Crown Ether Modified Magnetic Nanoparticles for the Selective Determination of ^{226}Ra from Aqueous Samples

by

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Abstract

Bare (unmodified) and crown ether modified Fe_3O_4magnetic nanoparticles (MNPs) were investigated for the rapid extraction of ^{226}Ra from water samples. Experimental parameters such as counter-ion choice, concentration and pH were optimized to achieve maximum extraction of ^{226}Ra on to the MNPs. ^{226}Ra content was determined using a Hidex 300SL liquid scintillation counter (LSC) with α/β separation capability, or a gamma spectrometric detection system. The bare MNPs showed an extraction of 95±1% and 100±1% at pH 9 in 0.1M NaCl and 0.1M $NaClO_4$ respectively, whereas an extraction of ~12-24% was obtained, over the range of 2-4.The modified MNPs yielded 94±1% extraction efficiency in the presence of 0.01Mpicric acid at pH4. This study demonstrates that while bare MNPs can be used for removal of undesirable radionuclides from water, surface functionalization of Fe_3O_4 MNPs with suitable ligand modification can offer a selective mode of extraction for a target radionuclide.

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1. Toxicology of Radium

On April 26, 1986, devastation swept the land when a reactor exploded at the Chernobyl Nuclear Power Station, sending large amounts of radioactive material into the atmosphere and surrounding regions. As a result, 400,000 people lost their homes, 90,000 died from cancer, and hundreds of billions of dollars in economic losses were incurred over the next 30 years. This event is considered the worst nuclear accident in history, with a level 7 rating on the International Nuclear and Radiological Event Scale (INES) [1] **[1,2]**. Within days, almost all of Europe had been affected by the nuclear fallout. Over 20 radionuclides were detected, contaminating a majority of the northern hemisphere. During the month of May alone, thousands were hospitalized daily and diagnosed with radiation disease of rapidly progressing severity. This event, along with previous incidents such as the bombings of Hiroshima and Nagasaki (Japan, 1945; estimated total casualties: 135,000) and more recent crises such as the Fukushima nuclear disaster in March 2011, estimated to have released 10% the amount of radiation observed in the Chernobyl accident as a result of damage from an earthquake-induced tsunami, are raising concern for the potential danger of radionuclear materials around the world **[1,3]**.

Radiological materials have been used in a variety of applications; Radium, for example, has been used in medicine for diagnostics and radiation therapy and in industry as a luminescent paint for clock dials. Radiological materials are also of great concern when it comes to terrorism and nuclear warfare. Radiation dispersion devices (RDDs) can spread radionuclides over a large area using explosives. Nuclear warheads, meanwhile use radioactive materials such as ^{235}U and ^{239}Pu to initiate nuclear fission or fusion reactions, resulting in devastating explosions accompanied by radio-nuclear fallout **[4,5,6]**.

^{226}Ra is a long-lived alpha emitting radioactive isotope in group II of the periodic table. As an alkaline earth metal, it has very similar chemistry to calcium and barium forming 2+ ions in solution. It forms soluble complexes with hydroxide, chloride, bromide and nitrate in ground water. The dangers of ^{226}Ra arise from its unique physical

[1] A level 7 INES rating is described as "a major release of radioactive material with widespread health and environmental effects requiring implementation of planned and extended countermeasures" **[47]**.

and chemical properties, long half-life, and short-lived daughter isotopes. For the same reason, radium is very dangerous if absorbed into the body as it can displace calcium in bones [**7**,**8**].

Based on historical disasters such as those mentioned above, the demand for novel analytical methods for the monitoring of radioisotopes has become very high among scientists [**1**,**2**,**3**]. The growing menace of both nuclear terrorism and industrial pollution, and the national security and environmental concerns that result from these issues are threatening Canada each day.

Although there is no data available regarding the effects of external exposure of ^{226}Ra by absorption through the skin, exposure is known to occur internally through inhalation and ingestion. When a cell is exposed to radiation it can emit a particle disrupting chemical bonds which may cause mutations. Depending on the dose and frequency, exposure can lead to marrow suppression, brain abscesses, bronchopneumonia, sarcoma, anemia, necrosis of the jaw and ultimately death. These ailments occur due to a decrease in immune cells or infection resulting from cell disruption caused by radiation [**9**]. ^{226}Ra specifically, is known to induce bone sarcomas and carcinomas in bones due to the accumulation of its gaseous daughter isotope (^{222}Rn). In 1986, following in-depth research on the employees who worked in the watch dial painting industry, it was estimated that the lowest dose of radium necessary to induce bone sarcoma is 38 kBq/kg in humans [**10**].

Once ingested or inhaled, radium cannot be metabolized in the body and instead decays over time. Elimination of radium occurs through urination (2-4%), fecal excretion (main process) and, in the case of inhalation exposure, release through the lungs. The excretion of radium occurs in 2 stages; the first is a rapid excretion of ~85% of the intake amount through the feces and urine (10:1 ratio of elimination) and the remaining 15% will be excreted very slowly due to retention in bones. It is estimated that some radium (<1%) will still be retained even 30 years after exposure [**10**].

1.1. Concerns

Radionuclear decay involves the emission of often harmful and persistent isotopes which can be absorbed into the body and the environment. Certain radionuclides are more dangerous than others and can cause serious health effects due to their unique chemical

structures and physical properties. Gamma (γ), beta (β) and alpha (α) radiation each pose their own threats to humans, depending on their dose and the pathway of exposure [**4**].

Gamma rays are high-frequency, high-energy particles which can penetrate deep into tissues causing external damage such as burns, blisters or redness. This type of exposure is halted once the subject moves away from the radiation source. Gamma decay involves the release of electromagnetic radiation from a nucleus in the excited state (E^*) as it relaxes back down to the ground state (E). This type of decay commonly occurs after α- or β-decay as they produce excited state daughter isotopes. Generally, γ-decay can be expressed by the following equation:

$$^{A}_{Z}E^* \rightarrow {}^{A}_{Z}E + \gamma$$

where γ is a gamma ray, A is the mass number and Z is the atomic number [**11**].

β particles are not as immediately harmful as γ rays due to lower energies and lesser penetration; however, if ingested or inhaled they can release radiation internally causing often irreversible damage to surrounding tissue [**4**]. In the case of β-decay, a negative or positive electron (referred to as a negatron (β^-)and a positron (β^+) respectively) is emitted from the nucleus of the parent radionuclide (E), resulting in a daughter (F) with a higher atomic number (Z) by 1 mass unit but no change in mass number (A). In this process, and antineutrino (υ*) or a neutrino (υ) will be produced in the case of negatron and positron decay respectively to balance the number of leptons on both sides of the equation. A gamma ray may also be released to lower the energy of the resulting daughter nuclide. The general reaction scheme for β-decay is shown below [**11**].

$$^{A}_{Z}E \rightarrow {}_{z+1}{}^{A}F + \beta^{-/+} + \upsilon^{*}(or\ \upsilon) + (\gamma_{rays})$$

Despite their low energies, α emitting radionuclides such as ^{226}Ra, taken into the body through inhalation, ingestion, injection or absorption, can be much more dangerous than their γ and β counterparts, and have been known to cause cancers over prolonged periods given sufficient dose and frequency of exposure [**4**]. In alpha decay, a particle containing 2 protons and 2 neutrons is emitted by a nucleus due to the coulomb repulsion between the nucleus and the α-particles ($^{4}_{2}He$). This results in a daughter isotope (F) with a mass number (A) which is 4 mass units lower than that of the parent isotope (E) and an atomic number (Z) which is 2 mass units lower. Similarly to beta decay, alpha decay may also be

accompanied by the emission of a γ-ray. This type of decay can be described by the equation below [**11**].

$$^{A}_{Z}E \rightarrow ^{A-4}_{Z-2}F + ^{4}_{2}He + (\gamma_{rays})$$

If non-penetrating sources such as α and β radiation enter the body, they can infiltrate into organs and cause disruption on the cellular level through energy transfer during decay. Unlike external exposure, internal exposure continues until the material is excreted from the body through metabolism or decay processes [**4**].

Environmental sources such as water, soil, animals and plants could potentially be contaminated by radionuclear materials in the event of a nuclear disaster or RDD attack [**6**]. These materials could then be absorbed into the body through various routes of entry, leading to sickness or even death [**12**]. For this reason, it is important to analytical methods for the rapid and accurate detection of radionuclides in the environment as well as in biological samples.

Due to its high radiotoxicity, ^{226}Ra is the most studied naturally-occurring radioisotope. The danger arises from its long half-life of 1600 years, high abundance, α-emitting properties (damaging cells), chemical properties (displaces calcium in the body), and short-lived daughter nuclei (resulting in quick accumulation of ^{222}Rn) [**7**].

1.2. Isotopes of Interest

Certain radioisotopes such as ^{236}Ra (an α-emitter), and^{90}Sr (a β-emitter) are of particular concern upon absorption into the body as they have similar chemical structures and properties to calcium ions which can be displaced in bones [**4**]. These two analytes also have higher absorption rates in the gastrointestinal (GI) tract in comparison (see Table 1) to other common radionuclides.

Table 1: Physical Effects of Radiological Exposure [4]

Americium-241	75% absorbed, 10% retained	Minimal, usually insoluble	Rapid in first few days	Skeletal deposition. Marrow Suppression. Hepatic deposition
Cesium-137	Completely absorbed. Follows potassium	Completely absorbed. Follows potassium	Completely absorbed. Follows potassium	Renal excretion. Beta and gamma emissions
Cobalt-60	High absorption. Limited retention	<5% absorption	Unknown	Gamma emitter
Iodine 131	High absorption. Limited retention	High absorption. Limited retention	High absorption. Limited retention	Thyroid ablation/carcinoma
Phosphorus-32	High absorption. Limited retention	High absorption. Limited retention	High absorption. Limited retention	Bone, rapidly replicating cells
Plutonium-238, 239	High absorption. Limited retention	Minimal, usually insoluble	Limited absorption. May form nodules	Lung, bone and liver
Plutonium-238, 239	High absorption. Limited retention	Minimal, usually insoluble	Limited absorption. May form nodules	Local effects from retention in lung
Polonium-210	Moderate absorption and retention	Minimal	Moderate absorption	Spleen and kidney
Radium-226	**Unknown**	**30% absorption, 95% fecal excretion**	**Unknown**	**Skeletal deposition. Marrow Suppression. Sarcoma**
Strontium-90	Limited retention	Moderate absorption	Unknown	Bone - follows calcium
Tritium Tritiated water-HTO	HT-minimum, HTO-complete	HT-minimal, HTO-complete	HTO-complete	Panmyelo - cytopenia
Uranium-238, 235 fluorides UO3, sulfates, carbonates	High absorption. High retention	High absorption	High absorption. Skin irritant	Renal. Urinary excretion
Uranium-238, 235 some oxides nitrates	Moderate absorption. High retention	Moderate absorption	Unknown	Nephro - toxic. Urinary excretion
Uranium-238, 235 high oxides, hydrides, carbides, salvage ash	Minimal absorption. Retention based on particle size.	Minimal absorption, high excretion	Unknown	Nephro - toxic. Urinary excretion
Urium-228, depleted uranium metal	Retention based on particle size	Minimal absorption, high excretion	Forms pseudo-cysts with urinary excretion. Limited absorption	Nephro - toxic. Deposits in bone, kidney and brain

^{226}Ra is a product of the ^{238}U decay series (see Figure 1). It forms a 2+ ion in solution, making it a threat to drinking water sources [7].

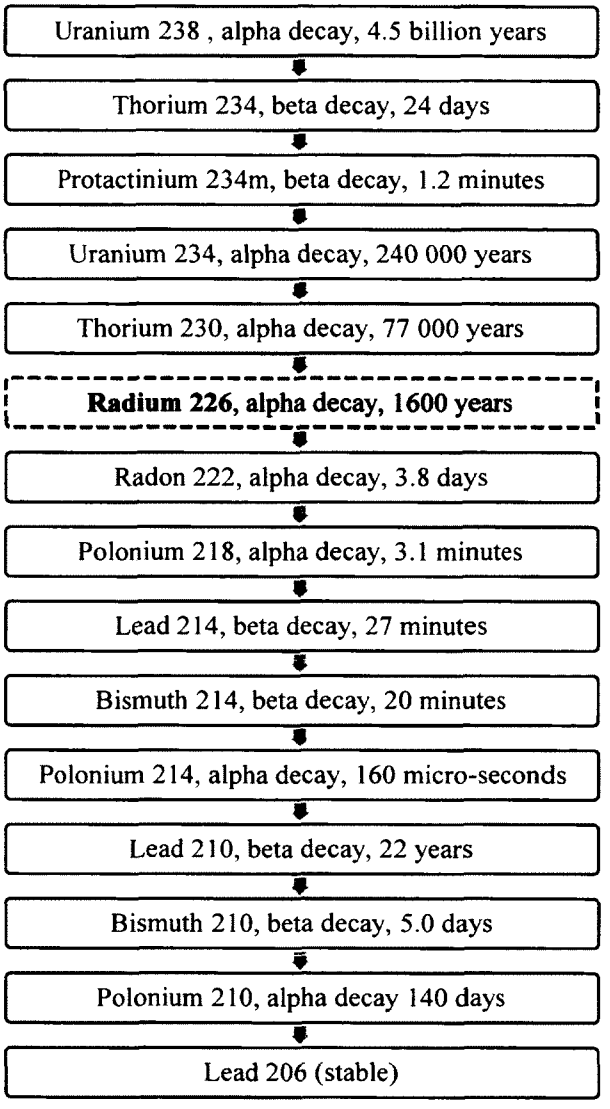

Figure 1: The 238-U Decay Series [7]

^{238}U is a naturally occurring radioactive element on our planet which is found in the form of a UO_2 mineral called pitchblende [**13**]. Due to uranium mining across the world, monitoring of radium (a daughter isotope of uranium) levels in ground water is a common occurrence [7]. According to government guidelines, sampling and analysis tests of individual radionuclides must be carried out frequently enough to accurately determine the annual exposure. The frequency in which these tests are performed is dependent on the fluctuations and level of radiation measured throughout the year. If levels remain below the maximum acceptable concentrations (MACs) and are consistent,

sampling may be carried out seasonally, semi-annually or even annually whereas if fluctuations or radiation levels are larger, testing must be conducted more frequently.

1.3. Actions

Health Canada has established a set of guidelines for the quality of drinking water in the country [**14**]. They have calculated Maximum Acceptable Concentrations (MACs) for the most commonly observed radionuclides (listed in Table 2) in Canadian water sources.

Table 2: Maximum Acceptable Concentrations of Commonly Detected Radionuclides in Canadian Drinking Water [14]

Total uranium[1]	0.02 mg/L	Tritium (^{3}H)	7000
Lead-210 (^{210}Pb)	0.2 Bq/L[a]	Strontium-90 (^{90}Sr)	5
Radium-226 (^{226}Ra)	**0.5 Bq/L**	Iodine-131 (^{131}I)	6
		Cesium-137 (^{137}Cs)	10

The levels of radionuclides in Canada's drinking water are monitored, and water treatment processes with removal efficiencies of up to 99% are used to ensure radiation levels remain within the guidelines.

These regulations were established in conjunction with the International Commission on Radiological Protection (ICRP), the United States National Research Council (USNRC), the American National Standards Institute (ANSI), the Standards Council of Canada (SCC), the United States Environmental Protection Agency (USEPA), the World Health Organization (WHO), the Ontario Ministry of Environment (OMOE), the Canadian Nuclear Safety Commission (CNSC), the International Commission on Radiological Protection (ICRP), and the International Organization for Standardization (ISO) [**14**].

The Australian Drinking Water Guidelines state a MAC of 0.5 Bq/L of gross α-activity[2] for ^{226}Ra and ^{228}Ra [**7**]. In drinking water, levels are below this value; however, ground water samples are known to reach levels up to the MAC, and thus monitoring of ground water is performed to ensure safety against contamination from uranium mining.

[2]The gross alpha activity is defined as the total activity of all alpha emitters in a dry sample once radon and uranium have been eliminated.

2. Extraction and Detection Methods

Quantitation of radioactive isotopes is performed by measuring the intensity of radiation emitted by the source. This value is normally expressed in disintegrations per minute (DPM) and can be converted to the standard radiation unit of Becquerel (Bq) using the following relationship [**15**]:

$$1\ Bq = 60\ DPM$$

Unfortunately, counting techniques are not perfect. The number of emissions emitted (DPM) and the number of emissions detected (expressed as the counts per minute or CPM) are often different as the counting efficiency expressed by Equation 1 below the detector is not 100% [**15,16**].

$$Counting\ Efficiency = (CPM/DPM*100\%)$$

Equation 1

For this reason, corrections must be applied to the CPM observed in order to obtain the DPM. In this study, this is done by applying the counting efficiency to the calculation of % sorption by taking into account consideration the effect of the matrix on the analytical signal. This procedure is described in more detail in chapter 3. Background radiation also affects the CPM hence a blank correction is also necessary.

2.1. Extraction Methods

In order to analyze environmental and biological samples using the techniques described in section 4, sample preparation steps must first be performed to ensure the analyte (^{226}Ra) is efficiently separated from impurities or interferences such as barium, strontium, calcium and uranium [**7,17**]. The two main types of extraction are liquid-liquid extraction such as dispersive liquid-liquid micro-extraction (DLLME) and solid-phase extraction (SPE) with the use of ion exchange chromatography resins or other solid-phase materials such as magnetic nanoparticles (MNPs) [**7**].

2.1.1. Ion Exchange Chromatography

Cation exchange chromatography is the process by which a solution containing a positively-charged analyte is passed through a column containing a solid cationic exchange resin in order to displace reversibly-bound ions on the resin for the eventual selective separation of the analyte

from other compounds in the sample matrix [**18**]. These resins are composed of amorphous organic particles containing negatively charged sites used to attract positively charge analytes. Polymeric materials such as polystyrene modified with sulfonate ($-SO_3^-$) groups are often employed for this purpose [**19**]. Once the analyte is bound to the column, it can be selectively eluted[3] based on its affinity for the column in relation to the affinity of the interferents [**18**]. Due to their small size and increased polarizability, ions of higher charge have a stronger affinity for the column and will thus be eluted last. In order to elute the more strongly bound analytes, a stronger eluent is often required to disrupt the interaction between the analyte and the stationary phase as the solvent molecules must compete with the analyte for sites on the column. Depending on the nature of the column, increasing or decreasing the polarity of the mobile phase may result in an increase in the eluent strength which could aid in the elution of more strongly bound analytes [**19**].

