohexano-18-crown-6 for the purpose of selectively capturing ^{90}Sr in urine. Finally, the fourth section discusses the use of a robotic magnetic separator (the Biosprint 15) as a scaled down proof of concept that robotic magnetic separation could be used during emergency radiobioassay.

The following is a list of all chemicals and equipment used during experimentation. All materials were purchased at the highest purity available. 99% pure ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) and 99.99% pure ammonium dihydrogen phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). 98% pure ferric chloride (FeCl_3) was obtained from Honeywell Riedel de Haen (Steelze, Germany). 97% pure sodium hydroxide pellets and reagent grade acetone were purchased from Caledon Laboratory Chemicals (Georgetown, ON, Canada). Azobisisobutyronitrile (98% pure) (AIBN) was supplied by Pfaltz & Bauer (Waterbury, CT, USA). Technical grade (80%) Divinylbenzene (DVB), 98% pure 4'4'5 ditertbutylhexano 18-crown-6 and 99% 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) were obtained from Aldrich (St. Louis, MO, USA). Acrylamide (99% pure) and Triton X-114 (>98% purity) was purchased from Sigma. Radioactive ^{90}Sr (332 Bq/g) in equilibrium with ^{90}Y in 1 M HCl with 100ppm inert ^{88}Sr and ^{89}Y carriers) was acquired from NIST (Gaithersburg, MA, USA). Nitric acid (70%) was

purchased from Anachemia (Montreal, QC, Canada). Optiphase Hisafe 3 liquid scintillation cocktail was purchased from Perkin Elmer (Woodbridge, ON, Canada). HCl (38%) was procured from Allied Chemical (Mississauga, ON, Canada) and methanol was obtained from Fisher Scientific (Fair Lawn, NJ, USA).

Deionized water was produced by a Millipore system from Milli Q, (Ottawa, ON, Canada) and was used to prepare all aqueous solutions. Urine was obtained from donors in conjunction with Health Canada. Preserved urine was acidified with 1% HCl, sonicated for 30 minutes and stored in the fridge at 4°C. Fresh urine samples were collected and used same day without further treatment. Beta counting was performed on a Perkin Elmer Tri-Carb 3180 TR/SL Liquid Scintillation Analyzer. A Varian scirnitir series 1000 Fourier transform infrared spectrometer (FTIR) and a Varian Cary 3 UV-Visible spectrophotometer were used for the FTIR of the p-acrylamde coated magnetic nanoparticles and the UV-Visible analysis of the transfer of magnetic nanoparticles. A grade N52 neodymium magnet measuring 10 cm X 10 cm X 1.3 cm (composed of NdFeB) was purchased from K&J's Magnetics Inc. A Biosprint 15 magnetic separator was purchased from Qiagen (Valencia, CA, USA).

2.1 Synthesis of functionalized magnetic particles

2.1.1 Ferromagnetic nanoparticle

Magnetic particles were synthesized as outlined by Yu and Chow in 2004³², and summarized by Hrdina et al, in the following manner: FeCl_3 (1.64 g) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.996 g) were dissolved in 30 mL of 0.17 M HCl. A three necked flask was filled with 50

mL of 1 M NaOH. The iron solution was added dropwise with mechanical stirring under an inert gas. The magnetic nanoparticles, which could be observed by the formation of a black precipitate, were formed instantly upon addition. Once the dropwise addition was complete, stirring was allowed to continue for 30 minutes before collection of the magnetic nanoparticles. The particles were then washed several times using deionized water and centrifuged before they were combined and dispersed in 250 mL of H₂O and stored at 4°C¹⁸.

2.1.2 Coating of magnetic nanoparticles with a polymer.

The magnetic particles were coated with one of two acrylamide polymers. Since the backbone of the polymers was identical, the procedure for the two polymerization reactions used was identical to that reported by Hrdina et al¹⁸. A 40 mL portion of magnetic particles was taken from the previously stored solution (containing ~ 7 mg/L of MNPs) and was washed with methanol and centrifuged. These particles were then re-dispersed into 20 mL of methanol. 40 mL of methanol solution containing the polymer reagents was then prepared in a 125 mL round bottom flask. The reagents included: AIBN (0.025 g), DVB (1.78 mL) and a certain quantity of acrylamide. For the p-AMPS polymer coated particles, 0.209 g of AMPS was added to the reaction vessel; for the neutral polymer coated particles, 0.0717 g of acrylamide was added. The flask was then sonicated until the reagents were dissolved and the magnetic particles were added. This solution was sonicated, bubbled with nitrogen to remove oxygen, and placed in a water circulation bath at 70°C for 15 hours. At this point, a white solid had begun to form and

the flask was sonicated for 15 minutes to disperse the solid in solution. The polymerization was resumed for another 9 hours for a total polymerization time of 24 hours at 70°C. After this time, more of the solid was observed in the reaction flask and the solution was sonicated once more for an additional 15 minutes. The solvent was evaporated and the dry particles were washed multiple times in acetone and then methanol using ultra sonication and magnetic separation. P-AMPS particles were light brown in colour whereas the p-acrylamide particles appeared darker in colour. Upon washing, the darker particles became progressively lighter resulting in light brown particles, similar in colour to the p-AMPS particles. The particles were dried overnight and the p-AMPS particles were re-dispersed in methanol (50 mg/mL). The p-acrylamide particles were tested to confirm the polymer's presence on the magnetic particle using FTIR and kept for further modification¹⁸.

2.1.3 Surface immobilization of 4'4'5 ditertbutylhexano 18-crown-6 on p-acrylamide coated MNPs

The dry nanoparticles coated with p-acrylamide were preconditioned by washing multiple times with acetone followed by multiple washings with methanol using an hour of ultrasonication and magnetic separation. This preconditioning step increases the affinity of the crown ether for the particles by raising their surface activity. The particles were dispersed in 50ml of methanol with 4'4'5 ditertbutylhexano 18-crown-6. The first batch used 0.47 g of 4'4'5 ditertbutylhexano 18-crown-6 and the second used 0.90 g 4'4'5 ditertbutylhexano 18-crown-6. The resulting mixture was sonicated for 30

minutes. After sonication, the methanol was removed using rotary evaporation. The resulting oil was allowed to dry in the fume hood overnight. The surface immobilized particles were washed by sonication in methanol and magnetic decantation. The product was then dispersed in methanol to create a 60 mM suspension of the surface immobilized crown ether¹⁸.

