UNIVERSITÉ DE NANTES
FACULTÉ DES SCIENCES ET DES TECHNIQUES

_____

ECOLE DOCTORALE 3MPL
MATIERE, MOLECULE, MATERIAUX EN PAYS DE LA LOIRE

Année 2013

Exploration de la chimie du polonium

_____

THÈSE DE DOCTORAT
Discipline : Chimie
Spécialité : Radiochimie et chimie inorganique

Présentée
et soutenue publiquement par

Ali YOUNES

Le 16 décembre 2013, devant le jury ci-dessous

Président	M. BUJOLI Bruno, Directeur de recherche, CNRS., Université de Nantes.
Rapporteurs	Mme. CUTLER Cathy, Professeur, Université de Missouri, USA
	M. AUPIAIS Jean, Directeur de recherche, CEA-DAM, Bruyères le Châtel.
Examinateurs	M. ANSOBORLO Eric, Expert international, CEA, Marcoule.
	M. BUJOLI Bruno, Directeur de recherche, CNRS., Université de Nantes.
	Mme. CHAMPION Julie, Maitre assistant, SUBATECH-Ecole des Mine de Nantes
Membre invité	M. MOKILI Marcel Bandombele, Ingénieur de Recherche, ARRONAX.

Directeur de thèse : M. MONTAVON Gilles

Co-directeur de thèse : M. DENIAUD David et M. ALLIOT Cyrille

In the memory of Marie Curie

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

Marie Sklodowska-Curie

(1867 – 1934)

Remerciements

This thesis is the end of my journey in obtaining my PhD. I have not traveled in a vacuum in this journey. This thesis has been kept on track and been seen through to completion with the support and encouragement of numerous people including my well wishers, my friends, colleagues and various institutions. At the end of my thesis I would like to thank all those people who made this thesis possible and an unforgettable experience for me. At the end of my thesis, it is a pleasant task to express my thanks to all those who contributed in many ways to the success of this study and made it an unforgettable experience for me. This work would not have been possible without being able to work with all the wonderful people at SUBATECH more specifically in the radiochemistry Group, ARRONAX and CEISAM.

I owe my deepest gratitude to my thesis director, Gilles MONTAVON, for his ideas and support of my PhD and research. Without his continuous optimism concerning this work, enthusiasm, encouragement and support this study would hardly have been completed. I would also like to thank him especially for having the confidence in me to present this work at several international conferences. Giving me the freedom and opportunity to go and defend the things we have done here may have been the best learning experience I could have. I quite simply cannot imagine a better adviser.

I am also very grateful to my co-directors Cyrille ALLIOT and David DENIAUD for their scientific advice and knowledge and many insightful discussions and suggestions.

Great thanks to all the members of my defense jury. To Professor Bruno BUJOLI (University of Nantes) who presided over the ceremony and for those who participated as members of my jury de thèse:

- Professor Cathy CUTLER (University of Missouri) and Mr. Jean AUPIAIS (CEA) who accepted to sacrifice by their time and bringing me tips to improve my manuscript.
- Mr. Eric ANSOBORLO (CEA) who participated in my commité de suivi de thèse, support and stand beside me in presenting part of my work in some international conference.
- Miss. Julie CHAPMION (SUBATECH) who participated in my work.

I cannot begin to express my gratitude. It was a great honor to be able to present my work in front of you who are so respected in the field.

My special words of thanks should also go to my research guide, Mr. Marcel Bandombele MOKILI for always being so kind, helpful and motivating. I owe a lot of gratitude to him for always being there for me and I feel privileged to be associated with a person like him during my life.

I want to thank Mr. Bernd GRAMBOW (Director of SUBATECH) for allowing me to do my thesis in SUBATECH laboratory.

I express my heart-felt gratitude to my colleague in teaching Miss. Tomo Suzuki. She has taught me another aspect of life that "Time is like a river, you can never touch the same water twice because the flow that has gone by will never go by again". The students are lucky to have a person like you.