For a single analyte, an isocratic elution can be performed using a single solvent to rapidly elute all components in the sample. In the event that ions of varying affinities are to be selectively eluted from the column, a gradient elution is often carried out. In this type of elution, the eluent strength is increased gradually to obtain consecutive fractions of eluates, each containing a different ion. Generally, to separate radium from barium, a $Ba(Ra)SO_4$ precipitation is carried out under basic conditions in the presence of $Pb(SO)_4$ which is employed to remove interfering ions (thorium, actinium, polonium and uranium) and the precipitate is dissolved using CyDTA[4] or DTPA[5] and passed through the ion exchange column eluting each compound independently [**13**,**17**].

Anion exchange resins work similarly to cation resins; conversely, they are used to retain and elute negatively charged ions. These resins commonly utilize ammonium ($-NR_3^+$) functionalities to retain negatively charged analytes [**19**]. Although anion-exchange resins do not retain radium, they can be applied to remove negatively charged interferents such as Th, Ac or U from sample solutions before analysis [**13**].

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[3] Elution is the process by which a compound is removed from the solid phase by a mobile phase [**19**]
[4] 1,2-Cyclohexylenedinitrilotetraacetic acid
[5] Diethylenetriaminepentaacetic acid

2.1.2. Chelating Ligands

A ligand is an ion or molecule used to bind an analyte such as $^{226}Ra^{2+}$ [**19**]. Chelating ligands bind metal ions through more than one atom to form a stable complex. These molecules are typically employed for the purpose of extracting charged species from the aqueous phase (such as a ground-water or urine sample) into an organic solvent. In order to do so, a selective, neutral chelating agent is often introduced into the organic phase during a liquid-liquid extraction to capture metal ions in the aqueous phase and transport them into the organic phase. For ^{226}Ra, crown ethers (CEs) have been reported for this purpose [**13**].

CEs are cyclic organic molecules with unique chemical structures and sizes which allow them to selectively bind metal ions to form stable complexes [**19**]. Since CE cavities are neutral, a counter ion is often required to allow for uptake of the metal ion into the CE cavity. Dicyclohexano-21-crown-7 has been reported to have extracted radium in the presence of organocarboxylic acid counter anions [**13**].

2.1.3. Dispersive Liquid-Liquid Microextraction

Dispersive liquid-liquid micro extraction (DLLME) is used for the extraction of an analyte from an aqueous sample into an organic solvent. A research group at Health Canada explored this technique in 2011 for the rapid extraction of ^{226}Ra from drinking water [**20**]. In this method, a phase transfer reaction occurs where the ligand, dibenzo-18-crown-6 (in the organic phase) comes into contact with the analyte, ^{226}Ra (in the aqueous phase) and traps it within the crown ether cavity, transporting it into the organic phase. Since the latter is less dense than water, it can be drawn off as the top phase for analytical measurement.

DLLME is a rapid extraction technique as it combines extraction and preconcentration steps. For DLLME of ^{226}Ra from a water sample, an organic extractant comprised of dibenzo-21-crown-7 and 2-theonyltrifluoroacetone in toluene and a disperser solvent (acetonitrile) are mixed together and rapidly injected into the ^{226}Ra-spiked sample using a solvent delivery pump at a flow rate of 6.6 mL/min creating an emulsion. The emulsion is then disrupted by introducing a de-emulsifier (acetonitrile) using the same pump to separate the organic and aqueous phases from each other. The organic phase can then be extracted off the top of the sample and measured by LSC [**20**]. DLLME can be performed on a variety of sample volumes (20mL-200mL), gives reproducible results (<6% relative precision and <-5% relative bias) due to automation of

delivered volumes (with the assistance of an automated solvent delivery pump), possesses a simple experimental set-up (see Figure 2 below), and is selective for the removal of ^{226}Ra.

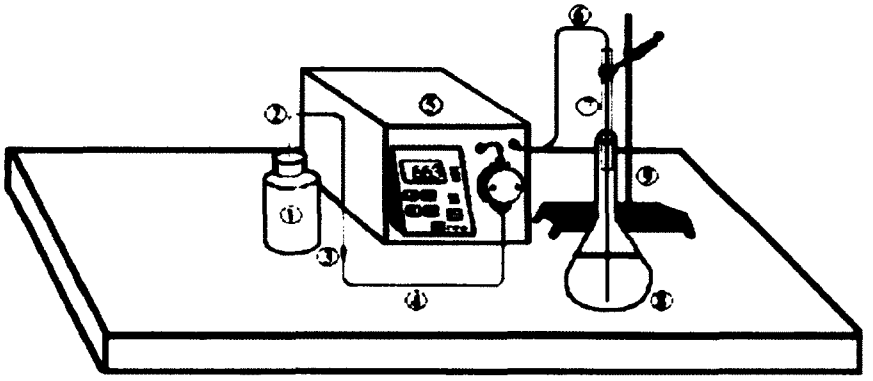

Figure 2: Schematic diagram of the experimental set-up for DLLME of ^{226}Ra from water samples. (1) emulsifier or de-emulsifier, (2) 1/16'' i.d. x 1/8'' o.d. Teflon tubing, (3) 1/8" to 1/16" adapter joint, (4) 1/32" i.d. x 1/16" o.d. PEEK tubing, (5) single piston solvent delivery pump, (6) 0.005" i.d. x 1/16" o.d. PEEK tubing, (7) guard insert (in order to align and support the PEEK tubing in the volumetric flask), (8) volumetric flask and (9) clamp and stand assembly (in order to hold the guard insert) [20]

An 89% extraction efficiency and a detection limit of 33mBq/L were reported for ^{226}Ra **[20]**. The main disadvantage of this technique is that large volumes of radioactive organic waste are produced.

2.1.4. Magnetic Nanoparticles

Magnetic nanoparticles (MNPs), as used in analytical extraction methods, offer many advantages over traditional SPE techniques; their small size and large, easily functionalized surface area along with their magnetic behaviour make them excellent candidates for both the extraction and separation of charged analytes **[21]**. Their surfaces can be made positive or negative simply by varying the pH of the sample in which they are suspended to provide optimal conditions for ion exchange (see Figure 3). Once the analyte is bound to the MNPs, they can be magnetically separated using an external magnetic field allowing for direct (measurement of the activity on the MNPs themselves) or indirect (measurement of the activity present in the supernatant) analysis.

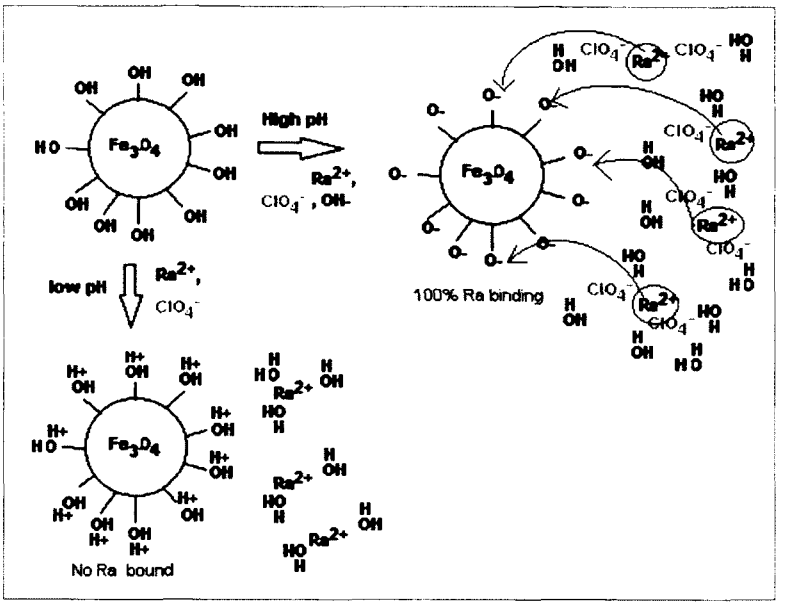

Figure 3: Predicted Mechanism of ^{226}Ra Binding to Bare MNPs in the Presence of ClO_4^-

The use of MNPs for SPE has been investigated for the extraction and pre-concentration of α-emitting radionuclides [**21**]. The %sorption (%S) for ^{226}Ra, ^{241}Am, ^{210}Po, and ^{233}U by the MNPs in spiked urine samples was tested. Each sample (50 mL) was spiked with 80-550 Bq of the radioanalytes. This method resulted in the rapid extraction (<5 mins) of certain radionuclides (such as ^{226}Ra) from urine and required low concentrations of MNPs (0.5mg/mL), resulting in an overall %S for ^{226}Ra of 77.2% using bare Fe_3O_4 nanoparticles and >97.6%S using Mn-Fe-O nanoparticles at pH 5.9. However, it is not selective for specific radionuclides, and consequently requires further separation processes in order to determine individual extraction efficiencies.

2.2. Detection Methods

Common analytical detection methods for the quantification of radioanalytes include: Liquid Scintillation Counting (LSC), Gamma-ray Spectroscopy and Alpha Spectroscopy [**13,22**]. The advantages and disadvantages of these methods can be examined by comparison of the detection limit, linearity, reproducibility and selectivity of each method. A good analytical technique is generally proficient in detecting very low concentrations (or activities in this case), produces data with a high correlation coefficient (as close as possible to 1.00), demonstrates excellent reproducibility among samples resulting in small standard deviations, and is capable of selectively determining a single analyte with little to no interference from other matrix components [**19**].

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) is a common detection technique used for the analytical quantification of radionuclides, however, this method cannot be used for the direct detection of radium since the half-lives of its isotopes are too short, resulting in concentrations below the limit of detection [**13**]. For this reason, radium is normally determined using alpha or gamma spectroscopy.

2.2.1. Liquid Scintillation Counting

Liquid Scintillation Counting (LSC) is a detection method used to analyze aqueous and/or organic isotopic compounds through quantitation of the number of emission events of a given isotope measured in the form of a photon of light [**15**]. In this method, samples are combined with a liquid scintillation cocktail which absorbs and re-emits the energy released during relaxation of an electron in its excited state. Primary and secondary phosphor molecules such as 2,5-diphenyloxazole (PPO) and 1,4-bis(5-phenyloxazol-2-yl) benzene, contained in the cocktail work together to convert the absorbed energy into light [**23**].These molecules absorb energy from emission events and re-emit radiation in the form of light through phosphorescence or fluorescence when they return to the ground state. Unlike fluorescent materials, phosphorescent materials must pass though an intermediate energy state before returning to the ground state and thus their photoluminescence is not as spontaneous. The light produced by the phosphor molecules is then sent toward the detector which uses 2 photomultiplier tubes to detect and record light pulses. More details regarding this process are described in the next chapter. This method is commonly used in the determination of α- or β-emitting particles [**13**].

LSC detection affords many advantages for the measurement of α-emitting radionuclides. It offers selectivity by allowing for discrimination between α and β signals, fast analysis times, the ability to analyze numerous samples sequentially without monitoring, reproducibility among samples and high sensitivity [**15,13**]. It also possesses low detection limits (0.05-0.2 Bq/L) and is thus adept in detecting very low concentrations of analyte [**13**].Although it has many advantages, LSC does present a few drawbacks, mainly pertaining to sample preparation. Quenching of the signal (detector response) is a significant issue when it comes to LSC [**15**]. Coloured samples or samples containing chemical quenchers[6] will interfere with the signal resulting in false results. During LSC detection, if the colour of the sample absorbs light of the same wavelength being

[6]Chemical quenchers: chemicals which cause a decrease in the signal outputted by the instrument [**19**]

emitted by the scintillator, or if the concentration of a specific chemical species (a quencher) in the sample matrix is too high, the signal will be quenched. Clear, colourless samples with a neutral pH and homogeneous composition give the most reliable results and thus samples must be prepared carefully to ensure they follow these guidelines.LSC with α/β-discrimination can detect ^{226}Ra with a MDA of ~10-40 mBq/L giving α-radiation detection efficiencies of up to 100% if samples are free of chemical and colour quenching [**13**].

2.2.2. Gamma Spectroscopy

Gamma ray spectroscopy directly measures γ radiation emitted from the analyte using lead shielding to minimize background radiation [**13**]. In order to measure gamma rays, the gamma detector must be capable of detecting fast-moving electrons which have come into contact with the photons emitted during gamma decay [**24**]. The energy of these electrons is equal to the energy of the γ-ray in which they interacted and thus by quantifying their energy, the detector can determine the energy of radiation as well as the count rate. Due to their high energies, the mean free path of the electrons which have come into contact with γ-rays is quite large and thus the size, shape and geometry of the detector can affect the shape and intensity of the gamma spectrum. The sample geometry must thus remain consistent from sample to sample for precise results. It offers several advantages over alpha spectroscopy and LSC for the analysis of ^{226}Ra. It affords specificity (by giving distinguishable signals for radium's peak and that of its progenies), lower instrumental interference and the ability to measure samples without purification or processing [**25**]. Low background High Purity Germanium (HPGe) γ-spectroscopy is able to detect ^{226}Ra with a minimum detectable activity (MDA) of ~2-40 mBq/L (within the national regulation limit of 37-40 mBq/L) [**14,13**].

Disadvantages of this technique include low counting efficiencies (due to the low γ-ray probability of only 3.28%) resulting in low intensity peaks, and ^{235}U interference at 185.74 keV (^{226}Ra is found at 186.1 keV). To resolve these issues, the concentration of uranium can be quantified using ICP-MS and the radium concentration or counting time can be increased to obtain higher peak intensities. Gamma counting is quite time consuming as it requires equilibrium between ^{226}Ra and its daughter ^{222}Rn (which can escape as it is in the gaseous phase). A minimum mass of 15g of sample is also required for analysis resulting in detection

limits of 2-3 Bq/Kg [**13**]. Nonetheless, it is generally the easiest and most cost effective analytical method for the determination of ^{226}Ra.

2.2.3. Alpha Spectroscopy

Alpha spectroscopy for the determination of ^{226}Ra works by measuring the α-radiation emitted from radium isotopes [**7**,**13**]. Each alpha particle which hits the detector will be absorbed and converted to an electronic pulse which is directly proportional to the particle energy [**26**]. Considering α particles are large, high energy particles with little penetrating power, very thin sources must be utilized to reduce interference [**7**]. For this reason, silicon surface barrier detectors are commonly used as they provide low spectral interference, present ease of use and afford high resolution and precision [**13**]. The samples and detector surface in the alpha spectrometer are place under high-vacuum to eliminate the interaction of the α- particles emitted by the sample with air molecules. Due to the geometry of the source and the detector, counting efficiencies typically range from 20-35% [**26**]. Unlike gamma counters, these instruments do not require high voltages or cooling conditions. Additionally, they are apt in detecting ultra-low radium activities with high sensitivity (due to high α-decay probabilities) and specificity. A MDA of 0.1 mBq/L can be achieved using α-spectroscopy. In conjunction with chemical separation, this method is capable of identifying concentrations 100 times lower than comparable γ-spectroscopy or LSC techniques.

3. Bare Magnetic Nanoparticles for the Extraction of Radionuclides

3.1. Introduction

Magnetite (Fe_3O_4) is a naturally occurring iron oxide mineral found in the earth's crust. It exhibits ferromagnetic behaviour due to unpaired electrons and magnetic dipoles which irreversibly align in the presence of a magnetic field [**27**]. The unpaired electrons in ferromagnetic materials are divided into magnetic dipole domains of $\leq$100nm.

Magnetite nanoparticles (MNPs) can be chemically synthesized via co-precipitation of Fe^{3+} and Fe^{2+} under basic conditions to form stable colloids [**28,29,30**]. Certain materials (known as paramagnetic materials) can be spontaneously magnetized in the presence of an external magnetic field. If the MNPs are small enough (10-40nm), they can be classified as superparamagnetic. This occurs when the particles are smaller than the size of their domain. A particle's domain is described by a small region (1-100's microns) in which magnetization is saturated. The net magnetic moment of superparamagnetic materials is zero in the absence of a field at $T > 0$ K however, with the application of even a weak external field, a net alignment of magnetic moments will result. Unlike paramagnetism, in which a single atom will align with the applied field, superparamagetism involves the alignment of all atoms in a single domain thus these materials have much higher magnetic susceptibility. Superparamagnetic iron oxide nanoparticles (SPIONS) are commonly used in MRI applications as they are easy to synthesize and form stable colloids with low toxicity. Electrostatic repulsion and van der Waals and magnetic dipole-dipole interactions between particles help stabilize the colloid.

In recent years magnetic nanoparticles have been widely used for many analytical and diagnostic applications in biology and medicine, including protein purification, drug delivery, sensors and medical imaging [**31,32,33,34**]. Due to their nanoscale size (smaller than many biomolecules) and large surface area, they are ideal candidates for use as MRI contrast agents or in extraction techniques for the preconcentration, separation and capture of analytes [**30,35,36,37,38,39,40,41,42,43**].

Bare MNPs have been employed for the extraction, pre-concentration and purification of α-emitting radionuclides (^{241}Am, ^{210}Po, ^{226}Ra and ^{233}U) and other cations (Y^{3+} and Sr^{2+}) in spiked urine samples [**21,44**]. Their extraction efficiency can be determined by direct measurement of the activity on the MNPs themselves or indirect measurement of the activity present in the

supernatant. Rapid extraction (<5 min) of ^{226}Ra from urine (50 mL spiked with 80-550 Bq) using a low concentration of MNPs (0.5mg/mL) resulted in an overall sorption of 77% for ^{226}Ra using bare Fe_3O_4 nanoparticles and 97% using Mn-Fe-O nanoparticles at pH 5.9. However, extraction on the bare MNPs was not found to be radionuclide selective.