2.2 Ion exchange SPE and selective elution

2.2.1 Phosphate efficiency

The phosphate efficiency was determined using 20 mL of preserved urine from a single donor spiked to 10 Bq of ⁹⁰Sr in equilibrium with ⁹⁰Y. The samples were adjusted to a pH of 9 and 0.1 mL of a 50 mg/mL suspension of magnetic nanoparticles coated with p-AMPS was added. The solution was placed onto a neodymium magnet for 30 minutes before the sample volume was reduced to 6 mL through removal of the supernatant using a 1-10 mL transfer pipet, ensuring the particles remained in the solution. It was noticed that the particles tended to remain in suspension in the last 5 mL of the urine sample. Once the supernatant was removed, an aliquot of 1.4 M ammonium dihydrogen phosphate in water was added in varying concentrations (between 75 mM and 200 mM). The particles settled at the bottom of the flask after a few minutes. An aliquot of 5 mL of the sample was taken from the flask and placed into a 20 mL LSC vial. The liquid scintillation cocktail (Optiphase Hisafe 3) was added to the vial to give a total volume of 20 mL. The scintillation vials were vortexed for 30 seconds

before being measured for 10 minutes in the Tri Carb LSC. The spectra were analyzed for the optimal analytical energy window and the highest ^{90}Sr recovery⁶.

2.2.2 Linear dynamic range (LDR)

The linear dynamic range was determined with similar experimental procedures as noted in Section 2.2.1. The differences between these extractions and those from Section 2.2.1 are that: the spike activities were varied between 12 Bq/L and 600 Bq/L and the phosphate concentration in urine during the elution step was kept at a constant 200 mM throughout all experiments herein. The operation window was kept within 3-125 keV⁶.

2.2.3 Accuracy, precision and minimal detectable amount (MDA)

The preserved urine samples were separated into 14 aliquots measuring 20 mL, consisting of 7 blank samples and 7 spiked to 190 Bq/L of ^{90}Sr in equilibrium of ^{90}Y . They were analyzed using the same methods noted above using 200mM phosphate for the elution and the operation window of 3-125 keV. The 7 blanks were measured to determine the MDA and the 7 spiked samples specified the accuracy and precision of the method⁶.

2.2.4 Robustness testing

Samples from 5 donors, taken in conjunction with Health Canada were taken and used directly for analysis to test the ability of this method to handle variations in urine samples from donor to donor. The particles of certain urine samples would not settle

low enough into the vial to siphon down to 6 mL. As much supernatant was removed as possible without disturbing the particles and the phosphate concentration was increased to 200 mM. After magnetic separation, the particles settled in the vial. A 5 mL aliquot of the urine was taken and measured for ^{90}Sr .

Two methods were utilized to decrease the interaction of the particles with the urine matrix. In the first method, a 1 mL aliquot of the non-ionic surfactant Triton X-114 was added to fresh urine samples which had been adjusted to pH 9. P-AMPS MNPs were added to the sample and magnetically separated. A non-ionic surfactant was chosen to reduce the interaction of the particles with the urine without interfering with the ion exchange³³. In the second method, fresh urine samples were acidified with 1% HCl and centrifuged to remove large biomolecules from the sample to emulate urine samples used in Sections 2.2.1-2.2.3, which had been stored for long periods of time. Afterwards, the acidified samples were adjusted to pH 9, 5 mg of particles were added, and the ^{90}Sr was extracted as in Section 2.2.3⁶.

2.3 Ion pairing SPE of ^{90}Sr using 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 and HNO_3

2.3.1 Crown ether loading

The loading on the particles was 33% (0.47g of crown ether per 1.5g of particles) for the experiments detailed in Section 2.3. In an attempt to reduce the amount of nanoparticles used in each experiment, Sections 2.3.3 and 2.3.4 had a 60% (0.9g on 1.5g) crown ether loading.

2.3.2 Optimal crown ether concentration

Aliquots measuring 3 mL were acidified with 1 mL of 16 M HNO_3 . Each sample was spiked with 16.4 ± 0.1 Bq of ^{90}Sr . Different amounts of the particles with the surface immobilized crown ether were added to each sample to determine the optimal concentration of crown ether in a urine sample for the extraction of ^{90}Sr . The mixture was sonicated for 15 minutes and placed on a neodymium magnet for 15 minutes. An aliquot measuring 5 mL of the supernatant of each sample was collected and transferred into a separate 20 mL LSC vial. The volume of solution was made up to 20 mL with Hi Safe 3 scintillation cocktail and the sample was counted for 10 minutes in the Tri Carb LSC. The supernatant was measured to count the amount of ^{90}Sr that was removed by comparing it to a sample extracted with 0 mM of crown ether.

2.3.3 Eluent

Two different eluents were tested during this project, deionized water and 10 mM Ethylenediaminetetraacetic acid (EDTA) in water. The EDTA solution was adjusted to pH 9 using solid NaOH. Urine samples measuring 3 mL were acidified to 4 M HNO_3 and spiked with ^{90}Sr . Crown ether immobilized onto the surface of polymer-coated magnetic nanoparticles was added to each sample for a concentration of 10 mM of crown ether. The particles were mixed into the solution and allowed to sit for 15 minutes and were then magnetically separated over 15 minutes. The supernatant was removed until only 1 mL remained, and a 5 mL aliquot of supernatant was measured for ^{90}Sr retention. The particles were mixed with 5 mL of either water or 10 mM EDTA

solution. After sonication for 5 minutes, the samples were magnetically separated over 10 minutes and the eluent was measured to determine how much ^{90}Sr was stripped from the particles.

2.3.4 MDA, precision and robustness

Five preserved urine samples, with volumes of 7.5 mL each, were treated as in Section 2.3.3, using the EDTA solution, with one slight change to the methodology: when using 7.5 mL of urine with 2.5 mL of HNO_3 , larger quantities of particles are required and a larger volume of the urine (~ 1.5 mL) remains after the supernatant is removed. When 5 mL of the 10 mM EDTA solution is added, the solution is too acidic and must be adjusted to pH 9 by adding sodium hydroxide. This required the addition of 1.5 or 1.6 mL of 30% NaOH solution, depending on the sample. This was accounted for by adding 0.1 mL of DI water to the samples that only required 1.5 mL of the NaOH solution.