My acknowledgement will never be complete without the special thanks to the members of SUBATECH, ARRONAX and CEISAM for their competence, kindness, for their collaboration and for the excellent atmosphere they create. Thanks Catherine, Massoud, Celine, Johan, Karine, Veronique, Ronald, Guy, Myriam, Nicolas, Andrea, Guillaume, Anne, Katy, Benoît, Ferid, Rémi, Nathalie, Freddy, Valérie, Nadia, Cécile and Laurent…

My heartfelt thanks to my fellow lab mates: Raba, Diana, Céline, Thomas, Zongyuan, Gohkan, Ning, Racid, Sivakumar, David… Thanks for the discussions, for the sleepless nights we were working together before deadlines, and for all the fun we have had in the last three years. Zongyuan you have to stop taking Vitamins.

I would like to acknowledge my other friends for their moral support and motivation, which drives me to give my best: Sary, Radwan, Charbel, Emilie, Kawther… I find myself lucky to have friends like them in my life.

A very special acknowledgement goes to my girlfriend Linglan, who loved and supported me during the final, critical months of my dissertation, and made me feel like anything was possible.

Finally, I would like to acknowledge the people who mean world to me, my parents, my brothers and my sisters. I don't imagine a life without their love and blessings. I

consider myself the luckiest in the world to have such a supportive family, standing behind me with their love and support.

Table des matières

Glossaire

TBP : Tributylphosphate

TOA : Trioctylamine

HDz : Dithizone

$N_2S_2O_2$: 2,2'-(2,2'-((5-(2-aminoethoxy)-1,3-phenylene)bis(methanylylidene))bis(1-(carboxymethyl)hydrazin-1-yl-2-ylidene))bisthiazole-4-carboxylic acid.

N_2S_2 : Diethyl2,2'-(2,2'-((5-(2-((tert-butoxycarbonyl)amino)ethoxy)-1,3 phenylene)bis(methanylylidene))bis(1-(2-(tert-butoxy)-2-oxoethyl)hydrazin-1-yl-2 ylidene))bis(thiazole-4-carboxylate).

ARS: acute radiation syndrome

BAL: British Anti-Lewisite or 2,3-dimercaptopropanol

CCl_4: Tétrachlorure de carbone

CETAMA: Commission d'établissement des méthodes d'analyse

DDTC: diethylammonium diethyldithiocarbamate

DDTC: diethyldithiocarbamate

DMPA: N-(2,3-dimercaptopropyl) phthalamidic acid

DMPS: 2,3-dimercaptopropane sulphonate

DMSA: meso-dimercapto succinic acid

DTPA: Diethylene triamine pentaacetic acid

EDTA: Ethylene diamine tetraacetic acid

FDA: Food and Drug Administration

HOEtTTC: N'N-di[2-hydroxyethyl]ethylene-diamine-NN-biscarbodithioate

HSAB: Hard-soft acid-base classification of Pearson

IAEA: International Atomic Energy Agency

IARC: International Agency for Research on Cancer

ICRP: International Commission on Radiological Protection

LET: Linear energy transfer

MIBK: Methyl isobutyl ketone

NCRP: National Council on Radiation Protection and Measurements

PIPS: polysilicon-insulator-polysilicon

PROCORAD : promotion des contrôles qualité en radiotoxicologie

RBE: relative biological effectiveness

TOPO: Tri-n-octylphosphine

ZnS: zinc sulfur

Introduction générale

Le polonium, dernier élément de la famille des chalcogènes (symbole Po, numéro atomique 84), se situe dans la classification périodique sous le tellure. Il a été découvert en 1898 par Pierre et Marie Curie dans le cadre de leurs recherches sur la radioactivité de l'uranium et du thorium dans la pechblende de Joachimsthal (Pologne)[1]. Ayant observé que la radioactivité des minerais d'uranium était supérieure à celle attendue, ils entreprirent des traitements sur la pechblende pour isoler les éléments inconnus, radioactifs et présents en quantité infime. Très rapidement, ils constatèrent qu'une fraction de l'activité se concentrait dans les précipités de sulfure en milieu acide et conclure sur l'existence d'un nouvel élément, homologue du tellure, auquel ils donnèrent le nom de polonium (en hommage au pays d'origine de Marie Curie).

Le polonium est un élément hautement radioactif et toxique. Même pour de faibles quantités (quelques microgrammes), la manipulation de l'un de ces isotopes, le polonium 210 (^{210}Po), est très dangereuse et nécessite un équipement spécial et des procédures strictes. C'est par ailleurs l'un des poisons les plus dangereux et il se retrouve naturellement dans le milieu naturel car il fait partie de la chaîne de décroissance de l'uranium 238. C'est l'isotope qui a été extrait par Marie Curie et qui a conduit à la découverte de l'élément Po.