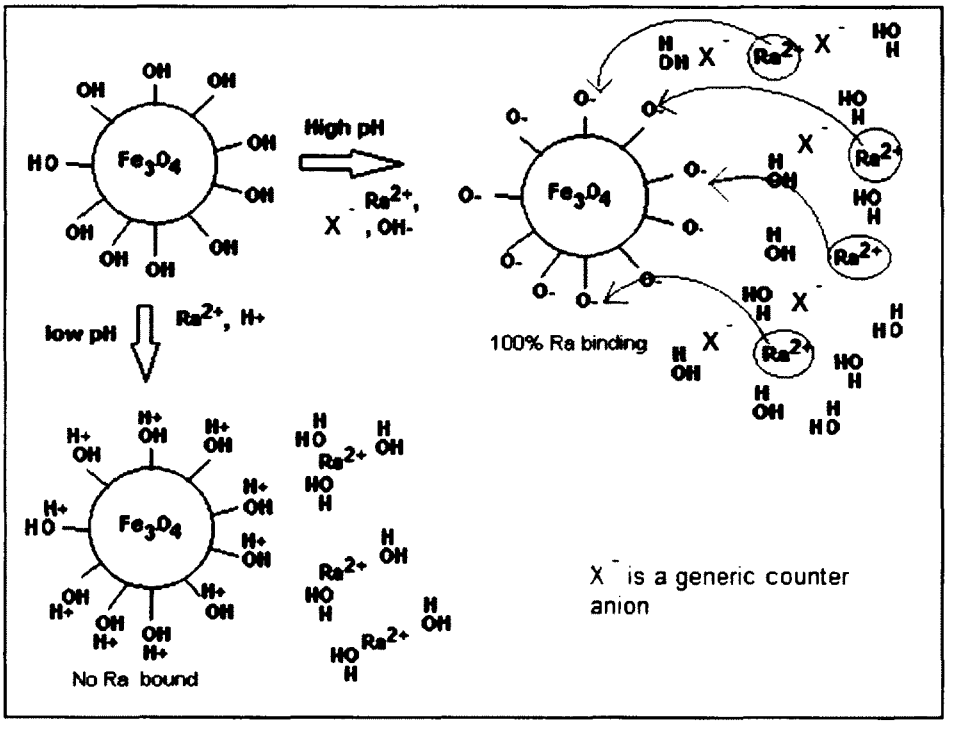

Figure 4: A schematic representation of the pH tunable surface of bare MNPs

Binding of cations such as those mentioned above is possible due to the tunable surface properties of the MNP surface. As shown in Figure 4 above, their surfaces can be made positively or negatively charged by varying the pH of their medium to provide optimal conditions for ion exchange [**45**]. In aqueous solution, once the analyte is bound to the MNPs, they can be magnetically separated using an external magnetic field.

At low pH, the surface becomes protonated making it positively charged as per chemical Equation 2 below.

$$FeOH + H^{+} \leftrightarrow FeOH^{+2}$$

Equation 2

At high pH, however, the surface becomes negatively charged,thus cations will be preferentially absorbed onto the surface according to chemical Equation 3 below:

$$FeOH + OH^- \leftrightarrow FeO^- + H_2O$$

Equation 3

3.2. Materials and Methods

3.2.1. Chemical Reagents

Sodium perchlorate (CAS# 7601-89-0, ACS reagent ≥98.0, MO, USA), sodium chloride (CAS# 7647-14-5, ACS reagent ≥99.0, USA), aluminum nitrate nonahydrate (CAS# 7784-27-2, 100.00%, USA), yttrium chloride hexahydrate (CAS# 10025-94-2, 99.99% trace metal basis, USA), sodium nitrate (CAS# 7631-99-4, ≥99.0, Japan), 0.03M Picric acid (CAS# 88-89-1, Reagent Plus ≥99%, MO, USA),sodium hydroxide pellets (CAS# 1313-73-2, Assay min. 99%, USA), methanol (CAS# 67-56-1, Assay ≥99.9%, USA), iron (III) chloride anhydrous, Assay min. 98%, Riedel-de Haën, Germany), and iron (II) chloride tetrahydrate (CAS# 13478-10-9, Reagent Plus, 99%, MO, USA) were purchased from Sigma Aldrich.^{226}Ra standard was obtained from the National Institute of Standards and Technology (SRM 4965, NIST, Gaithersburg, MD, USA), hydrochloric acid from Anachemia (36.5-38%, ACS reagent, Montreal, QC, Canada), anhydrous ethyl alcohol from Commercial Alcohols Inc.(Brampton, ON, Canada), Liquid Scintillation Cocktail (LSC) containing nonylphenolethoxylate from Perkin Elmer (CAS# 9016-45-9, OPTIPHASE 'HISAFE' 3, Massachusetts, USA), and Milli Q water from Millipore (0.22μm, 18MΩ·cm, MA, USA).

3.2.2. Instrumentation

5mL BD Luer-Lok™ plastic syringes were purchased from VWR International Inc. (Mississauga, ON, Canada), 125/box, NJ, USA) , 0.22μm PES Syringe filters from Tisch Scientific (Ohio, USA), VanGuard V6500 Centrifuge from Hamiltonian Bell Company Inc. (NJ, USA), Branson 2510 Sonicator from Branson (0-60 minutes, Danbury, USA), Hidex 300SL Liquid Scintillation Counter (α/β separation, Mississauga, ON, Canada) from Gamble Technologies Limited, Gamma Spectrometer Model No: LS-1116 (EG & G ORTEC, GA, USA) from Putnam Technology Inc., Vortex Genie 2 (NY, USA) from Scientific Industries, Lab Roller II (5-60 rpm, NJ, USA) from Labnet, 50mL Self-Standing Polypropylene Centrifuge Tubes (25 tubes/bag, NY, USA) from Corning Incorporated, Accumet Excel pH meter XL15 (±0.01, Singapore) from Fisher Scientific, Analytical Balance AT 201 (±0.01mg, Switzerland) from

Mettler Toledo, 15mL polypropylene centrifuge tubes from Corning Inc., variable automatic micropipette and tips from Eppendorf North America, USA (0.5-10μL, 10-100μL, 100-1000μL, 500-5000μL, and 1-10mL)), Nalgene 500mL narrow mouth bottles from Thermo Scientific (12/pack, USA), 125mL Erlenmeyer flask from Pyrex (USA), Circulating Heating Bath from HAAKE GH Fisons, and SH-1 Magnetic stir plate from Taisite (China). Finally, 20 mL plastic LS Vial (HDPE, UreaCap, PE Cone Lnr (#986706 1 CS is 500 EA, NJ, USA) and 20mL glass LS vial (22-400 Urea Cap with PE cone Ln (#986546 1 CS is 500 EA, NJ, USA) were obtained from Wheaton.

3.2.3. Synthesis of Bare MNPs

Bare MNPs were prepared using a common co-precipitation method described herein [**44**]. 1.2600g of $FeCl_3$ and 1.0356g of $FeCl_2 \cdot 4H_20$ were dissolved in 15mL of aqueous HCl (12.5mL of DDW + 2.5mL of 1M HCl). This solution was added drop-wise using a Pasteur pipet into a 125mL Erlenmeyer flask containing 25mL of 1M NaOH under vigorous stirring in the presence of Nitrogen gas. Once all the solution was added, it was stirred for 30 minutes. The resultant colloidal solution was evenly distributed into four 15mL plastic centrifuge tubes and centrifuged (VanGuard V6500 Centrifuge, Hamiltonian Bell Company Inc., NJ, USA) at 10,000 rpm and the supernatant was removed. The remaining magnetic nanoparticles were washed five times with DDW until the pH of the resulting supernatant was ~6. The clean MNPs were stored in 125mL of DDW in the fridge at 5°C to be used for bare colloidal MNP (35 mg of bare MNPs/mL of deionized water) tests later on.

A second batch of MNPs was prepared using the same procedure, however the final product was washed 3 times with methanol, transferred into a 50mL centrifuge tube and left to dry in the fumehood overnight. The resultant dry MNPs were transferred into a 20mL, glass liquid scintillation vial and stored at room temperature for further modification.

3.2.4. Extraction of ^{226}Ra using Bare MNP Suspension

Several experimental parameters were investigated in preliminary stages of research to determine the optimal experimental conditions for removal of radium from aqueous samples. The pH of all sample solutions was adjusted using 4M NaOH and 5M HCl respectively. A pH range of 2-10 was investigated to determine the pH for optimal removal. The mass of MNPs required for optimal removal was also investigated using both dry and colloidal MNPs. Masses

of 5-25mg were tested for the removal of ^{226}Ra from 20mL sample solutions. A variety of different salts were also tested at various concentrations to be used as possible counter ions in future experiments with CE-modified MNPs. For initial parameter optimization, $NaClO_4$ and NaCl were used at 0.1M concentrations. Later, YCl_3, $Al(NO_3)_3$ and picric acid were tested. All samples were analyzed using LSC detection or gamma spectroscopy depending on the nature of the sample.

In order to calculate the extraction efficiency of ^{226}Ra by the bare MNPs, instrumental parameters such as the pulse length index (PLI) limit and region of interest (ROI) must be determined. This was done by investigating the 2D plot of the alpha and beta signals given off by the sample (see Figure 5). The PLI limit determines whether a pulse is considered alpha or beta [**46**]. It is described by a horizontal line which can be drawn in between the alpha and beta regions to separate these pulses from one another. Long alpha pulses are separated into the alpha channels and shorter beta pulses are placed in the beta channels. In the case of the example shown below, the PLI limit was chosen to be 10. In order to separate these pulses, a photoelectric cell within the instrument called the photomultiplier tube (PMT) converts light into electric current and amplifies it to be sorted into channels. An electric circuit known as a multichannel analyzer (MCA) then sorts these currents into channels (bins) based on their pulse height and length and separates them to be measured by different detectors. The ROI is described by the region of the MAC where the counts are being collected. In order to determine the alpha ROI range for the analyte, vertical lines which encompass the alpha signal can be extrapolated from the 2D plot as shown below. The range (from left to right) can then be entered into the software to determine the counts under the analyte peak in the alpha spectrum within that range. In the spectrum below, the alpha ROI range is approximately 575-685.

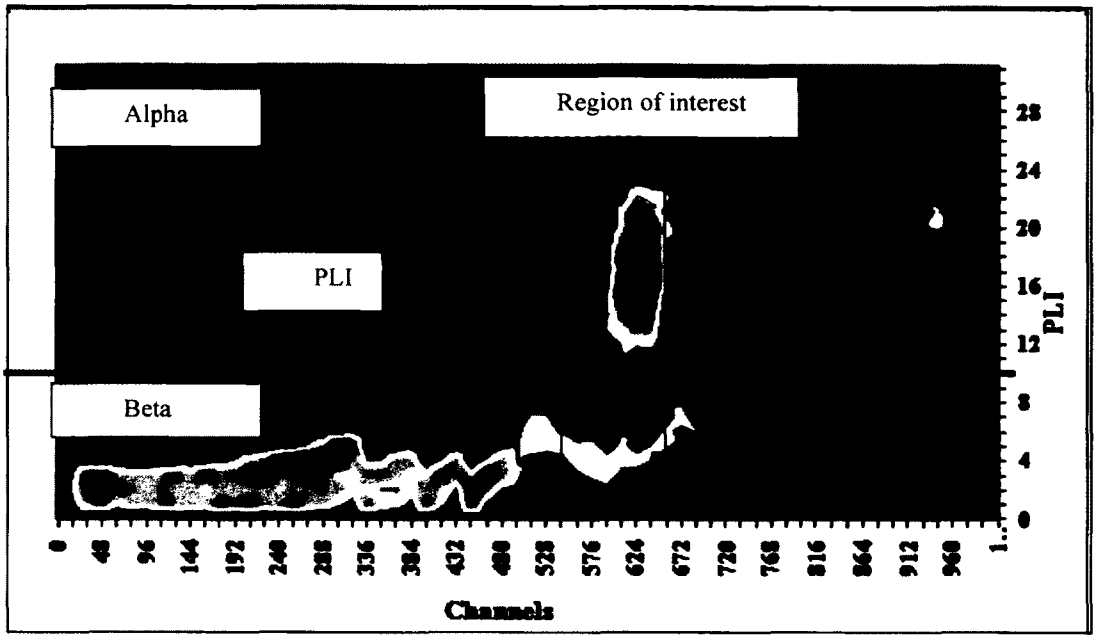

Figure 5: 2D Plot of the alpha and beta signals given off by a 20mL $NaClO_4$ sample spiked with 0.4Bq of ^{226}Ra

The extraction of ^{226}Ra using bare MNPs was carried out in pre-weighed, pre-labeled, 20mL, glass liquid scintillation vials. All samples were prepared in triplicate. Each vial (except the blank) was spiked with 0.8Bq of standard ^{226}Ra (using a 7.26Bq/g of ^{226}Ra intermediate standard solution prepared from NIST SRM 4965) using a 100-1000μL variable automatic micropipette (Eppendorf North America, USA). The mass of ^{226}Ra standard spiked into each vial was measured on an analytical balance (AT 201, ±0.01mg, Mettler Toledo, Switzerland) and recorded. 19mL of appropriate salt solution (NaCl, $NaClO_4$, YCl_3, $NaNO_3$, $Al(NO_3)_3$ and picric acid) prepared in deionized water with various concentrations were added to each vial and the pH of the solution was adjusted (using an Accumet Excel pH meter XL15 (±0.01, Fisher Scientific, Singapore) with 4M NaOH and 5M HCl) to the necessary value. After pH adjustment, a known mass of MNP suspension prepared in deionized water (5mg of MNPs yielded optimal results) was then transferred to all except the spike sample using a 100-1000μL variable automatic micropipette. The final volume of the solution was adjusted to 20mL with deionized water and the pH of the solution was recorded once more. The mass of the final sample was taken on the analytical balance and recorded as before. The samples were then agitated for 10 minutes using a Labnet lab roller II (NJ, USA) and ultrasonicated for 30 minutes using a Branson 2510 sonicator (Danbury, USA). They were then placed on a 4'' x 4'' x 1/2" thick NdFeB Grade

N52 magnet (K&J Magnetics, Jamison, PA, USA) to magnetically separate the MNPs from solution. 5mL of supernatant liquid were collected off each sample from atop the MNPs using a 5mL syringe and transferred through a 0.22μm PES syringe filter (to ensure no particles were passed through) into a pre-weighed, pre-labeled, 20mL, polypropylene liquid scintillation vial. The mass of supernatant was recorded. All samples were combined with 15mL of Optiphase Hisafe 3 liquid scintillation cocktail (Perkin Elmer, MA, USA) and vortexed (Vortex Genie 2, Scientific Industries, NY, USA) until homogeneous. Measurement of ^{226}Ra was carried out on a Hidex 300 SL automatic TDCR liquid scintillation counter (LSC) with alpha/beta separation option (Gamble Technologies Limited, Mississauga, ON, Canada) for a counting time of 1 hour per sample. An alpha delay time of 40, an alpha tail offset of 2, a pulse length index (PLI) of 6, a Y gain of 40 and a region of interest (ROI) between 500–800 channels in the α/β separation mode were the parameters for LSC analysis. A schematic representation of the experimental procedure is shown below in Figure 6.

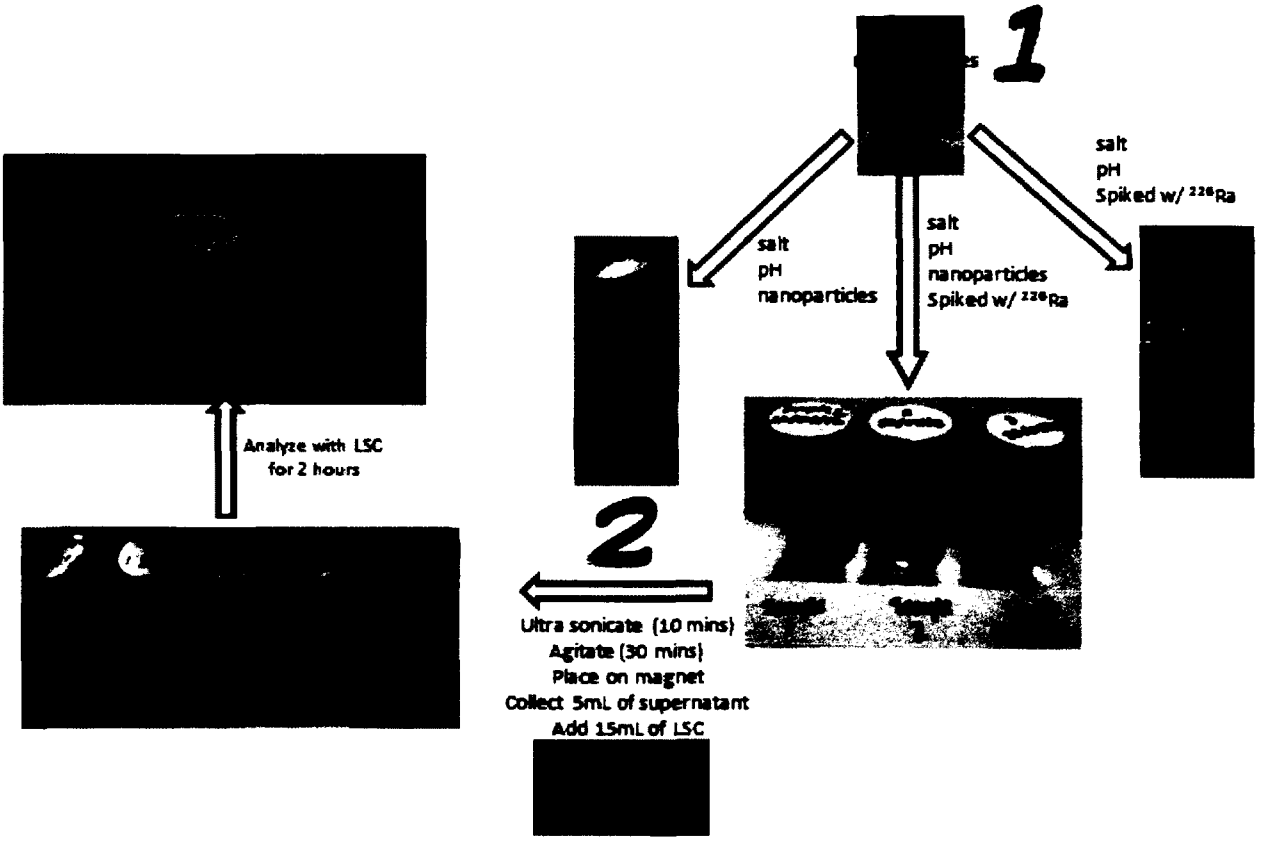

Figure 6: Experimental procedure for the extraction and detection of ^{226}Ra from aqueous samples using bare MNPs

3.2.5. Sample Preparation

Colloidal stability, nanoparticle distribution and separation of MNPs from solution were investigated through the introduction of ultrasonication, agitation and magnetic separation of the particles once they were exposed to the analyte. PES syringe filters were employed to filter out any MNPs from the solutions being analyzed to avoid signal quenching from the instrument. Tests were conducted with and without syringe filters to determine whether or not this sample preparation step was necessary. In order to determine whether or not the ^{226}Ra signal of a sample measured at the start of a run and a sample measured at the end of a run would be affected by the decay of ^{226}Ra over a typical experimental run time (2-24 hours), a single sample was measured every hour via LSC analysis over a period of 24 hours. This was done to determine if it was necessary to perform a decay correction on samples analyzed by this method.

