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Separation of no-carrier-added ^{203}Pb , a surrogate radioisotope, from proton irradiated $^{\text{nat}}\text{Tl}_2\text{CO}_3$ target using calcium alginate hydrogel beads

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Abstract: ^{203}Pb is a promising radioisotope in the field of medical science as an imaging surrogate of ^{212}Pb . In the present investigation ^{203}Pb was produced by proton irradiation of natural Tl_2CO_3 target and was separated from the bulk Tl target using calcium alginate (CA) hydrogel beads with a high separation factor (3.8×10^4 at 10^{-3} M HNO_3). During the separation process ^{203}Pb was encapsulated in CA beads and desorption of the radioisotope could only be achieved in 1M HNO_3 . Possibility of Tl uptake was also checked in Fe doped CA (Fe-CA) beads after oxidation of Tl(I) to Tl(III) by sodium bismuthate. No significant uptake of Tl(III) was noticed in the Fe-CA beads. The matrix is therefore suitable for isolation of ^{203}Pb from the target as well as its storage in the bead for therapeutic as well as diagnostic purpose.

Keywords: ^{203}Pb , no-carrier-added, calcium alginate, iron doped calcium alginate.

1 Introduction

Radiotherapy using different α -emitting radionuclides is effectively used for the treatment of different types of cancers and for prolonging the lives of patients suffering from this deadly disease. Metastatic melanoma, a type of advanced skin cancer, is a fatal ailment having low survival rate. Although melanoma accounts for only 4% of all skin cancers, it is identified as the most aggressive and found to cause approximately 80% of all deaths from skin cancer [1, 2]. Research with ^{212}Pb for targeted radiotherapy towards metastatic melanoma is developing in recent years. The beta-emitter ^{212}Pb ($T_{1/2} = 10.64$ h) decays to the radionuclide ^{212}Bi ($T_{1/2} = 60.55$ min) which eventually decays by β (64.06%) and α emission (35.94%) [3]. ^{212}Bi is therapeutically useful because of its suitable α -energies and intensities, 6.05 MeV (25.13%), 6.09 MeV (9.75%).

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Reports on animal experiments with ^{212}Pb labeled DOTA peptides [4] show that treatment of mice inoculated with melanoma cells significantly decreased tumor growth, resulting in extended survival times and in many cases (45%) complete remission of the disease. Application of matched pairs of radioisotopes for imaging and therapeutic applications in single step are currently being investigated [5–8]. This is in line with modern day's therapeutic approach. For ^{212}Pb melanoma therapy, clinical trials are under process with ^{203}Pb ($T_{1/2} = 51.92$ h) tagged as an imaging companion with recommendation from Federal Food and Drug Administration [9]. Investigations with clinical trials and animal studies directed that ^{203}Pb imaging is a viable substitute for the therapeutic ^{212}Pb isotope [4]. The actual immune conjugate for the therapy consists of a receptor-specific peptide labeled with ^{212}Pb that targets melanoma cells. After injection, the peptide acts as a vehicle to carry the radioisotope to the melanoma receptors on the surfaces of melanoma cells. Development of an imaging surrogate for patient-specific dosimetry and to monitor the tumor response to ^{212}Pb labeled peptides were studied with the ^{203}Pb -alpha-melanocyte stimulating hormone peptide as a matched-pair SPECT imaging agent for ^{212}Pb -DOTA-Re(Arg11)CCMSH [10]. ^{203}Pb emits 279.2 keV photons (81%) which facilitates imaging and measurement for biodistribution analysis, cancer imaging, and quantitative dosimetry. Low radiochemical purity of ^{203}Pb can dramatically reduce the radiolabeling yields due to the competition of the existing contaminants. Therefore, high specific activity and radiochemical purity of ^{203}Pb are essential for radiolabeling of peptides.

There are several routes for the production of Pb and Tl radioisotopes, some of them have been cited below.