Il est donc à l'origine d'une exposition naturelle dans les maisons en granite avec des niveaux de radioactivité naturelle élevée et dans certains tabac (Figure I). Des agences de tabac avaient même envisagé de réduire les quantités de Po dans les cigarettes. Il est également présent en quantité importante dans les déchets radifères qui sont issus de l'extraction de l'uranium. Il se pose ainsi le problème de la gestion à long terme de ces résidus et l'étude du comportement du polonium dans ces déchets. Enfin, il peut être utilisé comme poison et on peut citer les deux cas très médiatiques, l'empoisonnement de l'espion russe Alexander Litvinenko et plus récemment, l'hypothèse de l'empoisonnement de Yasser Arafat. Les traitements aujourd'hui envisagés sont basés sur l'utilisation d'agents de décorporation développés pour d'autres éléments.

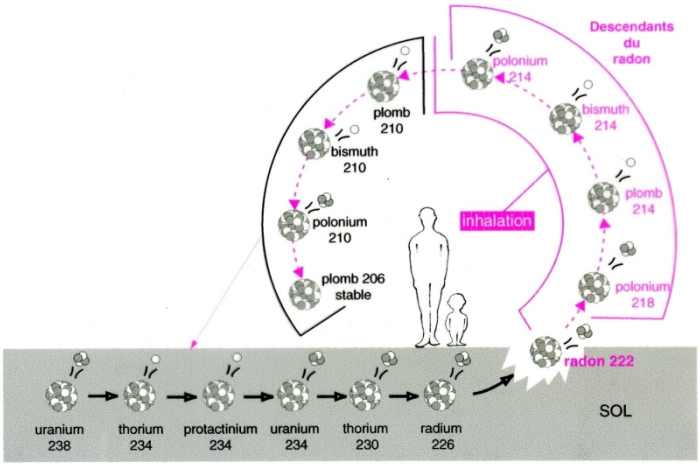


Figure I. Émanation du radon 222 et dispersion de ses descendants

Quel que soit l'enjeu social considéré, la connaissance des propriétés physico-chimiques du Po est primordiale pour comprendre son comportement et proposer des solutions pour gérer son impact si nécessaire. Malheureusement, la chimie du Po reste peu connue. Cela peut s'expliquer par deux raisons principales: (i) le polonium a quarante-deux isotopes connus avec une masse allant de 188 à 220, tous radioactifs [2] et (ii) sa faible disponibilité entrave les études de cet élément.

Lors de ce projet de thèse deux objectifs ont été visés : la production de polonium 210 (^{210}Po) au cyclotron ARRONAX à Nantes et la synthèse d'agents de complexation du Po(IV) en suivant une approche rationnelle dans le but de proposer de nouvelles molécules comme agents de décorporation en cas d'ingestion. Au-delà des objectifs appliqués, l'idée est également d'exploiter les données acquises dans le but de générer des données de base (détermination des espèces et les paramètres thermodynamiques associés) permettant de contribuer à mieux comprendre la chimie du Po(IV). Le document est divisé en deux parties.

La première correspond à une partie introductive dans laquelle je rappelle ce qu'est le polonium, quels sont les enjeux à mieux connaitre sa chimie ainsi que la méthodologie suivie dans la thèse et les principaux résultats. La deuxième partie, rédigée en anglais, reprend les résultats de cette thèse.

Une nouvelle méthode de production du Po-210 est proposée. Celle-ci est basée sur la réaction nucléaire $^{209}\mathrm{Bi}(\alpha, 3\mathrm{n})\,^{210}\mathrm{At} \xrightarrow{T_{1/2}=8.1h} {}^{210}\mathrm{Po}$. Une optimisation des conditions de séparation a été nécessaire. Une partie des données qui a servi à l'optimisation a notamment pu être exploitée afin de caractériser les espèces du Po(IV) en milieu acide chlorhydrique (HCl) et en présence de TBP (Tri -n-butylphosphate) ainsi que les paramètres thermodynamiques associés. En parallèle, des molécules ont été désignées et synthétisées pour une complexation *a priori* optimale du Po(IV). Leur capacité à complexer le polonium a été testée à pH 7,4 et une comparaison avec des molécules de référence proposées dans la littérature a été faite. Des résultats très prometteurs ont ainsi été obtenus.