3.3. Results and Discussion

3.3.1. Particle State

In the preliminary stages of this study, both dry and colloidal MNPs were investigated in order to determine which physical state would be most conducive for future tests. The dry MNPs showed inconsistency in the %Removal over replicate samples and did not give a linear trend in removal over increasing pH (see Figure 8below). The particles were not well dispersed and appeared to break down at lower pHs (less than 6) resulting in brownish coloured solutions which could not be directly analyzed by LSC (see Figure 7 below). The colloidal MNPs gave much more consistent results from sample to sample demonstrating a linear trend in extraction of ^{226}Ra over a pH range of 4-8 (see Figure 8). The lack of precision demonstrated by the dry MNPs is most likely due to the uneven size distribution which resulted from aggregation of the particles upon drying. They also demonstrated excellent dispersion at low pHs and gave clear and colourless supernatant solutions across all pH values (2-8) which were readily measurable by LSC after magnetic separation and filtration through the syringe filter.

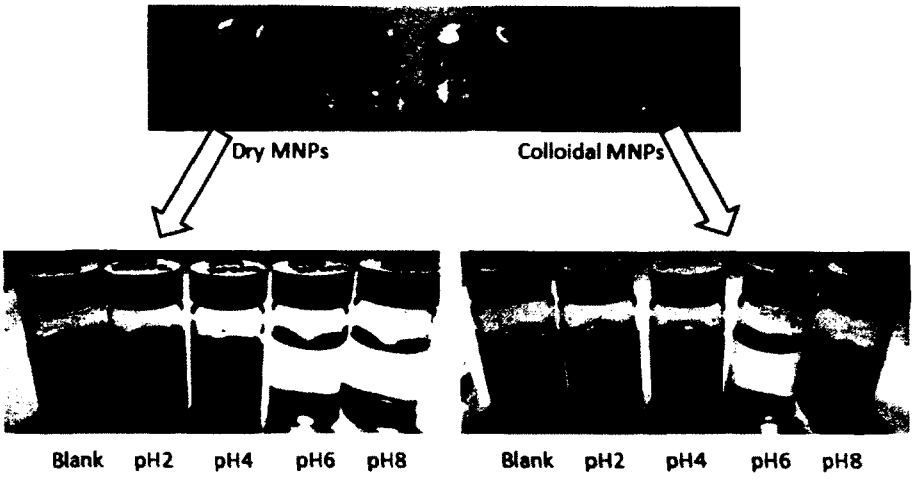

Figure 7: Dry and colloidal MNPs dispersed in 0.1M $NaClO_4$ solution at varying pHs[7]

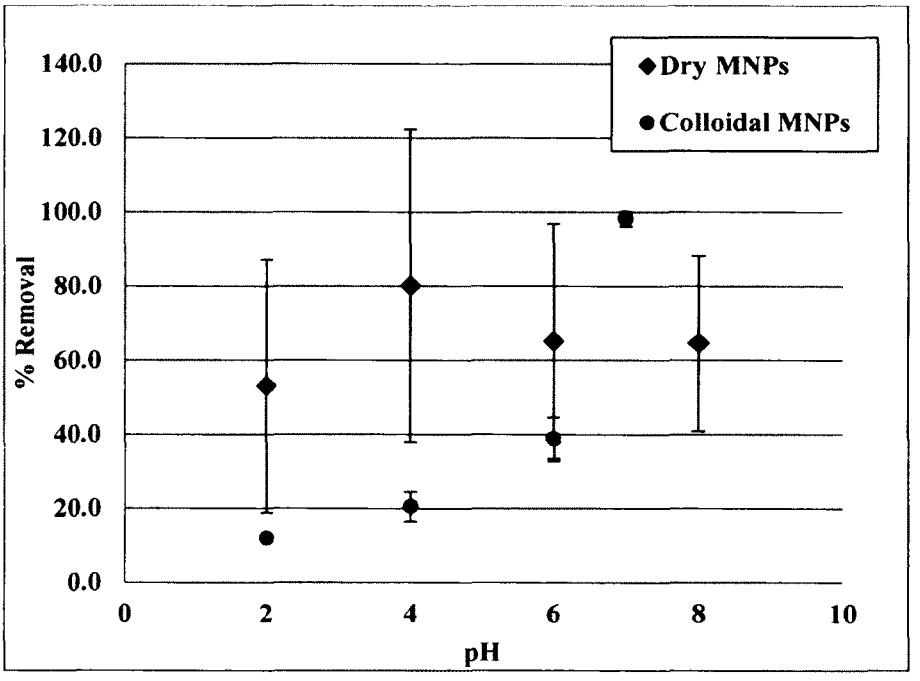

Figure 8: ^{226}Ra Extraction efficiency of dry and colloidal MNPs in 0.1M $NaClO_4$at pH 2, 4, 6 and 8

As can be observed in Figure 9 below, the separation and colloidal stability of the colloidal MNPs is very good. Before agitation, the particles settle nicely at the bottom (without the use of an external magnetic field) of each sample leaving a clear and colourless supernatant above them, however, once the particles are subjected to ultrasonication and agitation, a uniform

[7] The blank and spike solutions in this experiment were prepared with a pH of 5

distribution of suspended particles can be observed in solutions, allowing for suitable conditions for analyte-particle interactions to occur.

Figure 9: Effect of ultrasonication and agitation of MNP suspensions

3.3.2. Effect of Syringe Filter

In order to determine whether or not it was necessary to filter the supernatant from atop the MNPs to remove unwanted particles which could interfere with LSC detection measurements and extraction experiment was performed as per section 3.2.4 (Extraction of ^{226}Ra using Bare MNP Suspension) above. In this experiment, 5mg of colloidal MNPs were introduced into 20mL of 0.1M $NaClO_4$ solutions spiked with 0.8Bq of ^{226}Ra. One spike (containing ^{226}Ra but no MNPs), one blank (containing MNPs but no ^{226}Ra) and 4 sample solutions (of pH's 2, 4, 6 and 8 respectively) containing both MNPs and ^{226}Ra were prepared in 6 separate, pre-weighed, pre-labeled 20mL glass LCS vials. After magnetic separation, 5mL of the supernatant from each of the 6 samples were collected using a 5mL syringe and past through a 0.22μm PES syringe filter into 6 separate 20mL plastic LSC vials and combined with 15mL of liquid scintillation cocktail for analysis.

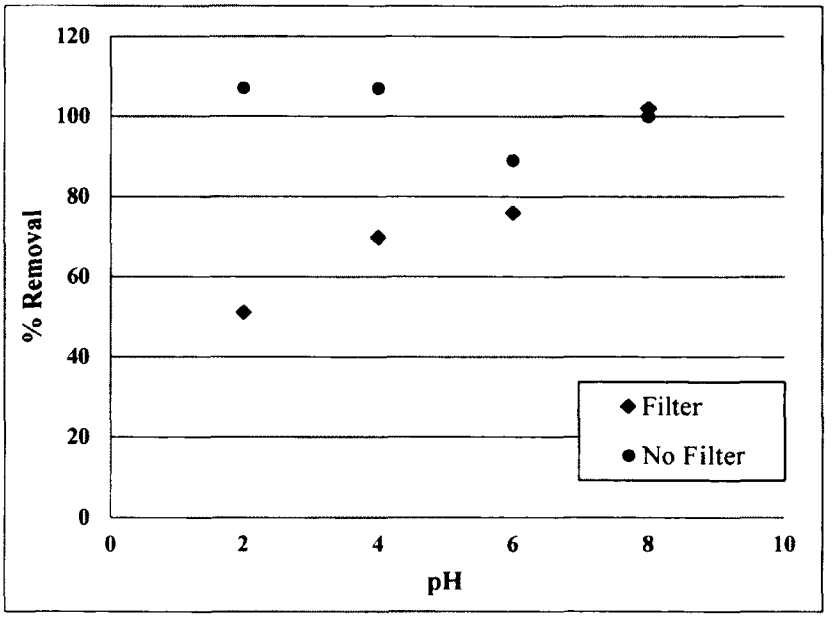

Figure 10: Effect of filtration of ^{226}Ra containing samples exposed to 5mg of bare colloidal MNPs in 20mL of 0.1M $NaClO_4$ at pH 2, 4, 6 and 8 on LSC detection

Similarly, 5mL of supernatant from each sample were transferred into 6 other 20mL vials without filtration. The results, shown in Figure 10 above, follow the predicted trend of increasing extraction efficiency with increasing pH in the case where the samples were filtered, however, a deceiving result of 89 - 107% extraction was observed in unfiltered samples. This false negative result for the detection of ^{226}Ra is most likely due to quenching of the LSC signal in the unfiltered sample solutions due to the presence of suspended particles or colour in the supernatant. To avoid this, all future samples analyzed through LSC were filtered before analysis.

3.3.3. Counter Ion Type and Concentration

When comparing $NaClO_4$ to NaCl, the extraction efficiency is not the only parameter the must be considered. One must also take into consideration the effect of the counter ion's properties on the detection signal. As is the case with most spectroscopic methods, it is important that the signal is sharp and intense enough to distinguish it from background interferences. $NaClO_4$ presents a higher extraction efficiency than NaCl, and looking at the alpha spectrum below (see Figure 11), it can be seen that the contribution of the daughter side peaks (to the right of the analyte signal) to the analytical signal is minimized in the presence of this salt making it easier to single out the ^{226}Ra contribution. These side peaks represent radium's alpha emitting daughter isotopes, Rn-222, P-218, Po-214, and Po-210 (see Figure 11).The spectrum given by the $NaClO_4$salt results in a sharper, cleaner spectrum which may give a better representation of the actual ^{226}Ra removal. For this reason, $NaClO_4$ was used in further tests with bare MNPs for the determination of optimal extraction conditions for ^{226}Ra from water samples.

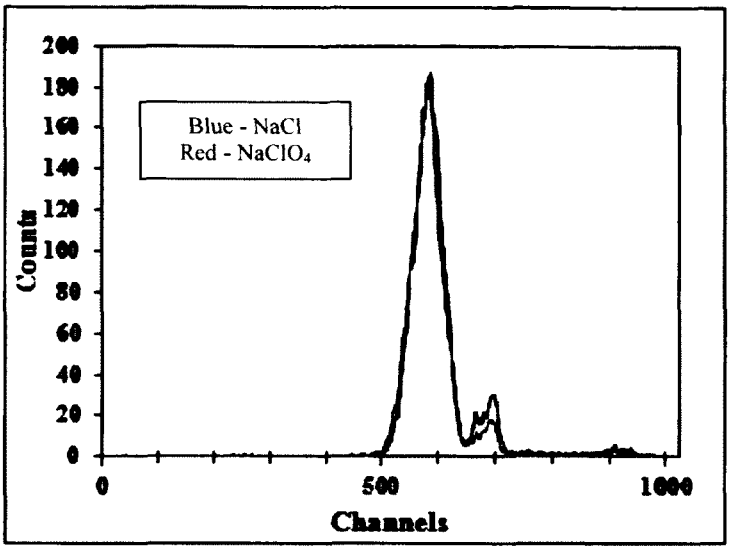

Figure 11: Alpha Spectrum for ^{226}Ra in 0.1M NaCl and 0.1M $NaClO_4$

The concentration of $NaClO_4$ was varied from 0.01M-0.5M to determine the effect of salt concentration on extraction of ^{226}Ra onto the bare MNPs at high pH (~8.5). The results (Figure 12) showed nearly quantitative extraction of ^{226}Ra (>99%) for $NaClO_4$ concentrations ranging from 0.01 to 0.1M, whereas, a linear decrease (to 80%; r^2> 0.999) in extraction of ^{226}Ra was observed as the concentration was increased from 0.1 to 0.5M.

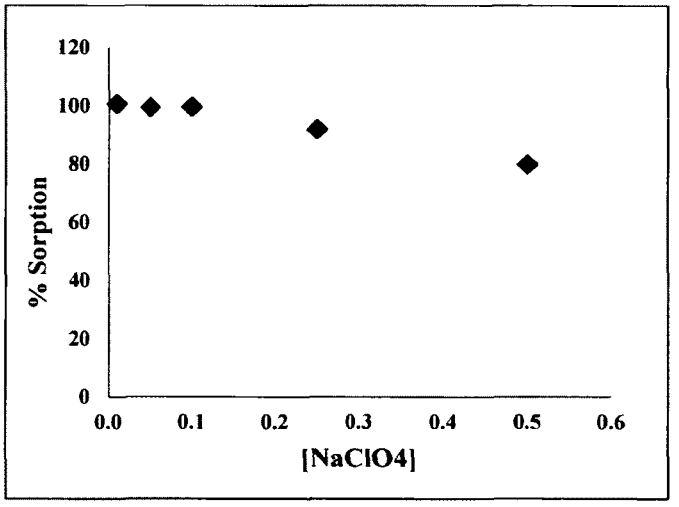

Figure 12: % Sorption of ^{226}Ra (0.2 Bq) from 20mL $NaClO_4$ solutions (0.01 M - 0.5 M) using 10mg of bare colloidal MNPs at pH 8.5

Similar sorption results (as shown in Table 3 were also obtained when 0.1M $NaClO_4$ was replaced by 0.1M NaCl and 0.1M $NaClO_4$:$Al(NO_3)_3$ (1:1). Unfortunately, $Al(NO_3)_3$, could not be tested at pH levels> 6 due to the formation of solid $Al(OH)_3$ precipitates. An inorganic salt (NaCl, $NaClO_4$, and $Al(NO_3)_3$) was added to the extraction solution to provide a counter anion that will be needed for future studies when crown ether modified MNPs will be used to study the selective uptake of ^{226}Ra. Since crown ether cavities are neutral, a counter ion is often required to allow for uptake of the metal ion into the cavity.

Table 3: % Sorption of ^{226}Ra (0.2 Bq) from 20mL of 0.1M salt solutions using 5mg of bare colloidal MNPs over a pH range 2-9

pH	salt					
	NaCl		$NaClO_4$		$NaClO_4$+$Al(NO_3)_3$ (1:1)	
	% Removal	Std. Dev.	% Removal	Std. Dev.	% Removal	Std. Dev.
2	15	6.0	11.9	0.8	19.1	6.7
3	21	4.8	24.2	3.0	18.9	4.2
4	19	14.1	20.5	4.0	13.9	5.3
5	7.0	4.8	20.8	3.2	16.7	3.9
6	31	9.2	38.7	5.9	21.5	7.1
7	51	20.0	69.8	13		
8	88	5.0	98.2	2.0		
9	95	0.0	100	0.7		

3.3.4. Effect of Sample Matrix on the LSC Signal

The alpha liquid scintillation spectra for an aliquot of a ^{226}Ra spiked sample (in presence of 0.1M $NaClO_4$) treated with MNPs at pH 4, a second aliquot of a ^{226}Ra spiked sample (in presence of 0.1M $NaClO_4$) treated with MNPs at pH 8, and a third aliquot of the spiked sample that was not treated with MNPs is shown in Figure 13.

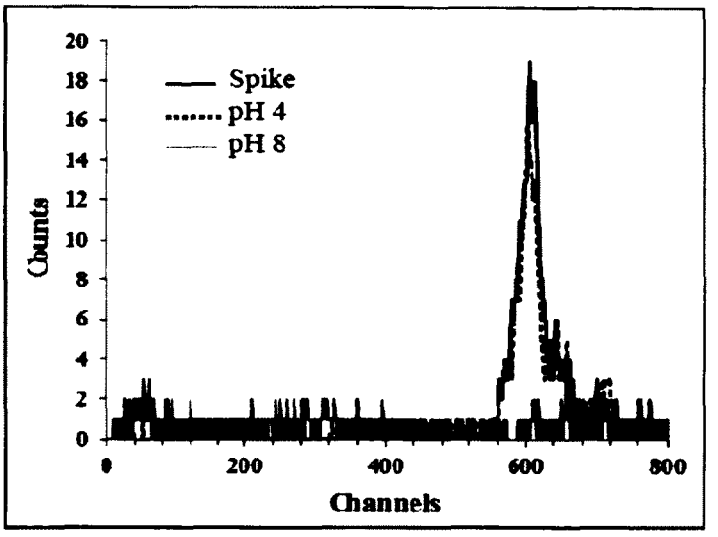

Figure 13: Alpha liquid scintillation spectrum of the supernatant after extraction of ^{226}Ra with bare MNPs from 20mL of 0.1M $NaClO_4$ spiked with 0.2 Bq (at pH 4 and 8) as compared to the spike only (no MNPs treatment)

The alpha liquid scintillation spectra in Figure 13 demonstrate the comparative extraction of ^{226}Ra at pH 4 and pH 8. In the ^{226}Ra standard, radium is in equilibrium with its daughter isotopes, ^{222}Rn, ^{218}Po, ^{214}Pb, ^{214}Bi and ^{214}Po. The alpha liquid scintillation spectrum produced by the LSC is not capable of distinguishing the ^{226}Ra peak from those of its alpha emitting daughter isotopes (especially ^{222}Rn and ^{218}Po) resulting in a broad peak (as shown in Figure 13) with up to three side peaks (corresponding to ^{222}Rn, ^{218}Po, and ^{214}Po, respectively) to the right of ^{226}Ra. As a result, calculation of extraction efficiency (as % sorption) of ^{226}Ra on bare Fe_3O_4 MNPs based on the measured activity (for a region of interest (ROI) under the major peak over 580-680 channels) and spiked activity (as described in Equation 4) will not be appropriate due to the contribution from the overlapping alpha emitting daughters of ^{226}Ra within the ROI that was not accounted for in the spiked activity.

$$\%\ Sorption = [1 - \frac{Measured\ activity}{Spiked\ activity}] \times 100\%$$

Equation 4

A better estimation of extraction efficiency (as % sorption) for ^{226}Ra on MNPs can be expressed by Equation 5:

$$\%\ Sorption = [1 - \frac{(CPS_{spike\ treated\ with\ MNPs} - CPS_{blank\ treated\ with\ MNPs})}{(CPS_{spike\ not\ treated\ with\ MNPs} - CPS_{blank\ not\ treated\ with\ MNPs})}] \times 100\%$$

Equation 5

where CPS is the counts per second.