Five fresh donor samples were collected and spiked to 190 Bq/L. All samples were analyzed using the same methods as the blank. This would determine the precision and robustness of the method.

2.4 Magnetic separation using robotic system

A Biosprint 15 was employed to test the concept of using a robotic arm to magnetically transfer ^{90}Sr bound to magnetic nanoparticles from one solution to another for measurements. The Biosprint 15 has 15 sections, each working in parallel.

Each section has a magnet on a robotic arm, a plastic sheath for the magnet and 5 wells for solutions which hold 1 mL. The Biosprint can either dip the magnet, while inside the plastic sheath, into one of the 5 wells to pick up the particles, dip the plastic coating into a well or shake the magnetic particles loose. However, samples cannot be transferred from one section to another and each section performs the same task. The Biosprint can be manually given a series of commands to fully automate the process. Figure 2.1 displays the layout of the wells and sections and Figure 2.2 illustrates the layout of the robotic arms and plastic sheaths for 3 sections.

Figure 2.1: Layout of 5 wells per section and 15 sections in Biosprint 15

Figure 2.2: Layout of magnetic arms and plastic sheaths for 3 sections. Going from left to right are magnetic bars descending into plastic sheaths to manipulate magnetic particles

First, the Biosprint 15 was tested for its transfer capabilities using polymer coated magnetic nanoparticles. Varying amounts of magnetic nanoparticles were dispersed into deionized water and placed into one of the wells. The Biosprint was instructed to move the particles from one well and depose them into a new well filled with deionized water. Both wells were collected and the amount of nanoparticles was measured using absorbance spectroscopy⁶.

The Biosprint was then employed for magnetic separations of ⁹⁰Sr from a urine matrix. This was done using both the p-AMPS particles as well as the neutral polymer particles.

For the ion exchange column, preserved urine was adjusted to pH 9 and mixed with enough p-AMPS magnetic particles to create a 0.25 mg/mL suspension. The first 4 wells of three different sections of a Biosprint 15 were filled with urine. The final well of these sections was filled with 1 mL of 200 mM phosphate. The Biosprint was instructed to take magnetic particles from each of the first four wells and transfer them to the fifth well. The particles were allowed to sit for a minute before being retransferred to their original well. Figure 2.3 illustrates the Biosprint 15 magnetic separation for ⁹⁰Sr extraction using p-AMPS.

Figure 2.3: Series of operations for magnetic separation using Biosprint 15 for extraction of ^{90}Sr in urine using p-AMPS

For the ion pairing particles, the first four wells were filled with urine acidified with 4 M HNO_3 . The fifth well was filled with a suspension containing 10 mM crown ether surface immobilized to magnetic nanoparticles. The particles were transferred from the fifth well to the first well and the machine was instructed to pause. The contents of the fifth well were removed and replaced with 10 mM EDTA adjusted to pH 9. The magnetic particles were then removed from the first well and deposited into the EDTA solution. The particles were then used to extract ^{90}Sr from wells 2, 3 and 4 with elutions in the 5th between each extraction. The contents of each well were measured using the Tri Carb to measure transfer of ^{90}Sr . Figure 2.4 illustrates the magnetic

separation with the Biosprint 15 for the extraction of ^{90}Sr using magnetic nanoparticles with surface immobilized crown ether.

Figure 2.4: Series of operations for magnetic separation using Biosprint 15 for extraction of ^{90}Sr in urine using surface immobilized crown ether on magnetic nanoparticles

3. Results and discussion

3.1 Ion exchange SPE and selective elution

3.1.1 Phosphate efficiency curve

Urine samples were analyzed using the method described in Section 2.2.1. The spectra for preserved urine samples showed that the ^{90}Sr peak ended at 125 keV. Therefore, analysis was performed using the 3-2000 keV and 3-125 keV operating windows. The spectra in Fig. 3.1 prove efficient washing of ^{90}Sr , and trapping of ^{90}Y onto the particles.

Figure 3.1: Spectra of beta counting for a) ^{90}Sr in equilibrium with ^{90}Y , b) phosphate eluent (200 mM) and c) magnetic cationic exchange particles after elution with phosphate (200 mM)

The recovery of ^{90}Sr at various phosphate concentrations are presented in Table

3.1 below.

Table 3.1: Percent of ^{90}Sr recovered after elution from magnetic cation exchange particles using various phosphate concentrations for analysis in the 3-2000 keV and 3-125 keV operating windows.

Concentration of phosphate (mM)	3-2000 keV operation window for ^{90}Sr and ^{90}Y		3-125 keV operating window for ^{90}Sr only	
	% recovery	%error	% recovery	%error
75	54%	1%	53%	1%
100	67%	3%	64%	3%
125	85%	5%	80%	4%
150	89%	3%	85%	3%
175	96%	5%	88%	4%
200	103%	5%	93%	5%

The gap in recoveries for ^{90}Sr between the two operating windows becomes larger as the concentration of phosphate increases. The cause of this difference is that the elution of ^{90}Y from the magnetic particles increases with an increase in phosphate concentration; ^{90}Y produces a signal in the 125-2000 keV range. By narrowing the operating window, the majority of the ^{90}Y signal can be excluded from the LSC measurements. The rise in the percentage recovery of ^{90}Sr or ^{90}Y from the magnetic cation exchange particles with an increase of phosphate concentration is demonstrated in Figures 3.2 and 3.3 respectively. With these results, 200 mM of phosphate was chosen for all further elutions of ^{90}Sr from magnetic nanoparticles and the 3-125 keV operating window was used for all further measurements⁶.

Figure 3.2: Percent recovery of ^{90}Sr from magnetic particles at varying phosphate concentrations based on counts from the 3-125 keV operating window⁶.

Figure 3.3: Percent recovery of ^{90}Y from the magnetic nanoparticles at varying phosphate concentrations based on counts from the 125-2000 keV operating window⁶.

3.1.2 Linear dynamic range

The linear dynamic range was determined between 12 Bq/L and 600 Bq/L using 7 data points. Each data point was measured in triplicate to ensure the precision of the measurements. The methodology was described in Section 2.2.2. The linear dynamic range reached below the desired detection limit (19 Bq/L) and rose by a factor of 50. The operating window was set to 3-125 keV. Figure 3.4 illustrates the linear dynamic range for this method⁶. The error bars are too small to be seen in the following figure.