Le manuscrit se termine par une conclusion dans laquelle les résultats les plus importants sont résumés ainsi que les perspectives qui découlent de ce travail.

Partie I : État de l'art, objectifs de la thèse et principaux résultats obtenus

I. Le polonium

I.1. Découverte du Polonium

Le polonium a un nombre atomique Z = 84, il est localisé dans le groupe IV et appartient à la famille des chalcogènes. Il s'agit d'un élément métallique [3] qui ne présente que des isotopes radioactifs. Il a été découvert en 1898 par Pierre et Marie Sklodowska Curie, lors de ses recherches sur les radioéléments présents dans la croute terrestre, l'uranium et le thorium.

Marie Curie a démontré que la radioactivité mesurée dans les minéraux de l'uranium (en particulier la pechblende) ne pouvait être uniquement imputée à la présence d'uranium et de thorium [4]. Elle arrive donc à la conclusion que la pechblende contient d'autres isotopes radioactifs qui présentent une activité spécifique supérieure de celles des isotopes de l'uranium et du thorium. En avril 1898, elle indiquait dans ses notes présentées à l'académie des sciences "*This fact is quite remarkable and suggests that these minerals may contain an element much more active than uranium itself*" [1].

Pour permettre d'identifier cette substance, il fallait surmonter deux challenges: l'isoler et le detecter. Marie Curie a dû ainsi traiter plusieurs tonnes de pechblende dans le but d'isoler une quantité minime de produit ; à titre d'information, une tonne de minerais d'uranium contient moins de 100 µg de polonium [5].

La méthode de détection du Po est basée sur la mesure de la conductivité dans l'air générée par les rayonnements émis par la substance *via* un électromètre. L'appareil a été

construit par Pierre Curie et son frère Jacques en 1880 et reposait sur la mesure de l'effet piézoélectrique de la substance lors de l'application d'un champ électrique (Figure II). Il est constitué de deux électrodes métalliques, entre lesquelles un champ électrique est appliqué. Les rayons émis par la substance produisent une charge électrique dans l'air qui crée un courant d'ionisation entre les électrodes. La charge dans la chambre est compensée par des charges opposées et ceci est contrôlé par un électromètre à quadrants. Ces charges opposées sont obtenues en appliquant un poids à une lame de quartz piézo-électrique. Les charges produites par le quartz sont calculées à partir du poids appliqué et la durée pendant laquelle la charge est appliquée. Le courant d'ionisation peut être calculé et l'émission des rayons peut être quantifiée. Avec l'aide de cet appareil, Marie et Pierre Curie ont ainsi pu déterminer la présence de polonium et de radium dans le minerai de pechblende.

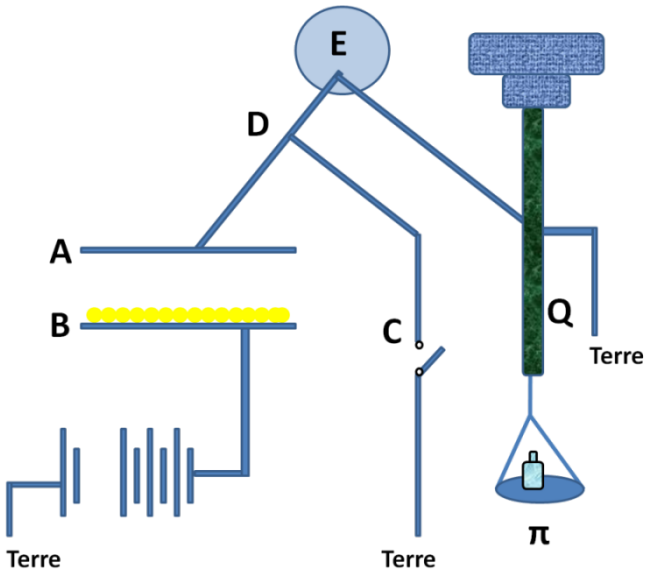


Figure II. Schéma de l'appareillage permettant la mesure de l'effet piézoélectrique

1- AB est la chambre d'ionisation. 2- CD relié à la terre. 3- E un électromètre à quadrants.