3.3.5. Counting Efficiency:

To determine whether or not the counting efficiency[8] of the LSC analysis was affected by colour quenching due to the presence of MNPs at high and low pH an experiment was conducted in triplicate at pH 4 and pH 9 respectively. Three blank samples were prepared for each pH. 5mg of colloidal MNPs were added to each of the 6 blank samples. The samples were made up to 20mL with 0.1M $NaClO_4$ and adjusted to pH 4 or pH 9 using 5M HCl or 4M NaOH respectively. The supernatant in the pH 4 samples was brown in colour whereas the supernatant in the pH 9 samples was colourless, however, after filtration all samples were colourless. 5mL of supernatant from each of the 6 samples were transferred into 6 separate pre-weighed, pre-labeled 20mL plastic LSC vials and spiked with 0.2 Bq of ^{226}Ra. Six spiked samples were prepared by adding 0.2Bq of ^{226}Ra and 5mL of 0.1M $NaClO_4$(adjusted to pH 4 or 9 respectively) into 6 separate pre-weighed, pre-labeled 20mL plastic LSC vials. All 12 samples were analyzed by LSC for a counting time of 2 hours and the signals of the samples which were exposed to MNPs were compared to those which were not. The counting efficiency was calculated as described by Equation 6 below.

$$Counting\ Efficiency = net\ CPS/spiked\ activity$$

Equation 6

The results (shown in Table 4) indicate a counting efficiency of 99+% in the case of all samples at both high and low pH. This shows that after filtration, in samples which have been exposed to MNPs, at both high and low pH's the nature of the supernatant in comparison to a sample which has not been exposed to MNPs has no effect on the analytical signal.

[8] Counting efficiency is described as the percentage of detectable pulses generated by photons given off by radioactive emissions. It is calculated by dividing the CPM by the DPM (see Equation 1 - Extraction and Detection Methods).

Table 4: LSC counting efficiency for ^{226}Ra in 0.1M $NaClO_4$ samples exposed to 5mg of colloidal MNPs

pH	Sample	Av. C.E.	Std. Dev.
4	1	99	2.94
	2		
	3		
	1*	105	7.2
	2*		
	3*		
9	1	110.9	6.4
	2		
	3		
	1*	107.8	6.0
	2*		
	3*		

3.3.6. Decay Correction

In order to determine whether or not a sample measured at the start or a sample measured at the end of a typical LSC analysis would be affected by the decay of ^{226}Ra, an experiment was conducted using a single ^{226}Ra spiked sample. A 20mL 0.1M $NaClO_4$ sample was spiked with 0.2Bq of ^{226}Ra was measured over a period of 24 hours to determine if it would be necessary to apply a decay correction to each sample measured after the first in a large batch of solutions.

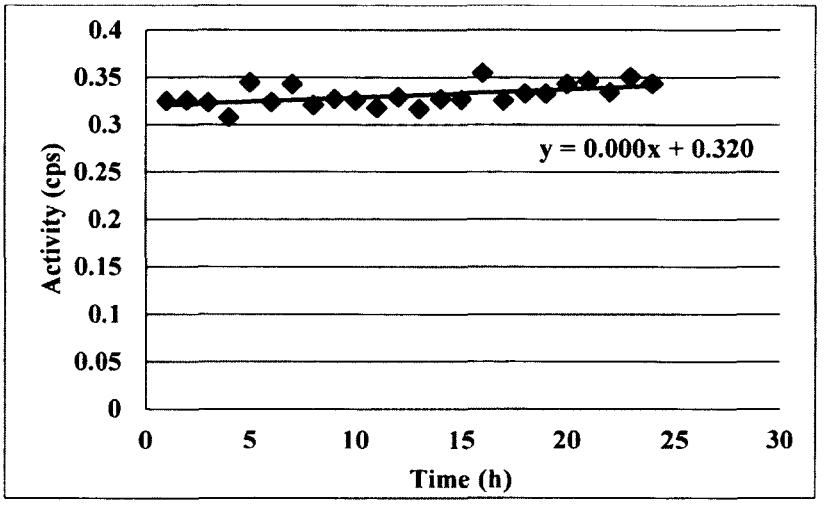

Figure 14: Decay Curve for 0.2Bq of ^{226}Ra spiked in 20mL of 0.1M $NaClO_4$ analyzed by LSC over a 24 hour counting time

As can be seen in Figure 14 above, the decay curve did not show any linear correlation between the observed activity (in counts per second) and the time elapsed (in hours) and there was no significant difference (0.014 ±0.012 cps) in measured activity between the first sample (0.303 cps) and the last sample (0.317 cps) and thus no decay correction was applied to samples analyzed by LSC.

3.3.7. Optimization of pH

The presence of hydroxyl functional groups on the surface of bare MNPs (as shown in Figure 4) allows them to behave as ion exchange media depending on the pH of the solution they are subjected to. At low pH the MNPs surface can become positively charged and can attract anions, whereas, at higher pH the surface can become negatively charged and can attract cations. Selective extraction of a cationic or anionic species on MNPs is restricted due to the generic ion exchange behaviour of the bare MNPs. pH dependent non-selective extraction of ^{210}Po, ^{226}Ra, ^{233}U, and ^{241}Am has been reported on bare Fe_3O_4 and Mn-Fe-O magnetic nanoparticles [**21**]. At a pH lower than the pK_a of the hydroxyl functional groups on MNPs, the majority of the surface should be positively charged (or neutral) and thus will not have the necessary electrostatic attraction for positively charged cationic species. This is supported by the lower extraction efficiency of the studied radionuclides at lower pH (2) when compared to that at a higher value (greater than or equal to 6) [**21**]. In order to gain further insight into the sorption of ^{226}Ra on bare Fe_3O_4 MNPs, the equilibrium pH of the solution during extraction was varied over the range of 2-9. As shown in Figure 15, a minimum sorption (≤20%) of ^{226}Ra was observed over a pH range of 2-5.

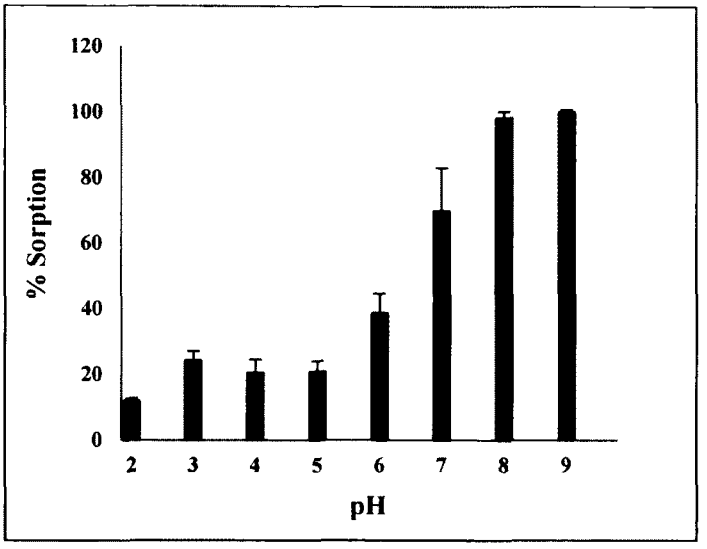

Figure 15: % Sorption of ^{226}Ra (0.2 Bq) from 20mL of 0.1M $NaClO_4$ solutions using 5mg of bare colloidal MNPs over a pH range of 2-9.

A gradual (almost linear) increase in sorption of ^{226}Ra was observed over the pH range of 6-8. Sorption of ^{226}Ra on bare MNPs was almost quantitative (~100%) at pH greater than or equal to 8. The extraction of ^{226}Ra over the pH range (2-9) presented in Figure 15 was carried out in presence of 0.1M $NaClO_4$. An interesting observation from Figure 15 was that bare Fe_3O_4 MNPs can be used as a pH tunablesolid phase substrate where radionuclides such as $^{226}Ra^{2+}$ can be extracted and pre-concentrated (at pH 9) out of an aqueous sample matrix while after matrix removal, the radionuclides can be stripped out of the MNPs at a pH range of 2-4.

4. Crown Ether Modified Magnetic Nanoparticles for the Selective Extraction of ^{226}Ra from Aqueous Solutions

4.1. Introduction

^{226}Ra is a product of the ^{238}U decay series. It is a long lived ($t_{1/2}$ = 1600 years) alpha-emitting radioactive isotope in group II of the periodic table. It forms soluble complexes with hydroxide, chloride, bromide and nitrate and is thus found in ground water along with these common species. It has higher absorption rates (~30%) in the gastrointestinal tract in comparison to other common radionuclides [**4**]. Internal exposure of radium can cause bone sarcomas and carcinomas of the perinasal sinuses and mastoid air cells (often called head cancers) [**44**]. Radium decay products, primarily radon and its short-lived daughters also have the potential to cause lung cancer. Concern for the radio-toxicity of ^{226}Ra has been reflected in a number of radiation protection guidelines and regulations, especially those established by the World Health Organization, U.S. Environmental Protection Agency, and Health Canada [**20**]. Health Canada's guideline for maximum acceptable concentration (MACs) of^{226}Ra in drinking water for human consumption is 0.5 Bq/L [**14**]. In addition to the naturally occurring radionuclides, drinking water could potentially be contaminated by radionuclides in the event of a nuclear disaster leading to harmful health effects or even death [**20**,**14**]. ^{226}Ra has been identified by the International Atomic Energy Agency as one of the radionuclides that can cause harm to human health if used in a malicious activity such as a terrorist attack using a radiological dispersion device (RDD) [**47**]. In order to monitor the ^{226}Ra radiation exposure risk along the water pathway, an analytical method with sufficient detection limit, accuracy, precision and sample turn-around time is required.

Generally, in order to analyze a sample for its radium content, sample preparation must first be performed to ensure the analyte (^{226}Ra) is efficiently separated from interferences such as barium, strontium, calcium and uranium [**47**,**7**]. Various separation techniques are available for this purpose, including solvent extraction [**48**,**49**,**50**], co-precipitation using barium sulphate or hydrous titanium oxide [**51**,**52**], solid phase extraction using MnO_2 resin [**53**,**54**] or Empore$^{(TM)}$ radium rad disks [**55**,**56**,**57**], ion exchange resins in combination with solid phase extraction [**58**,**59**,**60**], and dispersive liquid-liquid microextraction (DLLME) [**20**]. Van Leeuwen et al.

have summarized his review on different techniques available for selective extraction of naturally occurring radioactive Ra^{2+} [**61**].

Functionalized MNPs may be used for the selective preconcentration and magnetic separation of an analyte from an aqueous solution [**30,28,62,63,64,65,44**].Crown ethers have been used extensively in both liquid-liquid extraction and solid phase extraction methods for the capture of Ra^{2+} and Sr^{2+} due to their selective extraction properties [**20,44,66,67**]. Kawamura et al. showed that MNPs can be functionalized with crown ethers for use as catalysts [**63**]. Crown ether functionalized MNPs can be used for solid phase extraction to take advantage of the selectivity of the ligand and the magnetic property of MNPs for rapid and convenient separation of solid and liquid phases after extraction. In recent years, polymer coated MNPs modified with crown ethers have been investigated by our group for the removal of ^{90}Sr from aqueous samples. A method for the selective determination of ^{90}Sr in human urine samples was developed by Hrdina *et al* [**66**]. It involved coating the MNPs with a cation exchange polymer modified with crown ether. Polymer-coated MNPs were also used by Varve et al. for rapid bioassay of ^{90}Sr in urine samples [**44**].

Fe_3O_4 nanoparticles can also be coated with organic, inorganic or polymeric stabilizers to prevent aggregation and destabilization of the colloid thus increasing their shelf life. In the presence of certain ligands, the nanoparticles surface can covalently link certain species through intermolecular interactions while also passivating the surface [**28,68**].

Fe_2O_3 nanoparticles can be functionalized with silanes or siloxanes through ion exchange of -OH for SiO-M (where M is a metal atom such as Fe) on the particle surface. The reaction scheme is shown below in Figure 16 [**65**]:

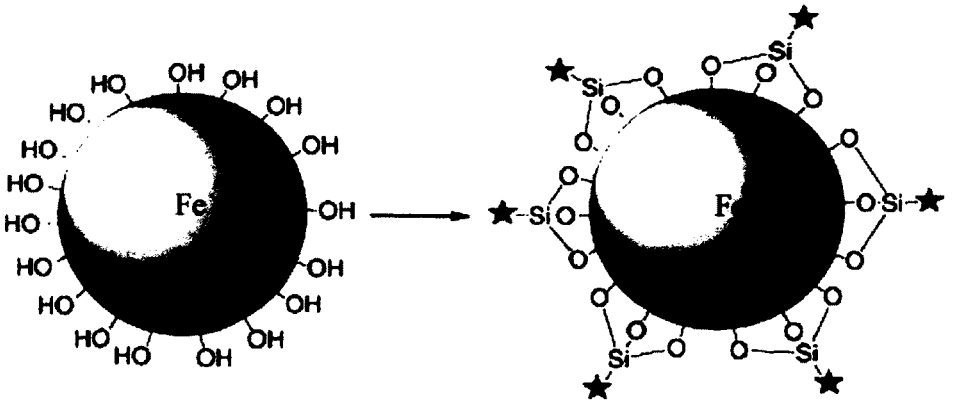

Figure 16: Silanization of bare iron oxide MNPs[9]

In general, to achieve this type of functionalization, a silane such as aminopropyltriethoxysilane (APTES) is sonicated in an organic solvent along with the nanoparticles. The mixture is heated over 24 hours and the MNPs are magnetically separated and washed to remove unbound siloxane.

In order to achieve further functionality, a ligand, such as crown ether (CE), can be added onto the MNP surface to extract the analyte selectively. CEs are cyclic polyethers which encapsulate positively charged metal ions. Within their cavities, the metal ions are solvated by oxygen atoms, forming a complex such as the $KMnO_4$·18-crown-6 complex shown below [**69**].

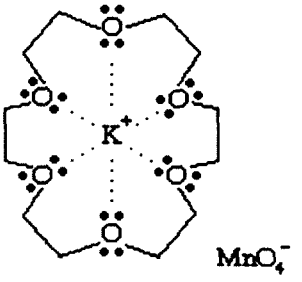

Figure 17: Structure of the KMnO4 • 18-crown-6 complex

Crown ethers are commonly used in the extraction of metal ions and radionuclides from aqueous solutions [**42,70,71,72,73,74,75,76,77**]. These molecules can be incorporated into separation columns to reduce the amount of material required. However, simpler methods, such as magnetic separation can more efficient. Fe_3O_4 nanoparticles can be modified with a crown ether to take advantage of their magnetic properties for separation as well as the selectivity of the

[9] Note: Stars represents any functional group

CE ligand [**78,75**]. This method has been used for the magnetic separation of cations such as Y^{3+} and Sr^{2+} from aqueous solutions by coating the MNPs with a polymer, impregnated with the CE ligand [**44**].

As described in the previous chapter, the bare Fe_3O_4 is known to be pH sensitive due to the presence of hydroxyl groups on the particle surface. At low pH, the surface becomes positively charged whereas at high pH, the surface hydroxyls are deprotonated allowing positively charged cations to interact electrostatically with the surface. In order to ensure that the selective properties of the crown ether are being utilized, the surface hydroxyl groups can be masked at low pH enabling the CE to selectively uptake the analyte ($^{226}Ra^{2+}$) in the presence of a counter anion to neutralize the cation charge. The mechanism for selective uptake of $^{226}Ra^{2+}$ by CE-MNPs is depicted in Figure 18 below.

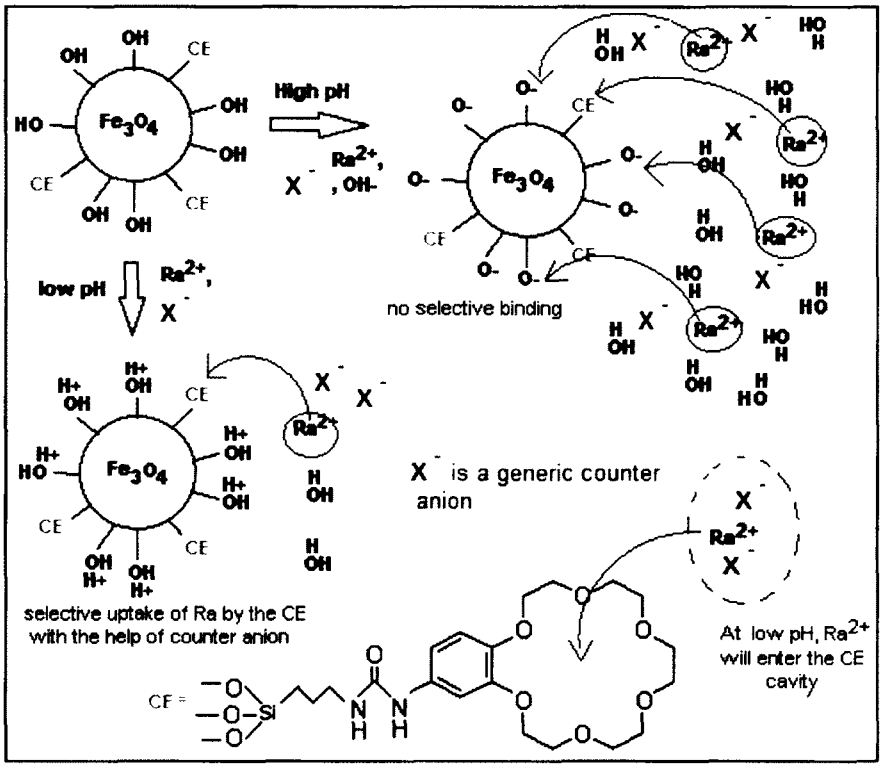

Figure 18: A schematic representation of the functional groups on MNP surfaces illustrating the non-selective (electrostatic) and selective (crown ether) binding of Ra^{2+} at high and low pH.