Figure 3.4: Linear dynamic range for extraction of ⁹⁰Sr in 20 mL of urine sample using p-AMPS coated magnetic nanoparticles⁶.

The 21 samples used to determine the linear dynamic range (LDR) were also used to calculate the efficiency of the extraction procedure. The recovery of ⁹⁰Sr was

found to be $87 \pm 10\%$. The LDR demonstrates linearity below the desired MDA of 19 Bq/L⁹, and the relative error was found to be well within the 40% set for this method. These requirements are dictated by ANSI N13:30^{6,8}.

3.1.3 MDA, accuracy and precision

The MDA was calculated using 7 unspiked urine samples from a single donor. The blank samples were prepared and measured as described in Section 2.2.3. For comparison, both the 3-2000 keV and the 3-125 keV operating windows were used for calculation of the MDA. During the LSC measurement, only 5 out of the 6 mL of eluent were used. The MDA equation was modified by multiplying the volume (V) by 83% to correct for the volume not used for counting. This was termed the percent volume measured and denoted by %V in the equation for the MDA, shown here^{6,8}.

$$MDA = \frac{(4.65 \times S_b + 3)}{E \times t \times V \times \%V}$$

Where S_b is the standard deviation on the number of LSC counts, E is the percent efficiency, and t is the counting time of the LSC in seconds. Table 3.2 presents the calculated values of various terms in the MDA equation^{6,8}.

Table 3.2: Determination of MDA for magnetic SPE of ^{90}Sr from urine, followed by elution using 200 mM phosphate, using both the 3-2000 keV and 3-125 keV operating windows^{6,8}.

Blank number	Counts (3-2000 operating window)	Counts (3-125 operating window)
1	190	127
2	169	99
3	168	113
4	151	113
5	179	115
6	158	106
7	164	113
Average	168	112
S_b	13	9
t (s)	600	600
E (%)	94%	87%
V (L)	0.02	0.02
%V (%)	83%	83%
MDA (Bq/L)	6.7	4.9
Error (Bq/L)	0.7	0.5

The efficiency of the method is higher while using the 3-2000 keV operating window because it includes the signal produced by ^{90}Y in the 125-2000 keV operating window. The MDA for the 3-125 keV operating window is lower than that of the 3-2000 operating window because the noise from light with energy above 125 keV is excluded. This is a second advantage for narrowing the operating window so it counts only the energy levels produced by the target analyte. The MDA is 3.8 times lower than required by Li and Kramer⁹, this method could be used with smaller volumes of urine (down to almost 5 mL if necessary) and still meet the desired detection limit⁶.

Using the method described in Section 2.2.3, seven urine samples were spiked, processed and measured for ^{90}Sr . The accuracy and precision of the method were calculated using the equations presented in Section 1.3.4, as summarized in Table 3.3,

where the B_{ri} is the relative bias of a sample, B_r is the average relative bias over all the samples and the $\%S_b$ is the relative standard deviation of the samples. The operating window for all subsequent measurements was set at 3-125 keV to avoid counting ^{90}Y decays⁶.

Table 3.3: Accuracy and precision of magnetic SPE of ^{90}Sr using preserved urine from a single donor, followed by elution⁶.

Sample #	^{90}Sr spiked in sample (Bq/L)	^{90}Sr measured (Bq/L)	B_{ri} (%)	B_r (%)
1	190	219	15%	12%
2	190	212	12%	$\%S_b$
3	190	218	15%	3%
4	190	206	8.5%	
5	190	206	8.3%	
6	190	205	7.8%	
7	190	218	15%	

The measured results were calculated using the equation of the line from Figure 3.4 in the Section 3.1.2. The positive relative bias is well within acceptable parameters, up to +50% set by ANSI N13:30⁸. The relative error is lower than the acceptable value of 40% by a factor of 10. This demonstrates a high degree of precision for this method when measuring multiple aliquots of the same sample. All experimental data demonstrates that the method has been developed to fall well within acceptable parameters. Its current setup can measure the first 9 samples in 180 min and a new sample every 15 min thereafter. Two limiting factors are the magnet size and the counting time required by the Tri-Carb LSC. An important advantage of this method is the low cost (\$0.01 per sample) of preparing the particles. Sample handling involves simple laboratory techniques including the use of a micropipette and a volumetric bottle top dispenser⁶.

3.1.4 Robustness

The robustness of this method was tested using urine samples from five donors, taken in conjunction with Health Canada. Each urine sample was collected and measured the same day. The processing and measurements were performed as described by Section 2.2.4. During magnetic separation, the particles would not settle to the bottom of the vial for all five samples. This is likely caused by the interaction between the particles and the urine matrix. For this reason, the volume of supernatant that could be drawn off from the sample (without disturbing the particles) varied between samples. The volume decanted was as low as 0 mL in some samples and as high as 14 mL in others. ^{90}Sr was eluted using 200 mM phosphate despite variations in the sample volume. During the elution step, the particles did not float. Therefore, the interaction between the matrix and the particles decreases in the presence of the phosphate salt. Therefore, taking a 5 mL aliquot from each vial, without disturbing the particles, for LSC measurement, was possible in all cases. The 3-125 keV operating window was used to exclude any signal from ^{90}Y eluted along with ^{90}Sr . The accuracy, precision, recovery and MDA as calculated by the equations in Section 1.3.4, are presented in Table 3.4 below.

Table 3.4: Accuracy, precision, recovery and MDA of magnetic SPE of ^{90}Sr in urine from five donors, followed by elution.

Sample #	Volume decanted (mL)	^{90}Sr spiked in sample (Bq/L)	^{90}Sr measured (Bq/L)	B_{ri} (%)	Recovery (%)	MDA (Bq/L)	B_r (%)
1	14	238	218	-19%	59%	8	6%
2	14	238	243	-8.1%	67%	7	%S _b
3	0	238	353	49%	108%	16	34%
4	14	238	187	-31%	50%	9	
5	11.4	238	368	40%	101%	6	

The “ ^{90}Sr measured” column in Table 3.4 was calculated using the linear regression equation in Figure 3.4. The relative error (%S_b) falls within the 40% as set by ANSI N13:30. The B_{ri} of all samples except for number 4 fall within an acceptable range between -25% and +50%⁸. The large variations come from the difference in urine matrix from donor to donor. Previous results in Section 3.1.3 used one donation of urine. The donation was spiked and separated into multiple sub samples, creating a low variation in results from sample to sample. In that case a slightly higher recovery than average was found over all samples, and hence the positive relative bias throughout all the sub samples. When analyzing urine from different donors, large differences in the urine matrix were noticed, resulting in a large variability in the relative biases. Using the calculated recoveries and the formula for MDA, samples containing ^{90}Sr at 19 Bq/L⁹ would show a detectable signal. The relative bias could be improved with the use of a spiked pair.