$^{203, 205}\text{Tl}(p, xn) \rightarrow ^{200, 201, 203}\text{Pb}$ [11–13],

$^{\text{nat}}\text{Pb}(p, xn) \rightarrow ^{203}\text{Bi}(\text{EC}) \rightarrow ^{203}\text{Pb}$ [14],

$^{\text{nat}}\text{Pb}(p, pxn) \rightarrow ^{203, 202m, 201}\text{Pb}$ and

$^{\text{nat}}\text{Pb}(p, 2pxn) \rightarrow ^{202, 201, 200, 199}\text{Tl}$ [15],

$^{203}\text{Tl}(d, 2n) \rightarrow ^{203}\text{Pb}$, and

$^{203}\text{Tl}(^3\text{He}, 3n) \rightarrow ^{203}\text{Bi} \xrightarrow{(\text{EC})} ^{203}\text{Pb}$ [for review cf. 13],

$^{197}\text{Au}(^7\text{Li}, xn) \rightarrow ^{198-200}\text{Pb}(\text{EC}) \rightarrow ^{198-200}\text{Tl}$ [16]

After the production of the desired isotopes, separation from the target matrix is mandatory for the application of the isotope as an *in vivo* radiotracer. Several separation methods have been reported for separation of no-carrier-added (NCA) Pb radionuclides from the target matrix. Few examples are liquid-liquid extraction (LLX) using diethyldithiocarbamic acid dissolved in chloroform [11], ion exchange chromatography using chelex 100 [14], co-precipitation with $\text{Fe}(\text{OH})_3$ followed by anion exchange separation [13], precipitation of bulk thallium as $\text{Tl}(\text{OH})_3$ after oxidation of Tl(I) to Tl(III) [17], complexation of Tl(I) using *n*-benzylaniline and followed by separation from its precursor ^{201}Pb [18]. Earlier ^{203}Pb was separated from Tl_2CO_3 target matrix using ion exchange resin [19], using a continuous ethyl ether extraction [20] and from Hg_2Cl_2 [21] and gold [16] target matrix by LLX using trioctylamine (TOA) dissolved in cyclohexane. Besides conventional techniques, separations were also performed via greener reagents like aqueous biphasic extraction using polyethylene glycol [22], complexation of Tl using poly (N-vinyl pyrrolidone) [23, 24] and by plant seed protein of *Erythrina variegata* Linn [25]. Separations were even achieved using algae of three different genera [26]. Each of the above methods has its own merits and demerits. The conventional LLX methods used are environmentally harmful organic solvents and reagents, while some of the greener methods like separation by algal genera is a time consuming process. In this paper we present yet another environmentally benign method for separation of NCA ^{203}Pb from proton irradiated Tl_2CO_3 target using calcium alginate (CA) hydrogel beads.

Hydrogels prepared from the alginate biopolymers found enormous applications in the field of separation science. A huge literature has been developed during recent years that reflects the wealth of evidences in favor of their wide applications [27–31]. Earlier NCA radioisotopes of $^{197-200}\text{Pb}$, $^{197-200}\text{Tl}$ and ^{197}Hg produced in ^7Li irradiated gold target were separated by CA beads [32]. Radioisotopes of all the three elements were found to have different adsorptions in the CA beads at different pHs of the medium. However, separation of NCA Pb isotopes from Tl target matrix by CA beads was not reported earlier. In the present work, CA beads are used for the first time to separate NCA ^{203}Pb from proton irradiated Tl_2CO_3 target matrix.

2 Experiment

2.1 Materials

Thallos carbonate was procured from Sigma-Aldrich. Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), ferric nitrate,

mercurous chloride (Hg_2Cl_2), trioctylamine (TOA) and cyclohexane were obtained from Merck, India. Sodium alginate ($\text{NaC}_6\text{H}_7\text{O}_6$) was procured from Sisco Research Laboratories Pvt. Ltd. (SRL), India, and all other chemicals used were of analytical grade and used as received.

2.2 Apparatus

A CANBERRA n-type high purity germanium (HPGe) detector of 3.25 keV resolution at 1.33 MeV in conjunction with DSA 1000 and Genie 2000 software was used for γ -ray spectroscopic measurement.

2.3 Preparation of calcium alginate (CA) and iron doped calcium alginate (Fe-CA) beads

Calcium chloride dihydrate solution (20% w/v) was taken in a beaker, and was kept in ice water ($\sim 5^\circ\text{C}$). Sodium alginate solution 3%, (w/v) was poured dropwise from a burette into the beaker containing the aforementioned calcium chloride solution with constant stirring using a magnetic stirrer. The beads were kept overnight in the mother liquor and then washed with deionized water prior to their use in the experiment [33].

For the preparation of Fe-doped Ca-alginate (Fe-CA) beads, a mixture of 0.1 M FeCl_3 and 0.1 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 20 : 80 (v/v) ratio was prepared. A 3% sodium alginate solution was added dropwise into the mixture of FeCl_3 and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution with constant stirring while maintaining the temperature at 5°C . The water-soluble sodium alginate was thus converted into water-insoluble Fe-CA beads [34, 35]. The resultant beads were washed with deionized water after 24 h to remove ferric chloride and calcium chloride from the surfaces of the beads.