Since it was observed in studies with bare MNPs that the sorption of $^{226}Ra^{2+}$ onto the bare MNPs due to electrostatic attraction is minimized at pH 2-4 (see Figure 15 - Chapter 3), uptake of $^{226}Ra^{2+}$ within this pH range could be resumed if a crown ether supported MNP is used.

The binding of cations such as Ra^{2+} is mitigated by electrostatic interactions such as ion-ion or ion-dipole interactions [**79**]. Polydentate ligands such as crown ethers exhibit macrocylic effects and are thus able to bind these ions with greater affinity [**80**]. Macrocyclic ligands contain donor atoms inside their ring structures which can donate electrons to metal ions which they encapsulate [**81**]. Due to their unique structures, macrocyclic ligands tend to form more stable complexes than their noncyclic polydentate counterparts [**81**]. The macrocyclic effect states that in a reaction between a noncyclic and a cyclic chelating ligand[10], the cyclic ligand will preferentially form a complex [**81**].

Crown ethers are known to selectively bind alkali metal ions based on their size, shape and arrangement [**67,82,83**]. Another ligand commonly used to complex metal ions is ethylenediaminetetra-acetic acid (EDTA) [**38**]. Polydentate ligands form much more stable complexes than monodentate ligands. A bidentate ligand for example has a formation constant 10x greater than a monodentate ligand [**84**]. EDTA is a hexadentate ligand which forms very stable complexes with metal ions with very high formation constants. Although Ra^{2+} does not normally form ion complexes, this ligand has the potential to encapsulate Ra^{2+} as it is known to do with Ca^{2+} forming the structure shown below in Figure 19.

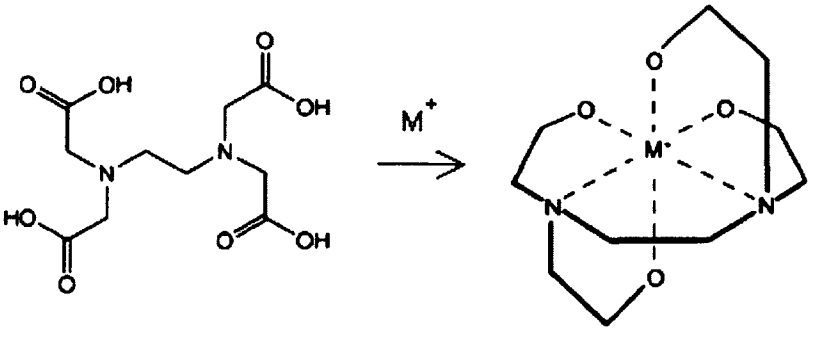

Figure 19: Complexation of a metal ion with EDTA [85]

[10] A chelating ligand is a polydentate ligand which binds a central atom through coordinate bonds formed by the donation of two electrons from a lewis base (such as oxygen or nitrogen) to a lewis acid (usually a metal ion).

In the present work, a novel application of crown-ether (18-crown-6) functionalized MNPs has been investigated for the rapid extraction of ^{226}Ra from aqueous samples. It involved synthesizing the surface functionalized MNPs, introducing them into the aqueous sample, ultrasonicating and agitating the sample, magnetically separating the MNPs from the sample, and measuring the ^{226}Ra content in the supernatant using liquid scintillation and gamma counting detection.

4.2. Materials and Methods

4.2.1. Chemical Reagents

Sodium perchlorate, sodium chloride, sodium nitrate, aluminum nitrate nonahydrate, picric acid, 4'-aminobenzo-18-crown-6, (3-isocyanatopropyl) triethoxysilane, 18-crown-6, dibenzo-21-crown-7, iron (III) chloride anhydrous, iron (II) chloride tetrahydrate, sodium hydroxide pellets, methanol, toluene anhydrous, sodium acetate trihydrate, and glacial acetic acid were purchased from Sigma Aldrich (Oakville, ON, Canada). ^{226}Ra standard was purchased from the National Institute of Standards and Technology (SRM 4965, NIST, Gaithersburg, MD, USA). Hydrochloric acid (36.5-38%) was purchased from Anachemia (Montreal, QC, Canada).Anhydrous ethyl alcohol was purchased from Commercial Alcohols Inc. (Brampton, ON, Canada). Liquid Scintillation Cocktail OPTIPHASE 'HISAFE' 3was purchased from Perkin Elmer(Massachusetts, USA).Finally, analytical grade anion exchange resin (1x8, 100-200 mesh chloride form) was purchased from Eichrom (IL, USA), and Milli Q water purification system (0.22μm, 18MΩ·cm) was obtained from Millipore (MA, USA).

4.2.2. Synthesis of Crown Ether Functionalized MNPs

Crown ether functionalized MNPs were prepared following the procedure outlined by Kawamura et al [**63**]. This is a two-step procedure in which the crown ether is attached to a silane group through a silanization reaction in the first step and the product of this reaction is covalently linked to the nanoparticle surface is the second step. The procedure is provided below.

For the silanization of the CE, 0.4257g of (3-isocyanto)propyltriethoxysilane were added to a solution of 0.4943g of 4'-aminobenzo-18-crown-6 in 10mL of toluene in a 20mL, glass liquid scintillation vial equipped with a magnetic stir bar. This mixture was heated with stirring for 12 hours at 80°C over a magnetic stir plate using a circulating water bath (HAAKE GH Fisons) with

the cap loosely screwed onto the reaction vessel. This reaction mixture was removed from the heat and kept in the fridge at 5°C overnight. The mixture was warmed to room temperature and concentrated under reduced pressure using a rotary evaporator (IKA RV0 digital) under water aspiration at approximately 40°C. The viscous, pale pink oil obtained was washed 5 times with hexane and dried in vacuo for 2 hours to give ~1.7g of the silanized crown ether reagent (a pale pink solid).

Synthesis of the CE-modified MNPs was initiated by combining a mixture of 1.3365g of bare, dry MNPs, 0.7694g of the CE reagent (as described above), 72μL of ethanol and 18mL of DDW in a 125mL, self-standing round flask. The reagents were sonicated (Branson 2510 Sonicator, Branson, Danbury, USA) for 1 minute and stirred at refluxing temperature (~78°C) for 12 hours. A 20mL, glass liquid scintillation vial containing 10mL of the solvent was equipped with a thermometer and placed aside the reaction flask to monitor the temperature of the reaction. The reaction scheme and experimental set up is shown below in Figure 20.

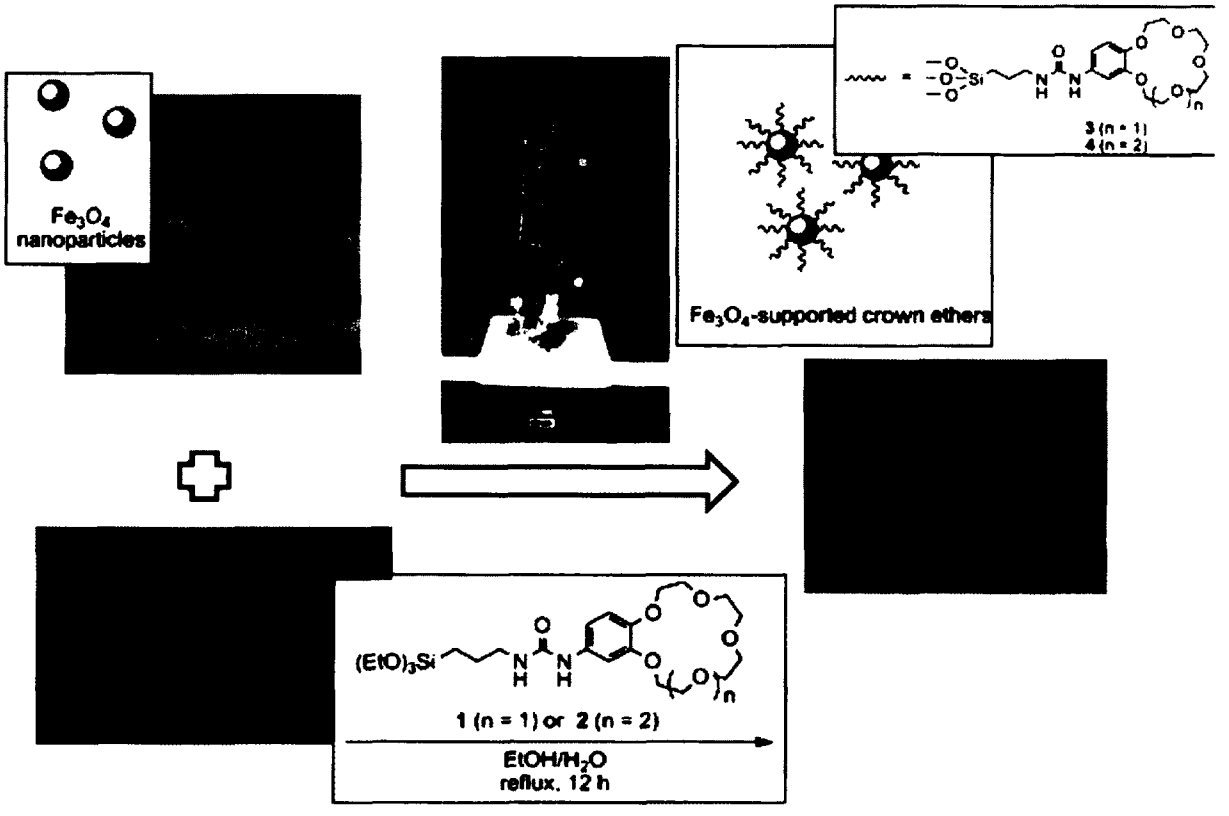

Figure 20: Reaction scheme and experimental set up for the synthesis of CE-MNPs

After the 12 hour reaction time, the sample was allowed to cool to room temperature and was dried under reduced pressure using a rotary evaporator. The particles were then washed 5 times with ethanol and stored in ~45mL of ethanol to give a colloidal suspension of 3mg of CE-modified MNPs/100μL of ethanol.

4.2.3. Characterization of CE-functionalized MNPs

Based on nitrogen analysis data given by their magnetically supported crown ethers, Kawamura et.al. concluded that there was approximately 6% CE surface coverage on their MNPs [**63**]. To ensure that the crown ether was actually bound to the MNP an FTIR spectrum of the 4'-aminobenzo-18-crown-6 reagent, the bare MNPs and the coated MNPs was taken and the three were compared. 1.0-3.0 mg of each substance were combined with 2 scoops of KBr to form the KBr pellet for analysis.

4.2.4. Binding Test of CE onto Bare MNPs

To test the binding capacity of the crown ether onto the bare MNPs directly without the need for a covalent siloxane linkage, an HPLC experiment was performed using a Gilson 115 UV Detector coupled with a Shimadzu LC-6A Liquid Chromatograph using a CE and a MeOH/H_2O/Acetonitrile (1:1:1) mobile phase. A 1mL CE standard solution with a concentration of 100ppm was measured by HPLC and exposed to 5mg of bare dry MNPs. The sample was then sonicated for 3 minutes and the MNPs were magnetically separated. The supernatant was measured by HPLC to determine the %CE bound to the MNPs. The sample was then left to react for 24 hours and the supernatant was re-measured to determine if a longer reaction time would result in further binding.

4.2.5. Extraction of ^{226}Ra using CE-functionalized MNP Suspension

The extraction of ^{226}Ra using CE functionalized MNPs was carried out in pre-weighed, pre-labeled, 20mL, glass liquid scintillation vials. Each vial (except the blank) was spiked with 15Bq of standard ^{226}Ra (using a 7.26Bq/g of ^{226}Ra intermediate standard solution prepared from NIST SRM 4965) using a 500-5000μL variable automatic micropipette (Eppendorf, USA). The mass of ^{226}Ra standard spiked into each vial was measured on an analytical balance and recorded. The volume was made up to 15mL using the appropriate salt solution using deionized water and sodium perchlorate or picric acid salt to yield concentrations of 0.1M or 0.01M respectively in a

final volume of 20mL. The pH of each solution was then adjusted to ~4. After pH adjustment, a known mass of colloidal MNPs suspended in ethanol (suspension containing 25mg of MNPs yielded optimal results) was transferred to each sample excluding the spike (which would be used to compare the extraction efficiency of CE functionalized MNPS) using a 100-1000μL variable automatic micropipette. An equal volume of ethanol was added to the spike solution to ensure its matrix composition was identical to the samples which were treated with CE functionalized MNPs. Finally, the volume of each sample was made up to 20mL using milli Q water (18MΩ.cm), the equilibrium pH of the solution was verified once again (it remained between 3.5 and 4.5) and the mass of each sample was recorded. The samples were ultrasonicated for 30 minutes and magnetically separated as above. 15mL of supernatant solution from each sample were transferred through a 0.22μm PES syringe filter using a 5mL syringe into its own pre-weighed, pre-labeled, 20mL, glass liquid scintillation vial and the mass of supernatant liquid was recorded. Each sample was analyzed using a Gamma spectrometric system (LS-1116 Gamma Spectrometer (with a hyper pure germanium detector), Putnam Technology Inc., GA, USA) for ^{226}Ra's 186 keV gamma peak using a counting time of 2 hours per sample.

4.2.6. Stripping of ^{226}Ra from CE-functionalized MNPs

A desorption experiment was performed to strip the extracted ^{226}Ra from the MNPs. This was done by removing the supernatant from atop the MNPs and leaching the ^{226}Ra-containing MNPs consecutively (five times) with 5mL portions of a 0.1M sodium acetate/acetic acid buffer solution at pH 4. Once the leaching buffer was added to the CE-modified MNPs, they were ultrasonicated for 10 minutes and magnetically separated before collecting the supernatant with a 5mL syringe. Figure 21 below shows the general procedure by which the particles were stripped using the buffer solution.

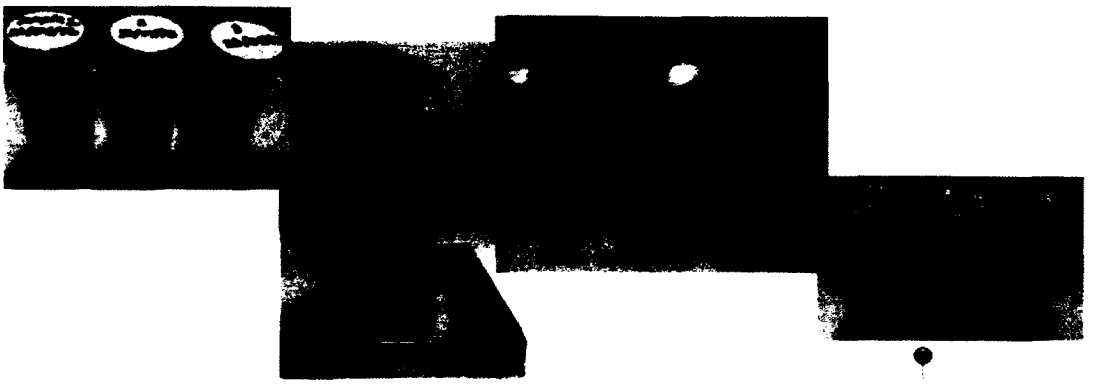

Figure 21: Stripping procedure for recovery of extracted ^{226}Ra from CE-modified MNPs

The supernatant was then transferred, through a 0.22μm PES syringe filter, onto an anion exchange column. After collection of the load solution from the anion exchange column, the column was washed 3 times using 5mL of the buffer solution and each wash was collected. The anion exchange column was prepared by forming a slurry of approximately 1 gram of anion exchange resin (Eichrom analytical grade anion exchange resin) with 5mL of 0.1M HCl and introducing it to an empty 20mL column (Eichrom, Part No. AC-20E-20M, IL, USA). The column was preconditioned with 10 mL of acetate buffer until the eluate was pH 4. Each 5mL fraction from the column was collected in a separate pre-weighed, pre-labeled, 20mL glass LS vial, combined with 15mL of LS cocktail, vortexed until homogeneous and analyzed for ^{226}Ra content using a Hidex 300 SL automatic TDCR liquid scintillation counter (LSC) with alpha/beta separation option (Gamble Technologies, Mississauga, ON, Canada) for a counting time of 2 hours per sample. The eluate was analyzed at various time periods (0, 7, 14, 21 and 28 days) to investigate the growth of radium's daughter peaks over time.

4.2.7. Preliminary Testing in Biological Matrices

Although the current method has been optimized for the removal of ^{226}Ra from water samples with an extraction efficiency of 94±1%, further testing in the presence of more complex matrices such as urine are proving to be more challenging. These types of matrices may contain further interferences such as Ca^{2+}, K^{+} and other cations which may compete with the analyte for a position within the CE cavity. Tests for removal of ^{226}Ra using CE-modified MNPs in urine are currently underway. Unfortunately, due to the intense colouration of the picric acid ion and the low CE surface coverage on the current MNPs, experiments are proving to be expensive and time consuming.

For urine testing, all samples were analyzed using the LS-1116 Gamma Spectrometer (Putnam Technology Inc., GA, USA) rather than LSC due to the coloured nature of the supernatant. Each sample was analyzed for a counting time of 2 hours.

4.3. Results and Discussion

To validate the extraction efficiency results using LSC, a number of extraction experiments were repeated where the measurement of ^{226}Ra was carried out using gamma spectroscopy with a counting time of 2 hours per sample. Due to the low relative intensity (3.6%) of the characteristic gamma peak of ^{226}Ra at 186.21 keV, the samples were spiked with a higher activity (15Bq of ^{226}Ra) in order to acquire a satisfactory signal to noise ratio. The extraction results obtained by gamma spectroscopic measurements were similar to those obtained with LSC measurements. However, due to the intense colouration of the picrate counter ion, in order to investigate selective uptake of ^{226}Ra using CE-functionalized MNPs, gamma spectroscopy was chosen for the measurement of ^{226}Ra.