A spiked pair involves the preparation of a second aliquot of the sample with the addition of a known quantity of standard. If a spiked pair was used to calculate the

recovery of each sample individually, a more accurate determination of ^{90}Sr could be obtained. The relative bias would be closer to zero for each individual sample³⁴. The disadvantage is that measuring a second aliquot of each sample would double the volume of sample required and cut the sample throughput in half. The sample throughput would be 40 min per donor for the first set of 8 donors and 30 min per donor thereafter. This is still more rapid than most other methods available for ^{90}Sr determination, such as those cited in Section 1.2. Whether a faster measurement or a more accurate measurement of urine samples, obtained with the use of a spiked pair, is more desirable will depend on the specific situations of the laboratory.

In an effort to reduce the matrix effect on the particles floating in the sample, two approaches were taken to pretreat the sample: a non-ionic surfactant (Triton X-114) and the use of HCl to prepare the urine samples in the same manner as the urine sample used for the previous sections in Chapter 3.1. The methodologies are described in Section 2.2.4. Neither of these methods was found to decrease the amount of particle floating.

3.1.5 Comparison to previously used analytical techniques

The method for separating and measuring ^{90}Sr from ^{90}Y in urine using magnetic separation, selective elution and LSC counting is described in Section 2.2 and in Figure 3.5. The advantages of using ion exchange SPE for the determination of ^{90}Sr compared to methods discussed in Section 1.2 include: meeting MDA requirements, rapid sample

turn around and ease of use. The disadvantage of this method is a lack of robustness during the magnetic separation stage.

Figure 3.5: Method for separating and measuring ⁹⁰Sr from ⁹⁰Y in urine using magnetic separation, selective elution and LSC counting

Ease and speed of operation is an advantage of the method based on ion exchange magnetic nanoparticles. Compared to analyses by Alvarez et al. and Antonio et al., the advantage of this method is that of same day analysis^{13, 14}. In comparison to other methods using traditional SPE resins, this method negates the need for preconditioning of the resin and constant monitoring. When using SPE columns, it is important to monitor them to ensure they do not become dry³⁵. A small improvement in rapidity over the work by Hrdina et al was attained. Their co-precipitation and centrifugation steps were replaced with an elution step, thus decreasing extraction time¹⁸. Faster methods developed by Li et al. and Sadi et al. using the traditional SPE resins require approximately the same amount of time for an analysis^{15,16}. The factor

limiting the speed in which samples can be placed in the Tri-Carb counter for automated counting is simply the number of samples that can fit on the magnet (10 cm x 10 cm).

The detection limit of this method can be as low as 5 Bq/L when 14 mL of the urine can be decanted and is below the required 19 Bq/L derived by Li and Kramer⁹ under all analytical conditions tested. All other methods with comparable analysis times have higher detection limits than the required 19 Bq/L^{13, 14, 15, 16}. The detection limit of 47 decays per minute boasted by the high performance extraction chromatography mentioned in Section 1.2.2 translates to 391 Bq/L, which is also too high for these applications¹⁷.

The developed method, however, varies from donor to donor, showing a lack of robustness. This disadvantage never raises the MDA above 19 Bq/L⁸; however it is of some concern. Other radiobioassay methods do not seem to have this issue^{13, 14, 15, 16}. It would be ideal if the robustness could be improved even though it is not a requirement for emergency radiobioassay. For ⁹⁰Sr separation, the use of magnetic particles for ion exchange SPE has at least one of the following advantages over any other method, namely speed, simplicity and MDA compatibility. With these advantages, this method could soon become the preferred emergency radiobioassay.

3.2 Ion pairing SPE of ⁹⁰Sr using 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 and HNO₃

3.2.1 Confirmation of polymer coating

The indicators of a successful polymerization are the color change of the magnetic particles and the FTIR analysis. The color of the uncoated magnetic nanoparticles was black while the magnetic particles collected after polymerization and washing were light brown. The following FTIR spectra (Figures 3.6 and 3.7) also demonstrate the coating of the polymer onto the particles.

Figure 3.6: FTIR spectrum for bare magnetic nanoparticles

Figure 3.7: FTIR: of magnetic particles coated by p-acrylamide cross-linked with DVB

The peaks of interest are noted in the following table

Table 3.5: Peak correlations for FTIR of DVB cross-linked p-acrylamide coated magnetic nanoparticles³⁶

Peak wavenumber (cm ⁻¹)	Peak correlations
3018	Aromatic C=C-H
2923	Methylene (H-C)
1446 1509 1602	Aromatic C=C
1629	Carboxylic acid derivative (amide)
3448	Amine

The bare magnetic nanoparticles show only a peak 3434 cm⁻¹. The extra peaks on the FTIR for the polymer coated magnetic particles show that some other components are present in the dry particles. These peaks indicate the presence of the polymerized species is present in the dry particles. After multiple washes with methanol and magnetic separation to collect the magnetic particles, all of the monomer and any polymer not attached to the magnetic particles would have been removed from the particles.

3.2.2 Optimizing crown ether concentration for ⁹⁰Sr uptake

The optimal concentration of crown ether in urine for the trapping of ⁹⁰Sr was tested by measuring the amount of ⁹⁰Sr removed by the magnetic particles from the supernatant in a 3 mL urine sample acidified with 1 mL of 16 M HNO₃. The method is described in Section 2.3.2. A linear trend was found between the amount of ⁹⁰Sr trapped by the crown ether and the amount of crown ether-immobilized magnetic particles used during the extraction, as demonstrated in Figure 3.8.

Figure 3.8: Percent of ⁹⁰Sr trapped on ion pairing SPE particles with respect to the concentration of crown ether present in the urine samples

The selective removal of ⁹⁰Sr is confirmed by the spectra collected by Tri Carb LSC measurements. The following spectra (shown in Figure 3.9) demonstrate the decrease of analytical signal in the ⁹⁰Sr region, without a decrease in the ⁹⁰Y region.