2.4 Irradiation and radiochemical studies

For the present study, $^{\text{nat}}\text{Tl}_2\text{CO}_3$ target was prepared by pelletizing Tl_2CO_3 powder on the support of thin Al foil of thickness 1.5 mg/cm². The target was irradiated with 19 MeV proton beam for 8.5 h with 130 nA beam current at the TIFR-BARC pelletron facility, Mumbai, India. After bombardment, the target was cooled for 2 h. Before dissolution of the target for radiochemical separation γ -ray spectral analysis of the product radionuclides was carried out with the help of a HPGe detector. The irradiated Tl_2CO_3 target was collected from the aluminum foil by careful

scratching with the help of a spatula followed by mild sonication in 2 mL triple distilled water in the same glass container. Minimum amount of conc. HNO_3 was added to dissolve the Tl_2CO_3 powder. The solution was evaporated to dryness followed by re-dissolution in 0.001 M HNO_3 . The process was repeated twice.

Again ^{203}Pb target was prepared by pelletizing of Hg_2Cl_2 powder on the support of thin Al foil of thickness 1.5 mg/cm². The target was irradiated with 19 MeV proton beam for 6 h with 300 nA beam current. The target was cooled for 2 h and then dissolved in aqua regia, evaporated to dryness and was finally taken in 0.001 M HNO_3 for radiochemical separation studies. ^{200}Tl radioisotope was separated from the bulk mercury by earlier established LLX method using trioctylamine (TOA) [21]. This ^{200}Tl radioisotope was spiked with bulk Tl to monitor its fate during separation of ^{203}Pb using CA and Fe-CA beads.

The adsorptions of NCA Pb and of bulk Tl into CA and Fe-CA beads were examined by batch mode. Each batch containing CA and Fe-CA beads was exposed to 2 mL of different pH solutions of HNO_3 (pH 1–5) and 0.1 mL radioactive stock solution containing ^{203}Pb and bulk Tl spiked with ^{200}Tl . The radionuclides were monitored by choosing the photo-peaks of 367.99 keV and 279.2 keV for ^{200}Tl and ^{203}Pb , respectively. The mixture was shaken at room temperature (25 °C) for 10 min and allowed to settle for another 10 min. A 2 mL fraction of the supernatant liquid from each set was taken in eppendorf and assayed for the radionuclides by means of γ -ray spectrometry with the HPGe detector. The number of beads was also varied to get the optimum separation condition. The percentage of adsorption was calculated by comparing the counts with the initial solution before addition of beads.

Considering the possible speciation of thallium as Tl(I) and Tl(III), the solution containing the bulk Tl spiked with ^{200}Tl was treated with sodium bismuthate to convert Tl(I) into Tl(III) and the subsequent possibilities of Tl(III) sorption on Fe-CA beads were checked expecting an exchange between the trivalent Tl and Fe present in the Fe-CA beads.

3 Results and discussion

Calcium alginate hydrogels are known to possess an egg-box like molecular arrangement where the Ca^{2+} ions occupy the interstitial spaces between the alginate polymer chains [36]. The biosorption of any cation in the gel matrix is expected to be dominated by the ion change phenomenon with Ca^{2+} ions. Thallium may exist as Tl(I) or

Tl(III) in chemical systems, or as a combination of both. As per calculations obtained from CHEAQS software [37], Pb is found to exist as a combination of free Pb^{2+} ions and $\text{Pb}(\text{NO}_3)_2^+$ species at pH 1. However, at lower acidity, free Pb^{2+} ions dominate which undergo ion exchange with Ca^{2+} ions present inside the CA gel showing thereby a high adsorption of ^{203}Pb at lower acidity which effectively leads to substantial separation of ^{203}Pb from bulk Tl. The adsorption profile of NCA ^{203}Pb and bulk Tl by CA beads against HNO_3 concentration is shown in Figure 1. Tl shows no uptake by the CA gel at any of the pH conditions studied whereas high adsorption of ^{203}Pb was observed at low acidity (i. e. 10^{-5} – 10^{-2} M HNO_3), which suddenly decreases at 10^{-1} M HNO_3 . The highest separation factor between ^{203}Pb and bulk Tl under this condition with 30 beads was found to be 3.23×10^3 at 10^{-3} M HNO_3 concentration.