4.3.1. Crown Ether Choice

21-crown-7 and 18-crown-6 were tested through a typical DLLME experiment [**20**] for the extraction of ^{226}Ra from the aqueous phase (0.1M $NaClO_4$ + 0.02M sodium citrate) into the organic phase (2-thionylfluoroacetone + toluene + CE) and the extractant was analyzed using LSC detection. 21-crown-7 and 18-crown-6 gave almost identical extraction efficiencies of 95±1% and 94±1% respectively. The alpha spectra for both crown ethers exhibited a clean, sharp peak with little to no interference from ^{226}Ra's daughter progenies (see Figure 22 below).

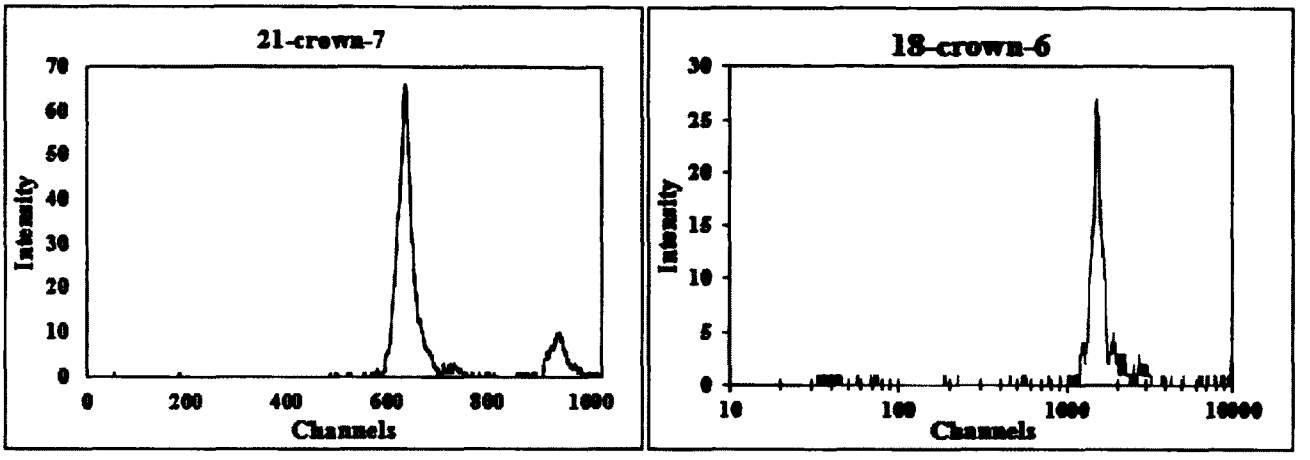

Figure 22: Alpha LSC spectrum for 20mL aqueous samples spiked with ^{226}Ra extracted using 21-crown-7 (right) and 18-crown-6 (left) by DLLME

Although the 21-crown-7 was cheaper than the 18-crown-6, only one method of synthesis was found in the literature for direct binding of the later onto bare MNPs [**63**]. Therefore 18-crown-6 was chosen over 21-crown-7 despite its higher cost.

4.3.2. Characterization of CE-functionalized MNPs

In Figure 23, 3 peaks (outlined in purple dotted boxes) were found to be characteristic of the nanoparticles as they appeared in both the bare and coated MNPs spectra but not in the crown ether spectrum. These peaks were located at 3398 cm^{-1}, 2908 cm^{-1}, and 2869 cm^{-1}.

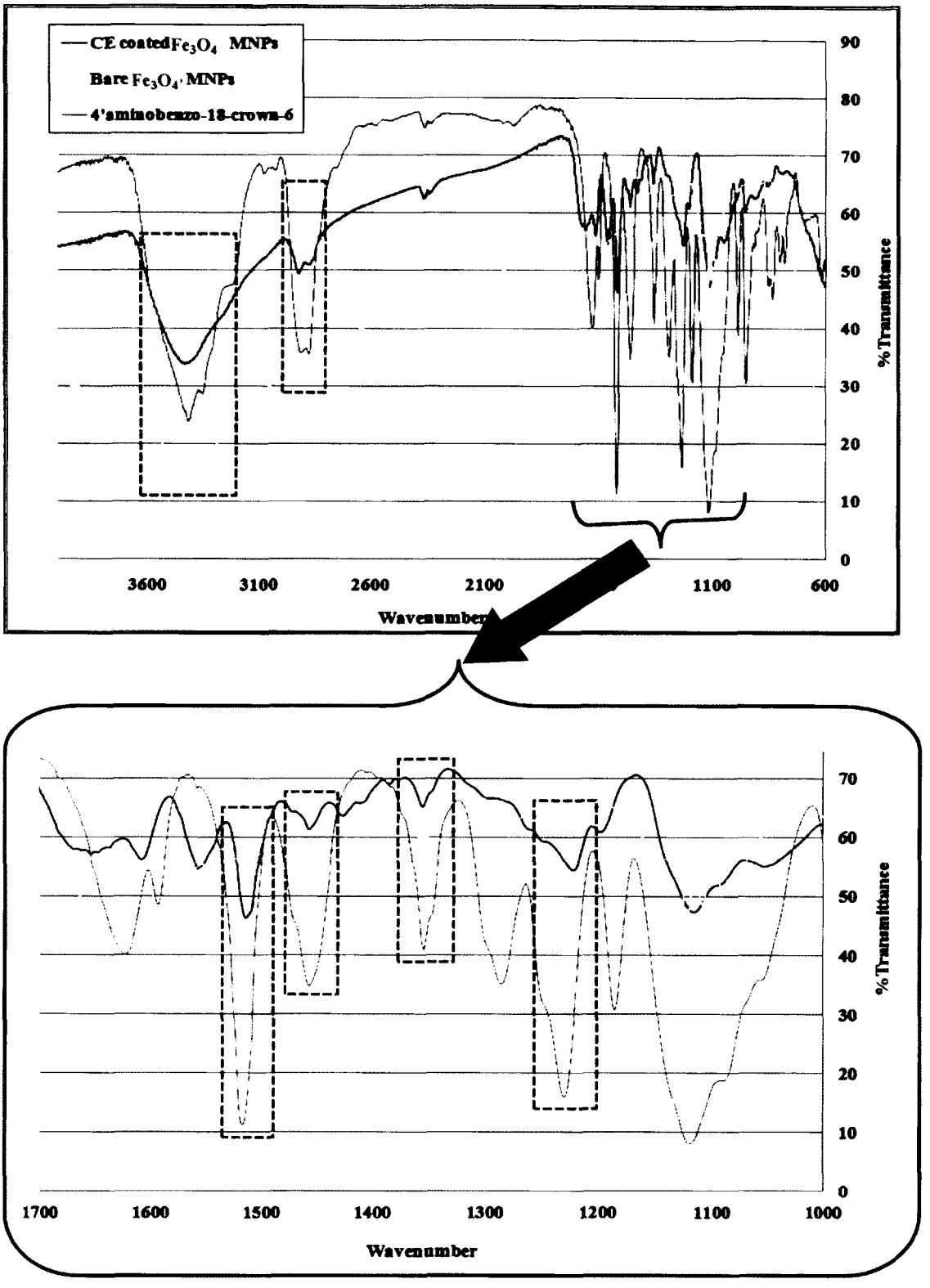

Figure 23: FTIR spectrum for bare and CE-modified MNPs and the 4'aminobenzo-18-crown-6 reagent

Similarly, 4 peaks were found to be common between the 4'-aminobenzo-18-crown-6 and the CE-MNP spectra. These peaks (outlined in red dotted boxes in Figure 23), found at 1518 cm^{-1}, 1458cm^{-1}, 1352 cm^{-1} and 1230 cm^{-1}, represent an N-H bend (1640-1500 cm^{-1}), a CH_2 bend (~1465 cm^{-1}), a C-N (1350-1000 cm^{-1}), and a C-O-C dialkyl stretch (1300-1000 cm^{-1}) respectively [**86**]. This data shows that the crown ether is in fact bound to the MNP surface.

4.3.3. Binding Test

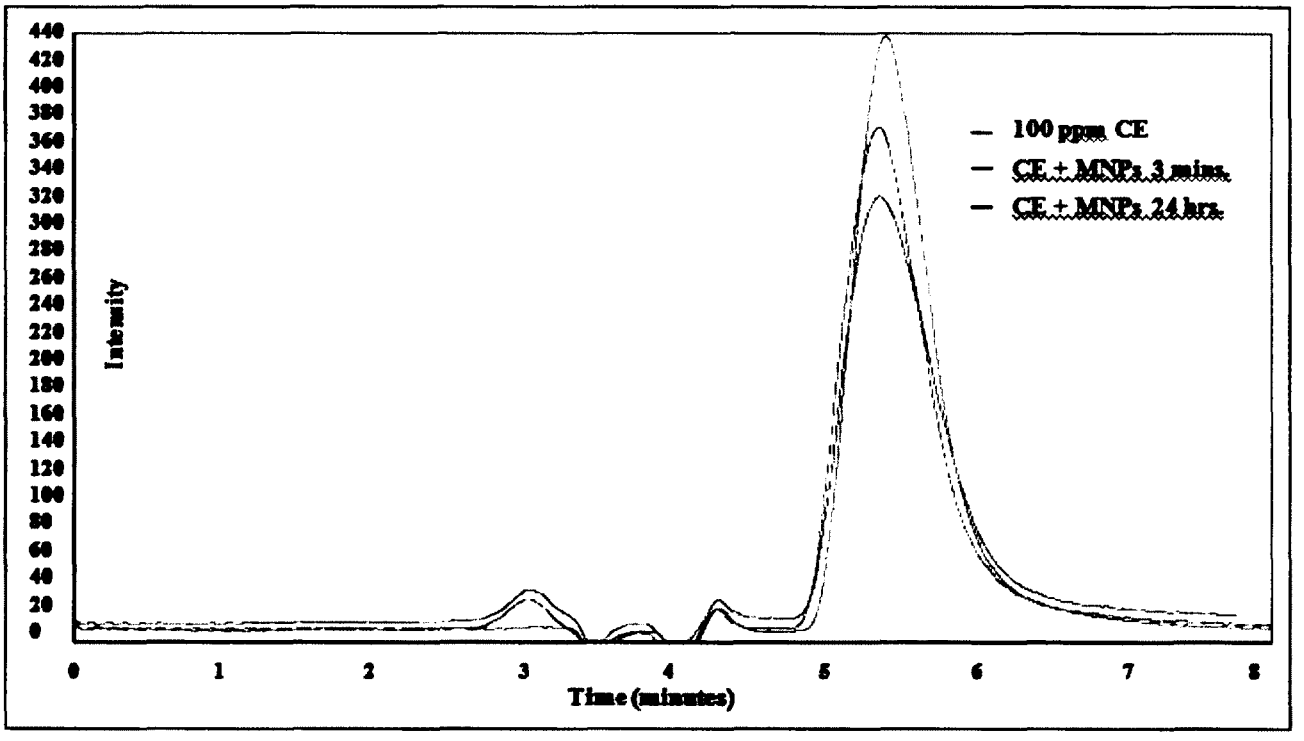

Figure 24: HPLC binding test for 5mg of bare dry MNPs exposed to a 100ppm solution of CE

The results in Figure 24 show a decrease in the peak height for the sample solutions which were exposed to MNPs. By dividing the peak area of the sample solution by the peak area of the standard CE solution and multiplying it by 100%, the amount of CE remaining in solution can be calculated. The %CE bound onto the MNPs can then be determined by subtracting the extracted amount from 100%according to Equation 7 below. Interestingly 9.4% and 10% of CE were bound to the bare MNPs after 3 minutes and 24 hours, respectively.

$$\%CE\ bound = 100\% - \frac{sample\ peak\ area}{standard\ peak\ area} \times 100\%$$

Equation 7

The minor increase in binding (<1%) after a long reaction time of 24 hours indicates that 3 minutes is adequate for adsorption of the CE to functionalize the MNP surface. Since a large batch of CE-functionalized MNPs had already been synthesized via the procedure described in section 4.2.2, in depth investigation of this method was not continued. Further investigation of the strength of this bond (formed by simple physical adsorption) could be beneficial for future research as the current synthesis method is quite time consuming.

4.3.4. Extraction of ^{226}Ra using CE-functionalized MNPs

Before beginning tests with gamma spectroscopic detection, a calibration plot (Figure 25 below) was created to determine the suitable spiking activity and linear dynamic range for quantitative measurement of ^{226}Ra based on the 186.21 keV gamma peak.

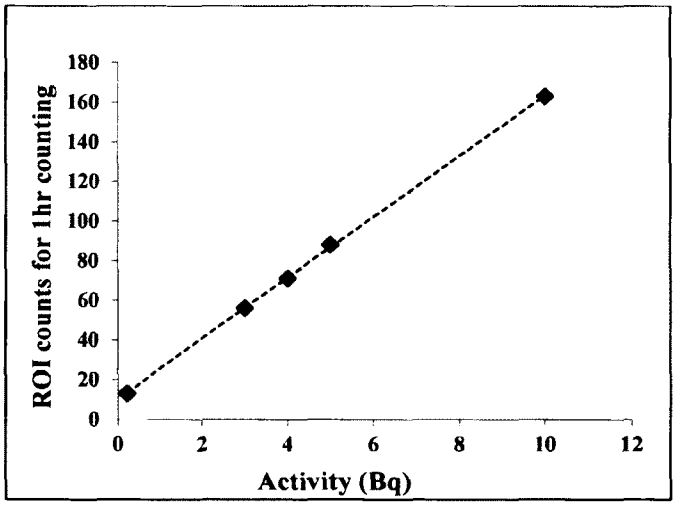

Figure 25: Calibration plot for the counts corresponding to the 186.21 keV gamma peak with increasing ^{226}Ra activity

^{226}Ra activities of 0.2Bq, 3Bq, 4Bq, 5Bq and 10Bq were tested and a linear relationship between the activity and counts per hour with a correlation coefficient of $r^2 = 0.9998$ was obtained.

For CE functionalized MNPs, the extraction conditions (0.1M $NaClO_4$ and pH 4) were chosen, so that, any sorption of ^{226}Ra (and its daughter products) on the MNP surface due to electrostatic attraction (by the residual hydroxyl functional groups after CE modification) was minimized. However, no uptake of ^{226}Ra was observed on the MNPs under this condition using 100mg of CE functionalized MNPs. This indicated that the necessary condition for crown ether

to form a complex with ^{226}Ra has not been satisfied. In order to increase the concentration of counter ion (perchlorate), the concentration of $NaClO_4$ was increased to 0.5M. ^{226}Ra has been reported to be extracted on Sr resin (a Sr selective extraction chromatographic resin from Eichrom Technologies LLC, Lisle, IL, USA) using a loading solution of ^{226}Ra containing 0.5M perchlorate (as perchloric acid) [**87**]. The extractant system in Eichrom's Sr resin is 1.0M 4,4'(5')-di-t-butylcyclohexano 18-crown-6 in 1-octanol. A 40% (w/w) loading of this organic solution is loaded onto an inert chromatographic support to prepare the extraction chromatographic resin. An increase in concentration of perchlorate ion from 0.1M to 0.5M did not provide any sorption of ^{226}Ra on our CE functionalized MNPs. A 3M solution of nitrate as $NaNO_3$ (3M HNO_3 is typically used for loading onto Eichrom's Sr resin) was also tested but no sorption of ^{226}Ra was observed under this condition either. The acid forms of perchlorate (perchloric acid) and nitrate (nitric acid) were not used as they start to decompose the MNPs under highly acidic ($pH \leq 1$) conditions. Drastic loss of ^{90}Sr extraction on the Sr resin in presence of high concentration of Na^+ (>0.4M), NH_4^+ (> 0.2M) and K^+ (>0.03M) has been reported [**88**]. While 0.1M $NaClO_4$ could not provide adequate concentration of perchlorate ion (0.5M as used by McAlister et al.), the presence of higher concentration of Na^+ (3M for $NaNO_3$ and 0.5M $NaClO_4$) in the solution could be preventing the extraction of ^{226}Ra on CE functionalized MNPs. Selective liquid-liquid extraction of strontium using crown ether has been reported in presence of picric acid [**89**]. As shown in Figure 26, when picric acid (0.01M) was used in deionized water (pH 4) a significant sorption (> 80%) of ^{226}Ra on to the CE functionalized MNPs was observed in comparison with the bare MNPs (~13%) under the same conditions.

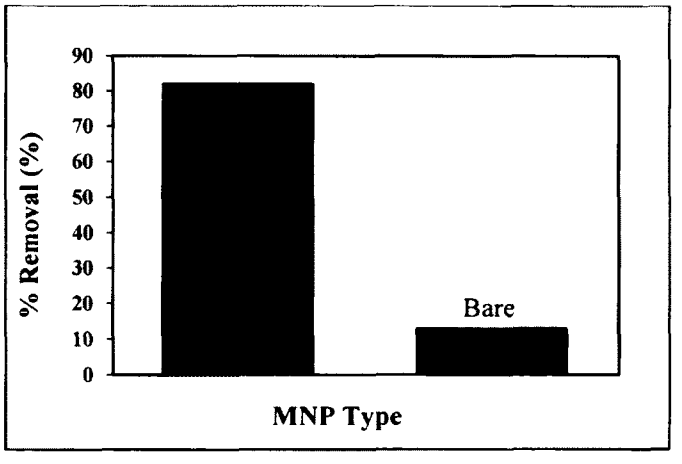

Figure 26: Comparison of the extraction efficiency using bare and CE-modified MNPs for ^{226}Ra

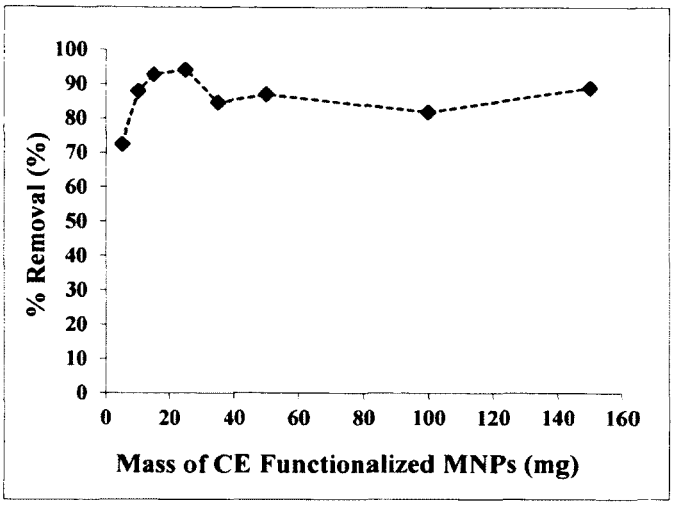

Figure 27: Optimization of the mass of CE-modified MNPs for ^{226}Ra extraction

In order to further optimize the extraction conditions, the mass of CE functionalized MNPs and the concentration of picric acid were investigated. Masses of 5mg, 10mg, 15mg, 25mg, 35mg, 50mg, 100mg and 150mg and picric acid concentrations of 0.005M, 0.01M, 0.015M, 0.02M and 0.03M were tested. As shown in Figure 27, 25mg of CE functionalized MNPs provided maximum sorption (~94%) for ^{226}Ra when 0.01M picric acid was used. When the concentration of picric acid was varied from 0.00M to 0.03M, maximum sorption of ^{226}Ra (~94%) was observed at 0.01M using 25mg of the CE functionalized MNPs (see Figure 28).