Figure 3.9: LSC spectra of supernatant after extraction of ^{90}Sr from urine using various crown ether concentrations: A) 0.0 mM, B) 1.6 mM, C) 3.1 mM, D) 4.5 mM, E) 7.2 mM and F) 11 mM.

3.2.2 60% loading

When a new batch of magnetic particles with surface-immobilized 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 was made with a 60% by weight loading of crown ether, the maximum percentage of ^{90}Sr (7.5 mL sample of urine spiked to 190 Bq/L) which could be removed from a urine sample was $69\% \pm 3\%$. This was down from $101\% \pm 5\%$

at a loading of 33% by weight under similar analytical conditions. Attempts to wash the magnetic particles of free crown ether using magnetic separation and 3 separate solvents did not improve the percent removal of ^{90}Sr during extraction. The solvents were methanol, 1:1 water/methanol and urine acidified with 4 M HNO_3 . The following table details the results of the washes.

Table 3.6: Percent of ^{90}Sr removed from urine matrix using ion pairing magnetic SPE particles after being washed with 3 different solutions

Washing solvent	% removal
None	69%±3%
Methanol	71%±8%
Water	70%±3%
Acidified urine	68%±3%

This signifies that the crown ether is bound to the particles and that the decreased effect from the overload of particles cannot be fixed with a washing solvent. The particles were used despite overloading to test two eluents and the robustness of the crown ether for trapping ^{90}Sr .

3.2.3 Eluent

Two different solutions were used to find an efficient method for eluting ^{90}Sr from the magnetic nanoparticles: deionized water and 10 mM EDTA in deionized water. The 10 mM solution of EDTA was adjusted to pH 9. The supernatant was measured for ^{90}Sr removal as described in Section 3.1. The recovery for the eluent was calculated using the amount of ^{90}Sr trapped on the particles by measuring the ^{90}Sr activity in the supernatant. The results showed that water eluted 40%±2% of the bound ^{90}Sr whereas 10mM EDTA was able to elute 86%±3% of the bound ^{90}Sr . For all further analyses, 10

mM EDTA was used as the eluent. The selectivity of this method is confirmed by Figure 3.9 through the observation of a single large peak in the ^{90}Sr region as seen in the second spectrum, compared with the 2 peaks in the first. The shift in the first spectrum was caused by color quenching in the urine matrix. With these results, the operating window of 3-165 keV was used to decrease background noise.

Figure 3.10: LSC spectra showing A) ^{90}Sr and ^{90}Y in equilibrium and B) EDTA eluted ^{90}Sr from surface immobilized crown ether magnetic particles

3.2.4 MDA, precision and robustness

The MDA for this experiment was calculated using the results from 5 preserved urine samples and the method of which is described in Section 2.3.4. The calculated values are presented in the Table 3.7.

Table 3.7 Determination of MDA of ion pairing magnetic SPE particles and elution of ^{90}Sr from urine.

	counts
Blank	90
	66
	59
	46
	35
Standard deviation	21
Counting Time (s)	600
Efficiency (%)	60
Volume (L)	0.0075
%V (%)	83
MDA (Bq/L)	45
Error (%)	3

The MDA is higher than the requirements for emergency radiobioassay, set at 19 Bq/L, and would need to be improved before these particles could be used for that purpose. The MDA is set at 19 Bq/L because it represents 10% of the level that, if found in urine excreted 3 days following inhalation, would require immediate intervention⁹. Optimization parameters discussed in Section 3.2.5 could help to improve the MDA of this method. These include lowering the dilution factor, and improving the efficiency of the method.

Urine samples from 5 different donors were collected and analyzed same day for ^{90}Sr using the method described in Section 2.3.4. The urine samples were spiked to 210 Bq/L. The following table presents the precision and robustness of the analysis and it

demonstrates that the ion pairing of Sr^{2+} and NO_3^- is unaffected by the variations in the matrix.

Table 3.8: Accuracy and precision of ion pairing magnetic SPE and elution of ^{90}Sr using fresh urine from five donors

Sample #	LSC Counts	Activity (Bq)	Expected activity (Bq)	Recovery ($\pm 5\%$)
1	475	1.1	1.58	64%
2	422	0.91	1.58	55%
3	476	1.0	1.58	64%
4	404	0.87	1.58	53%
5	455	1.0	1.58	61%
Average				60%
Relative Error				8%

The relative error of 8% is well within the acceptable experimental error set by ANSI N13:30⁸. The only variation in this method from sample to sample was the amount of hydroxide required for Samples 4 and 5. An extra 0.1 mL of NaOH solution was required to reach a basic pH of 9.

3.2.5 Future method optimization

This data proves the concept that surface immobilized 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 on magnetic nanoparticles can be used to selectively bind ^{90}Sr and that EDTA will elute the bound analyte into a solution for measurement using a Tri-Carb LSC counter. The method has demonstrated robustness by the measurement of different urine samples. This method now requires optimization and validation before it can be used in emergency radiobioassay situations. Parameters to be optimized include: surface immobilization of crown ether, ion pairing, elution conditions, sample volume, and production method.

As mentioned in Section 4.2.2, a crown ether loading of 60% by weight is too high and would need to be scaled back. It is known from previous results that a 33% by weight loading of crown ether achieves $101\% \pm 5\%$ recovery. From the results obtained, in Section 4.2.2, from extraction using the particles with a 60% by weight loading (0.9 g), it can be deduced that there is a 31% loss of ^{90}Sr during removal of the supernatant. A loading of 44% by weight (0.64 g) would approach the optimized amount, since this corresponds to a 31% decrease in crown ether. Since washing does not remove the extra crown ether from particles once it is surface immobilized, the crown ether cannot be loaded in excess without decreasing the efficiency of the method. Finding the highest weight percentage of crown ether which can be loaded without suffering a decrease in extraction efficiency would allow for a minimum amount of particles to be used. This would offer the advantage of lower extractant volumes and decrease the volume of sample to be collected after magnetic separation. It has been noted that a higher number of particles decreases the amount of supernatant which can be removed during magnetic separation, observed in Section 2.3.4.