Figure 2 shows the effect of variation of number of beads on the adsorption of NCA Pb and bulk Tl keeping the acidic condition fixed at 10^{-3} M HNO_3 . It was found that the uptake of Pb increases to a certain extent (70%–96%) upon increasing the number of beads from 20 to 50 and thereafter it becomes more or less constant. No uptake of Tl by CA beads was observed even when 60 beads were used. A slight increase thereafter is probably due to exchange of Tl^+ with protons of the gel matrix.

As the exact speciation of the Tl present in an irradiated sample matrix is not known, we performed a similar experiment with Fe doped CA gel beads (Fe-CA) where a certain portion of the Ca^{2+} ions has been replaced by the Fe(III) ions during the preparation of the gel. The experiment was performed expecting an exchange between the probable Tl(III) and Fe(III) of the Fe-CA gel. A plot of the adsorption percent with molarity of HNO_3 (Figure 3) clearly shows a trend similar to that in Figure 1 which

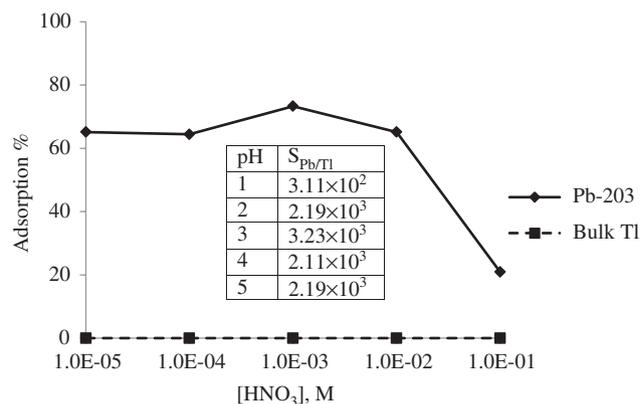


Figure 1: Adsorption profile of ^{203}Pb and bulk Tl by CA beads with variation of HNO_3 concentration (at 30 beads, 10 min shaking and 10 min settling time).

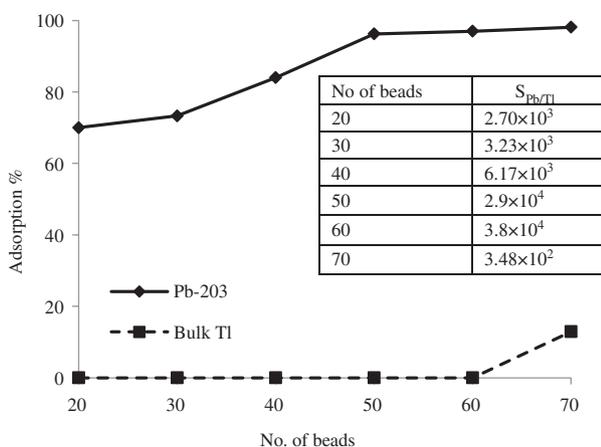


Figure 2: Adsorption profile of ^{203}Pb and bulk Tl with different number of CA beads keeping HNO_3 concentration fixed at 10^{-3} M (at 10 min shaking and 10 min settling time).

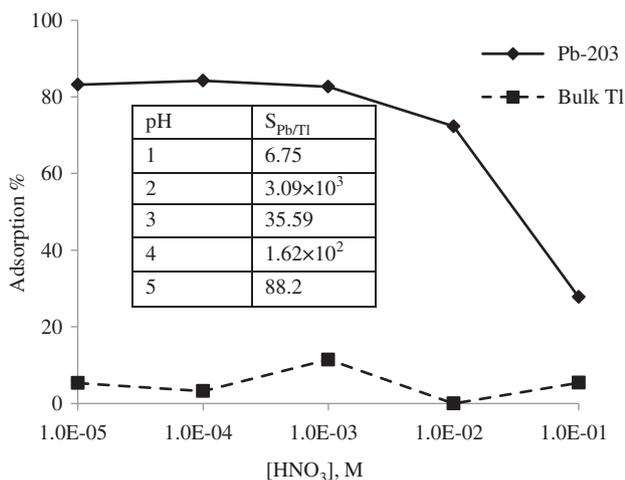


Figure 3: Adsorption profile of ^{203}Pb and bulk Tl by Fe-CA beads with variation of HNO_3 concentration (at 30 beads, 10 min shaking and 10 min settling time).

indicates that the existence of Tl in the radioactive solution is dominated by the presence of Tl(I) species over and above that of Tl(III).