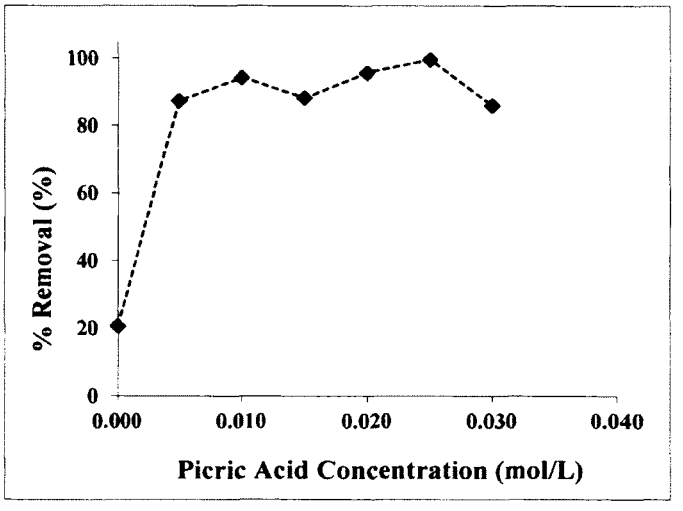

Figure 28: Optimization of concentration of picric acid for ^{226}Ra extraction

4.3.5. Stripping of ^{226}Ra from CE-functionalized MNPs

To verify that after treatment with CE functionalized MNPs the depletion of ^{226}Ra in the supernatant (measured via gamma spectroscopy) was actually due to its sorption on to the MNPs, the MNPs were sequentially leached using a pH 4 sodium acetate/acetic acid buffer solution to strip out the extracted ^{226}Ra. Introduction of this buffer solution would decrease the concentration of picric acid disabling the CE chemistry and releasing^{226}Ra from the particles into the buffer. The buffer (at pH 4) would also prevent further sorption of ^{226}Ra on to the MNPs due to the electrostatic attraction that was observed for the bare MNPs at elevated pH levels ($\geq$6).

LSC was used for the measurement of ^{226}Ra in the leach buffer that was used to strip the ^{226}Ra from CE-modified MNPs. LSC was chosen (over alpha or gamma spectroscopy) as the detection technique to avoid additional alpha spectroscopic source preparation (through micro-precipitation or electro-deposition) and to avoid lengthy counting time associated with both techniques. Unfortunately, due to the intense colouration presented by the picrate anion in solution, the ^{226}Ra activity could not be measure directly using LSC. To decolourize the solutions before analysis, an anion exchange resin was used to remove the picrate ion. The solutions were transferred onto the column through a syringe filter to avoid introducing any stray MNPs into the solution to be measured. A sample of CE- functionalize MNPs which had been subjected to a 0.01M picric acid solution (pH 4), containing 15Bq of ^{226}Ra was used for this investigation. The resulting supernatant was removed and analyzed using the gamma spectroscopic method showing a removal of 94±1% of the original spiked ^{226}Ra. The remaining MNPs were leached 5 times using a pH 4 acetate buffer (0.1M) solution (as described above) and each of the 5 supernatant solutions collected were passed through an anion exchange column which was then stripped using 3 consecutive 5mL portions of the same buffer solution. The results (see Figure 29) indicate that most of the ^{226}Ra is leached out in the first 2 leaches and a total of 5 leaches are necessary to recover all the ^{226}Ra present in the particles.

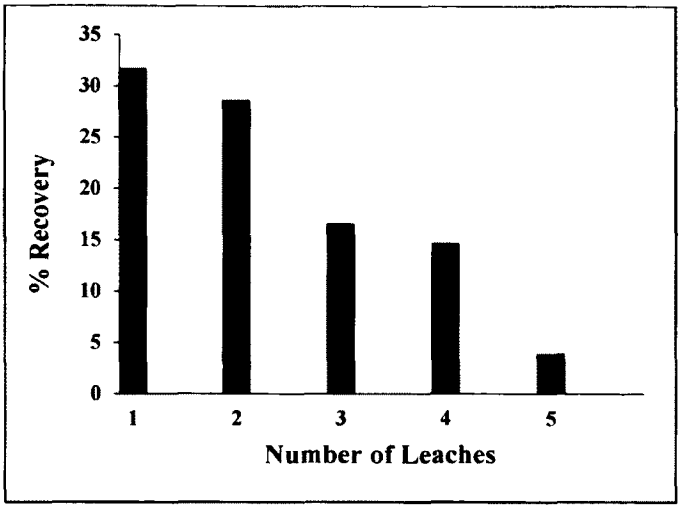

Figure 29: Desorption of ^{226}Ra from CE-MNPs using pH 4 sodium acetate/acetic acid buffer

The total recovery obtained from five consecutive leaches was found to be 95±1% confirming the results presented by the loss of activity in the supernatant as determined by gamma detection.

To verify the selectivity of the CE-modified MNPs for ^{226}Ra from its daughter progenies, a sample from the above desorption test (leach number 1) was measured immediately after leaching and again at 7, 14, 21 and 28 days of storage to observe the growth of radium's daughter peaks over time and illustrate their absence initially. The results (shown in Figure 30) demonstrate a sharp peak in the sample solution directly after leaching with no side peaks indicating that only radium is present in the sample.

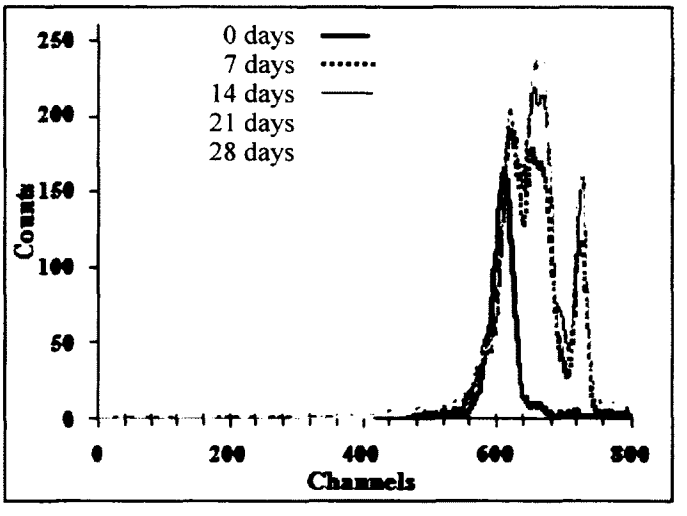

Figure 30: Selective extraction of ^{226}Ra from deionized water using CE-MNPs in the presence of 0.01M picric acid at pH 4 with LSC detection

In comparing this peak to that in a spike sample which has not been exposed to any MNPs (see Figure 13- Chapter 3), the selectivity of our CE-functionalized MNPs for ^{226}Ra in the presence of its daughters is confirmed in the alpha spectrum. Subsequent measurements over time reveal the growth of radium's daughter progenies to the right of the initial peak.

4.3.6. Preliminary Tests in the Urine Matrix

Results (shown in Table 5) indicate that in the more complex urine matrix, with so many interferences, a much higher mass of MNPs is required in order to obtain comparable results to those obtained in water samples. Increasing the mass of CE-MNPs from 100mg-500mg resulted in a ~40% increase in extraction efficiency for removal of ^{226}Ra from a 50mL urine sample, yielding an overall extraction of 102±1% in the presence of 0.01M picric acid at pH 4.

Table 5: Extraction of ^{226}Ra from 50mL urine samples using CE-modified MNPs

0.05	100.0	49.85
0.01	100.0	38.30
0.01	250.0	20.28
0.01	500.0	-1.79

To ensure that it was not the counter ion concentration that was preventing ^{226}Ra uptake into the CE cavity, the concentration of picric acid was increased from 0.01M to 0.05M using 100mg of CE-MNPs for extraction. The results (see Table 5) showed an ~11% decrease in extraction at the higher concentration.

5. Conclusion and Future Directions

Radioisotopes such as ^{226}Ra are often naturally occurring in the environment and technically exploited in industry. Radionuclear terrorism, warfare and accidents are the main concern to the Canadian government and people as exposure to their harmful radiation can lead to severe health effects (such as cancer) and even death. On these grounds, the development of a rapid analytical method based on the improvement of current technologies is highly desirable in order to monitor radioactivity levels in the environment and identify victims requiring immediate treatment.

Ideally, a combination of methods could be used for the extraction of ^{226}Ra from environmental or biological fluids. Ion exchange chromatography (IC) would eliminate any interfering ions such as Ba^{2+} or Ca^{2+}, resulting in decreased sample complexity and chelation could then be employed in a DLLME process to selectively extract the radium from the eluate fraction [**19,7**]. Similarly SPE could be used to exploit ligand-modified MNPs to selectively extract radium from the eluate. In order to obtain selectivity for a specific radionuclide such as ^{226}Ra, MNPs could be potentially modified using a crown ether for removal of the analyte [**67,13,20,90**].

For ^{226}Ra determination, LSC would allow for the most rapid analysis with the lowest sample volumes. The use of CE-modified MNPs for SPE of ^{226}Ra from aqueous samples would minimize the volume of radioactive and organic waste produced, eliminate the need for added sample preparation steps and provide a simple physical separation for removal of ^{226}Ra from solution [**21**].

Since at a high pH, negatively charged O^- groups would form on the bare MNP surface (see Figure 4), the non-specific binding of $^{226}Ra$and other positively-charged ions would be strong (due to coulombic attraction) [**21**]. For this reason, the reaction (conducted using crown ether modified MNPs) would be best performed at lower pHs in the presence of a counter ion to allow for the selective uptake of $^{226}Ra^{2+}$ into the crown ether cavity

Further investigation of this hypothesis and optimization of experimental parameters (such as counter ion type and concentration, pH, amount of MNPs, ligand concentration, spiking amount and choice of detection method) are necessary before a procedure can be established and validated for use in the selective extraction of ^{226}Ra from aqueous samples for accurate quantitation.

For quantitation of the analyte, a combination of LSC and γ-spectroscopy is desirable. Large scale analyses for parameter optimization during preliminary testing would be best performed using LSC as it allows for the successive analysis of multiple samples in very little time **[91,13]**. For small-scale method validation experiments, verification of results (through discrimination of the radium peak from its daughters), and investigation of more complex matrices such as urine or other biological fluids, gamma spectroscopy would be preferential, as it offers specificity and the ability to analyze unprocessed samples with very little background interference and low cost of analysis **[13]**.

For the analysis of ^{226}Ra, during preliminary testing or for a large number of samples, LSC would be best suited, as it allows for analysis of multiple samples sequentially and provides rapid, precise measurement with high sensitivity and selectivity **[91,13]**.

Alpha Spectroscopy offers high resolution, precision, specificity and sensitivity (100 times better than LSC) and would be preferable for the detection of ultra trace amounts of analyte. Both LSC and γ-spectroscopy require time consuming sample preconcentration and purification steps before samples can be analyzed **[7,13]**.

For more robust analyses, without the need for extensive sample preparation techniques, γ-spectroscopy is the most viable option **[13]**. It is a good tool for validating results obtained through LSC or α-spectroscopy measurements and can be combined with ICP-MS for more specificity for ^{226}Ra analysis. Although γ-spectroscopy analysis times are longer than those for other techniques and only one sample at a time can be assessed, direct measurement with little interference and low cost is possible. Furthermore, the added benefit of observing the activity of daughter isotopes (such as ^{222}Rn - see Figure 1) as well as the parent (^{226}Ra) produces more specific results.

For removal of ^{226}Ra from water samples, bare MNPs were tested under various experimental conditions. The colloidal particle state was chosen as opposed to using dry particles due to the increased stability of the particles in sample solutions, the augmented consistency in experimental results, the more efficient separation of the particles from the sample and the colourless nature of their supernatant solution. Syringe filters were shown to effectively remove unwanted analytical interferences in samples to be analyzed using LSC detection. The effect of radioactive decay of ^{226}Ra over a typical experimental analysis time had little to no effect on the detector signal and the counting efficiency of samples exposed to MNPs in comparison to MNP-

free solutions was greater than 99% exhibiting no loss of efficiency at low pH (4) or high pH (9). At lower pH, ^{226}Ra was not absorbed onto the bare MNPs surface, however, at higher pH, an extraction efficiency of ~100% was achieved in the presence of 0.1M $NaClO_4$ using only 5mg of MNPs. $NaClO_4$ was chosen at a concentration 0.1M due to its higher extraction efficiency in comparison to other salts tested along with the clean, sharp nature of the ^{226}Ra peak in the alpha spectrum at this concentration. Under optimal experimental conditions, ^{226}Ra can be extracted from water samples using bare colloidal MNPs almost quantitatively and determined using LSC detection. For selective removal of ^{226}Ra from water samples, further testing could be conducted at the lower pH range using ligand modified MNPs to allow for deactivation of the bare MNP chemistry, enabling ligand-analyte interactions.

The use of a selective extraction and preconcentration using CE-modified MNPs with LSC detection would allow a rapid radioanalytical method for measurement of ^{226}Ra. The use of CE-modified MNPs for SPE of ^{226}Ra from aqueous samples would minimize the volume of radioactive, inorganic and organic waste produced, eliminate the need for initial purification steps and provide a simple physical separation for removal of ^{226}Ra from solution [**21**].

Quantitative removal of ^{226}Ra using bare MNPs at higher pH (8-9) indicates that MNPs could also be used for decontamination of multiple positively charged radionuclides. Since at higher pH, deprotonation of hydroxyl (-OH) functional groups results in negatively charged O^- groups on the bare MNP surface (Figure 18), $^{226}Ra^{2+}$and other positively-charged ions would non-selectively be extracted by the MNPs due to electrostatic attraction [**21**]. For this reason, the reaction (conducted using crown ether modified MNPs)would be best performed at lower pHs ($\leq$ 4) in the presence of a counter ion (anion) to neutralize the positive charge on radium and allow for the selective uptake of $^{226}Ra^{2+}$ into the crown ether cavity. An extraction efficiency of 94±1%was obtained using 0.01 M picric acid at pH 4 with 25mg of CE-modified MNPs. After extraction of ^{226}Ra on to CE-modified MNPs, 5 consecutive leaches of the nanoparticles (with 5mL of 0.1M acetate buffer at pH 4) were used to strip the radionuclide. An anion exchange column was used to decolourize the leach buffer (by removing the picrate anion) after striping ^{226}Ra from CE-modified MNPs. A recovery of 95±1% was obtained from the stripping of ^{226}Ra from CE-modified MNPs. This value confirms the extraction efficiency determined indirectly using gamma spectroscopy (94±1%) to measure the activity in the supernatant solution under the same experimental conditions. The selectivity of the CE functionalized MNPs was confirmed

using LSC detection by measuring the eluate obtained from the first leach in the desorption experiment which demonstrated a single sharp peak in the region of interest representing the ^{226}Ra isotope. This sample was re-measured after 7, 14, 21 and 28 days exhibiting daughter peak growth over time. Further method development is underway to apply the CE-modified MNPs for determination of ^{226}Ra from drinking water and urine samples.

Due to the selective nature of the ligand, CE-modified MNPs have the potential for selective removal of ^{226}Ra in the presence of interfering radionuclides such as ^{235}U, ^{90}Sr and ^{241}Am. Due to the α/β discrimination capability of the current LSC detection technique, even very close fitting ions such as $^{90}Sr^{2+}$ which are known to be taken up by the 18-crown-6 ligand chosen for removal of $^{226}Ra^{2+}$ in the current research project, can be determined separately through detection rather than extraction. This would allow for the simultaneous determination of both radionuclides.

Using 500 mg of CE-functionalized MNPs in the presence of 0.01M picric acid, 15 Bq of ^{226}Ra spiked into a 50 mL urine sample were quantitatively removed with a 102±1% extraction efficiency. Further optimization in the urine matrix and exploration of other matrices such a cerebral spinal fluid and blood should be explored for complete method validation.

Decolourization methods through the use of an anion exchange column along with investigation of a stripping condition (see Figure 29 – Chapter 4 for results with pH 4 sodium acetate/acetic acid buffer solution) for direct measurement of the ^{226}Ra extracted onto the particles rather than indirect measurement of the activity in the supernatant are underway. EDTA diammonium salt and EDTA disodium salt at pH 10 are currently being investigated for extraction of the analyte from the CE-MNPs based on a current ion exchange method developed by at Health Canada's Radiation Protection Bureau [**92**].If these methods can be optimized, the advantages of LSC detection can be exploited to increase the sample turn-around time for further applications [**13**].

To further perfect these methods for future applications of CE-MNPs for selective removal of ^{226}Ra from aqueous solutions, the CE-MNP synthesis should be further investigated and optimized to reduce cost and time of particle preparation. The current synthesis method (described above in section4.2.2) is a long and expensive one which involves the use of a pricy CE reagent (4'-aminobenzo-18-crown-6) and several long and inconvenient waiting periods, yielding a very small ligand surface coverage of only 6% [**63**]. Physical adsorption of the CE onto the MNP surface as described in section 4.3.3 above, with a reaction time of only 3 minutes,

may be a much more feasible alternative and should be further investigated to determine the strength of the CE bond onto the MNP. Variation of the CE:MNP ratio may also be an avenue worth exploring for this optimization.

Once all preliminary tests are completed and the method is fully developed, determination of the analytical figures of merit such as the minimum detectable activity (MDA), the accuracy and the precision can be establish to further validate the use of CE-MNPs for selective extraction of ^{226}Ra and possibly even ^{90}Sr from aqueous samples.

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