The ion pair used would also need to be optimized for two reasons: first, nitrate is a known chemical quencher, which decreases the analytical signal, and second using nitric acid dilutes the urine samples. Currently, the method can handle 7.5 mL of urine diluted to 10 mL to add the required nitrate ions. Bahraini et al. found that sodium nitrate salt quantitatively paired and bound ^{90}Sr to 4'4'5 dtertbutylhexano 18-crown-6 on p-acrylamide⁷. Without the dilution, either lower quantities of crown ether could be used to have a 10 mM solution or the volume of urine can be increased to 10 mL. Other

ion pairs which are not quenchers should be explored to reduce the quenching. Any new methods should also explore using a solid salt as opposed to a solution. This would reduce the dilution factor, which would allow for a cheaper analysis or the sample size could be increased from 7.5 mL to 10 mL, thus increasing the analytical signal, by 25%.

The elution of ^{90}Sr from the crown ether immobilized particles with 10 mM EDTA has an 86% recovery. This discrepancy could be associated with quenching from the nitric acid, or a suboptimal concentration of EDTA. The EDTA concentration could be changed to increase the recovery of the method. Another area for optimization for the elution step was the need to add sodium hydroxide to the eluent. The amount of hydroxide required fluctuated slightly from sample to sample. If a buffer solution could be used, this would help create better control of the elution conditions, such as volume of eluent added and consistency of the pH of the resulting solution. These factors should be considered during method development for practical applications.

The current production method for the coated magnetic particles does not yield a large enough batch for an effective production of the extracting compound. While using a 33% by weight loading of the crown ether onto the particles; only ~85 mL can be analyzed by one batch of the surface immobilized crown ether particles. A much larger scale manufacturing procedure would need to be designed before this method would be feasible for analytical use on a large scale.

3.2.6 Comparing this method to literature methods

The method for using ion pairing SPE on magnetic particles would have the following general procedure (Figure 3.10).

Figure 3.11: General procedure for ^{90}Sr analysis using ion pairing SPE with magnetic separation

This method, while not completely validated, does show promise as being a potential replacement for traditional SPE resin methods discussed in Section 1.2. The speed of analysis and ease of using the crown ether immobilized particles far exceeds that of the ion pairing extraction columns and cartridge methods. The traditional methods discussed in Section 1.3.2 require overnight sample preparation, and the present use of crown ether magnetic nanoparticles requires only 45 minutes.^{13,14} All preconcentration methods used for the traditional resins can also be used with these magnetic particles. The important decision of choosing which method to use depends on the time available for analysis and sample volume. For larger sample volumes, the particles may become less practical as the mass of particles and crown ether required for the analysis increase linearly, as seen in Figure 3.7. This will increase the cost linearly

as well as increase the volume of magnetic particles after magnetic separation. In large volume cases, it may be preferable to use a traditional SPE resin. However, at low volumes, a method developed using the magnetic separation technique could prove to be faster and more cost effective than a column based SPE counterpart.

When compared to the non-traditional SPE resin methods discussed in Section 1.2.3, these particles show the promise of a faster method. This is because the particles do not require a centrifugation step to remove ^{90}Y . The crown ether particles themselves are able to selectively extract the analyte, diminishing the number of steps required¹⁸.

3.3 Magnetic separation using robotic system

3.3.1 Magnetic particle transfer

Magnetic transfer of particles was observed visually as the transfer occurred. Some particles were left in the original well, some were stuck to the plastic coating of the Biosprint 15's mechanical arms, but most were transferred into the destination well. This was quantified by measuring absorbance of the deionized water from the first well at a wavelength of 400 nm. The response of absorbance compared to concentration of particles is seen in the Figure 3.11⁶. The linear regression was used to calculate the mass of particles transferred in Table 3.9.

Figure 3.12: Absorbance at 400 nm vs concentration of magnetic particles (mg/mL)⁶

Table 3.9: details the transfer of magnetic particles by the Biosprint 15.

Table 3.9: Using absorbance at 400 nm to determine the percentage of magnetic nanoparticles transferred using Biosprint 15⁶

Original amount of magnetic particles (mg)	Absorbance at 400 nm after transfer	Amount of magnetic particles left after transfer (mg)	Percent transferred (%)
0.141	0.137	0.015	89
0.141	0.137	0.015	89
0.351	0.227	0.025	93
0.703	0.401	0.043	94
1.054	0.487	0.053	95
1.265	0.569	0.062	95
1.405	0.745	0.081	94

The average percentage of particles transferred was $93\% \pm 3\%$. This would decrease the efficiency of the methods from 87% to 81% and increase the experimental error. However, it would eliminate the V% term from the MDA calculation resulting in an overall improvement of the MDA of the two magnetic extractions designed above.

3.3.2 Magnetic separation of p-AMPS

The magnetic separation was performed as before; however, only 1 mL of sample was used per vial. When the Biosprint magnetic arm was placed in a vial containing urine at pH 9 combined with 2.5 mg of magnetic particles, the magnet would attract all the particles in solution. When attempting to remove the particles from the basic urine solution to another well, the particles would fall off of the magnet and back into the solution. No number of attempts to remove the particles would allow the magnet to transfer the magnetic particles from the urine solution. It is surmised that the interactions within the aqueous phase are stronger than the magnetic forces being used to extract the particles. This is possibly due to the distance between the magnet and the particles. Using a robotic arm with these particles under these conditions is thus not a viable option for creating a faster, more automated method for removing magnetic particles from the matrix.

3.3.3 Magnetic separation of ion pairing SPE magnetic particles

When the magnetic separation was performed using the Biosprint 15 under the same analytical conditions as described in Section 2.3, the magnetic transfer was observed visually. The supernatant from the urine samples and from the elution sample were both measured for ^{90}Sr . The following spectra (Figure 3.12) show the results of the LSC measurement of both the eluent and the supernatant.

Figure 3.13: Spectra of A) EDTA eluent in well # 5 and B) supernatant from well # 2.

As can be observed, very little, if any, ^{90}Sr was transferred from the urine wells and into the eluting well. As can be seen in the spectra, any transfer is more likely due to the particles being wetted with urine spiked with $^{90}\text{Sr}/^{90}\text{Y}$. This is shown by the signal having a higher energy than a simple ^{90}Sr signal. Removing the magnetic particles from the aqueous phase seems to rob the particles the ability to trap the ^{90}Sr . This is not a viable option for magnetic separation of ^{90}Sr bound to MNPs in urine.