Keeping in mind the pharmaceutical aspects of Tl, an attempt was also made to study its uptake properties in the gel matrix. To elucidate the possibility of exchange of Tl(III) with Fe(III) in Fe-CA beads, the radioactive solution was subjected to oxidation of the Tl(I) species to Tl(III) using sodium bismuthate. Figure 4 shows the extraction profile of the bismuthate treated NCA ^{203}Pb and bulk Tl in the Fe-CA beads at different acid concentration. It is clearly seen that the uptake of Pb follows a similar pattern as in the earlier experiment (Figures 1 and 3). However, 20%–30% uptake of Tl is observed in 10^{-1} and 10^{-2} M

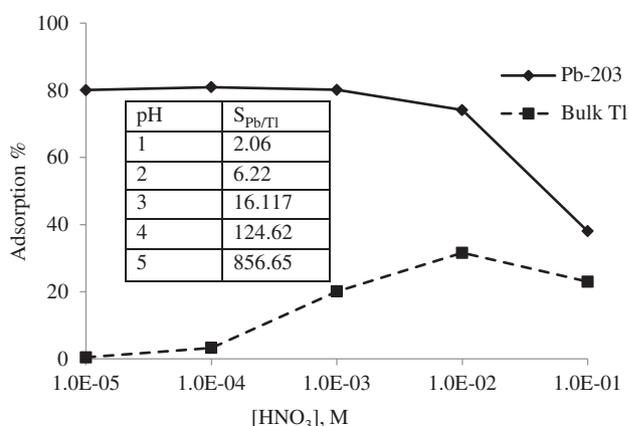


Figure 4: Adsorption profile of ^{203}Pb and bulk Tl by Fe-CA beads with variation of HNO_3 concentration after treating the beads with Na-bismuthate (at 30 beads, 10 min shaking and 10 min settling time).

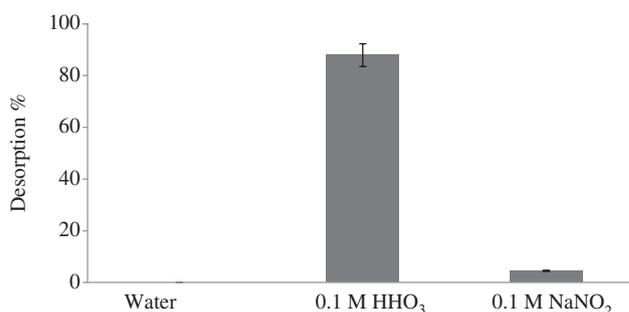


Figure 5: Desorption profile of ^{203}Pb in different desorption media from CA beads.

HNO_3 which slowly diminishes at lower acidity. This can be explained on the basis of higher charge interactions of Fe(III) with the carboxylate moieties present inside the interstices of the alginate polymer. This higher interaction of Fe(III) with alginate beads forbids an easy exchange between Tl(III) and Fe(III), hence little or no uptake of Tl is observed by Fe-CA beads in this case.

Desorption of ^{203}Pb from the CA beads was studied in water, 0.1 M sodium nitrite and 1 M HNO_3 media (Figure 5). The three desorption media were chosen on the basis of the following criteria: (i) water: as universal solvent, (ii) sodium nitrite: commonly used as preservative and (iii) 1 M HNO_3 : for achieving a drastic condition. It was found that considerable desorption of ^{203}Pb could only be attained using the drastic condition with 87.95% yield. When both adsorption of ^{203}Pb at best condition (97%) and desorption are considered, the yield of the overall process was found to be 85.31%. The present extraction process provides a distribution ratio (D_{pb}) of 32.33 for ^{203}Pb and (D_{Tl}) of 8.7×10^{-4} for ^{200}Tl indicating insignificant contamination by Tl radioisotope in the final solution. Therefore,

the CA beads are eligible for encapsulation and storage of NCA Pb which is suitable for therapeutic and imaging purpose required for targeted therapy and dosimetry.

4 Conclusion

Calcium alginate hydrogel was used for the separation of NCA ^{203}Pb radioisotope from proton irradiated bulk Tl target. A complete separation of NCA ^{203}Pb from Tl was achieved using CA beads. NCA ^{203}Pb could only be released from the CA beads upon treatment with 1 M HNO_3 . This is also an advantage for use in targeted therapy from an external source and for avoiding toxicity due to renal accumulation of the free radionuclide. The chances of uptake of Tl were also explored using the Fe-CA beads after conversion to Tl(III) upon oxidation by sodium bismuthate. However, Tl(III) uptake was found negligible. The present method is a fast and easy method for isolation of NCA ^{203}Pb using an environmentally benign biocompatible matrix and encapsulation of ^{203}Pb in the CA beads.

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