3.3.4 Future considerations for robotic magnetic separation

Attempts of particle transfer using a robotic arm have highlighted the two main issues governing the use of a robotic arm to move analytes bound to magnetic particles

from one vial to another. Firstly, the strong interaction of the particles and the aqueous phase in comparison to the magnetic interaction with the particles presents a challenge in particle transfer. Secondly, the interaction of the analyte and the aqueous phase may be stronger than that of the analyte and the magnetic particles.

The first obstacle could be overcome through the use of a less hydrophilic polymer while the second may require the formation of a chemical bond between the analyte and the particle. A bond of that nature would be stronger than the interactions between the analyte and the liquid phase. The analyte would need to be either bound selectively to the magnetic particles or any interferents would need to be removed prior to binding. Removing ^{90}Y from solution independently from ^{90}Sr in urine was previously demonstrated by Hrdina et al, as discussed in Section 1.2.3¹⁸.

3.4 Comparing ion pairing SPE and ion exchange SPE using magnetic particles

Comparing these methods to literature, it was found that both SPE methods could be useful for emergency radiobioassay. However, it must also be determined which one would be superior analysis technique for this application. Figure 3.14 shows the steps for both SPE methods which offer similar speed, ease and potential for recovery. They both have the potential for an 87% recovery. The p-AMPS resin particles are cheaper, easier to produce and have the potential to work with lower volumes, down to 5 mL. Once optimized, the surface immobilized 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 resin offers a more robust method with better control over selectivity.

Figure 3.14: Step by step guide for p-AMPS and crown ether methods for extracting ^{90}Sr from urine. For split cells, the left describes conditions unique to p-AMPS method and the right describes conditions unique to crown ether method.

The advantages of both methods over other techniques include the speed and simplicity of analysis. Both methods offer faster sample preparation than the traditional SPE resin preparation techniques. They also require simple laboratory techniques and only seven steps. These advantages allow for multiple samples to be prepared quickly and efficiently. Sample preparation can also be done in the field for immediate analysis, as only micropipettes, a magnet and disposable glassware are required, and all solutions can be prepared prior to an emergency. The other similarity is the potential to achieve a recovery of ^{90}Sr of 87%. The p-AMPS method had a recovery of $87\% \pm 10\%$ over 21 samples. The surface immobilized crown ether showed a 60% recovery during the analysis of five urine samples, but this was with a crown ether loading that was too high.

It has been demonstrated that with a 33% by weight loading of crown ether onto the particles that 100% binding of ^{90}Sr can be achieved and the EDTA eluent demonstrated a recovery of 87%. With these similarities in mind, the chemist's choice of method will be determined by cost per analysis, volume of urine and method robustness.

The advantages of the p-AMPS over the surface immobilized 4'4'5di-tert-butyl-cyclohexano-18-crown-6 are cost and ability to work with very low volumes. The cost of preparing one batch of the p-AMPS particles is 0.03\$ more than the particles coated with p-acrylamide. However, this does not take into account the need for crown ether or the large amounts of particles required for analysis. The p-AMPS particles can also be synthesized 1-2 days sooner as they do not require the addition of the crown ether. Following the current experimental procedure, 200 analyses of 20 mL urine samples can be performed from every batch of particles. This is a huge improvement over the quantity of samples that can be processed with the current production of p-acrylamide particles with the crown ether. Due to the low standard deviation in the blanks, the MDA for the p-AMPS is much lower than that of the crown ether coated polymers. This allows for lower sample volumes to increase the MDA above 19 Bq/L, down to 5 mL. These advantages would be highly sought after by a laboratory attempting to analyze a large quantity of samples with low volumes. If the detection limit is not found to be low enough for their purposes, the counting time can be increased to accommodate it.

The advantages of using the p-acrylamide coated particles with 4'4'5di-tert-butyl-cyclohexano-18-crown-6 immobilized to the surface are a more robust method, potential for higher volumes and better control over selectivity. It was demonstrated in

Section 3.2.4 that the effect of the matrix is minimal and is well within the acceptable range, with a relative error of 40%. This is unlike the p-AMPS particles, where the differences in the matrix create large variations in the volume of sample capable of being analyzed. This robustness leads to the ability of a laboratory to analyze much larger volumes of urine. This will lead to a potentially lower and more reliable MDA for the crown ether captured onto the magnetic particles⁸. Also, work performed by Horwitz et al. has shown selectivity of the binding of the Sr spec resin, similar to the particles created for this paper. Only potassium, gold, technetium, molybdenum and mercury show any affinity for the crown ether. The supernatant carries with it other potential analytes which can be extracted by other selective means. As discussed in Section 1.2.1, Sr spec resin can also be used to capture lead and selectively elute it from any daughter ions present. This allows for good control over the selectivity of the measurements¹⁰. This would be an advantage for laboratories looking to analyze large volume samples or wishing to have a specific method during an emergency radiobioassay. However, this requires a full method development of the 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 magnetic particle method.

4. Conclusion

The development of a novel method and proof of concept of another novel method for using SPE attached to magnetic nanoparticles have been achieved. Ion exchange SPE using p-AMPS was shown to be capable of handling some 20 mL samples with 87% recovery at an MDA of 4.6 Bq/L. However, the lack of robustness is an issue that was not overcome. A proof of concept that surface immobilized 4'4'5 di-tert-butyl-cyclohexano-18-crown-6 on magnetic particles can be used for rapid radiobioassay analysis of ^{90}Sr in urine was demonstrated. However, this method requires many parameters to be optimized before it can see use for emergency radiobioassay. The use of magnetic particles increases the speed and ease of using SPE compared to traditional SPE resins.

Future work for the p-AMPS particles would include finding a solution for the issue of the urine matrix causing the p-AMPS particles to float during extraction. One possible solution would be to use centrifugation, instead of magnetic separation, for the extraction step. This could cause an increase in analysis time, but should still be quicker and more sensitive than other methods previously developed. Future work for the particles with the surface immobilized crown ether include a finding the optimal crown ether loading onto the particles and choosing and optimizing the concentration of the most advantageous ion pair and some others, as discussed in Section 3.2.5.

The automation of the magnetic separation was not achieved using a Biosprint 15 mechanical arm and the SPE magnetic nanoparticles explored in this work. Particles

with a lower affinity for the aqueous phase and a stronger binding of the analyte would be required to achieve automation using this route.

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