4- Q lame de quartz 5- π : masse utilisée pour la quantification [6].

En juillet 1898, Marie écrivait:*"We thus believe that the substance that we have extracted from pitchblende contains a metal never known before, akin to bismuth in its analytic properties. If the existence of this new metal is confirmed, we suggest that it should be called polonium after the name of the country of origin of one of us."* Avec le radium, le polonium était le premier élément découvert à partir d'une méthode radiochimique.

Figure III. Photo d'un article d'un journal dans lequel le polonium est qualifié d'élément radioactif au pouvoir mystérieux

En 1902, Marckwald indiquait la découverte d'une nouvelle substance qu'il appela "radio tellurium" [7, 8]. Plus tard, entre 1904 et 1905, Rutherford montrait que la substance identifiée par Marie Curie et le radio-tellurium étaient une et même substance qu'il identifia par le terme « radium F » [9-11]. Après les mesures réalisées par Meyer and Schweidler [12], cet élément est définitivement appelé "Polonium" en l'honneur du pays d'origine de Marie Curie (Figure IV).

$$^{210}_{82}\mathrm{Pb}\,(RaD) \xrightarrow[22.3\,ans]{\beta} {}^{210}_{83}\mathrm{Bi}\,(RaE) \xrightarrow[5\,j]{\beta} {}^{210}_{84}\mathrm{Po}\,(RaF) \xrightarrow[138.4\,j]{\alpha} {}^{206}_{82}\mathrm{Pb}\,(RaG)$$

Figure IV. Schéma de désintégration du plomb-210 selon Rutherford.

I.2. **Les isotopes du polonium.**

Le polonium présente 42 isotopes radioactifs avec une masse atomique qui varie entre 186 et 227 u. Selon le modèle HFB-14 [2], 45 isotopes de polonium supplémentaires

pourraient exister (Figure V).

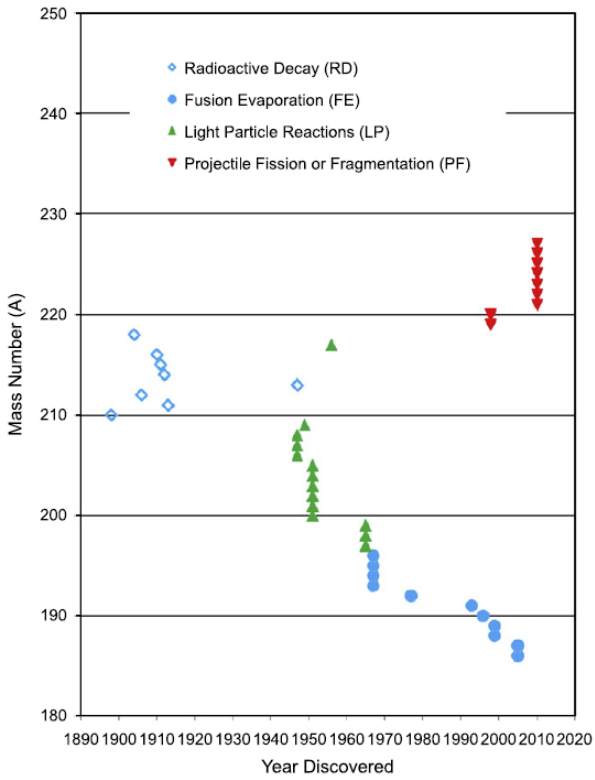


Figure V. Découverte des isotopes du polonium dans le temps [2].

La plupart des isotopes présentent des courtes périodes. Sept d'entre eux (^{210}Po, ^{212}Po, ^{214}Po, ^{215}Po, ^{216}Po and ^{218}Po) sont présents dans les trois grandes familles radioactives naturelles (Figure VI). Les autres radioisotopes du Po sont produits de manière artificielle.

Le ^{209}Po est l'isotope qui présente la plus longue période ($t_{1/2}$ = 102 ans). Il a été démontré que la valeur tabulée pour ^{209}Po est incertaine (de l'ordre de 25%) et la détermination de $T_{1/2}$ reste une donnée fondamentale à déterminer [13]. Il a été identifié par Kelly et Segre en 1949 [14] *via* le bombardement d'une cible de bismuth avec des deutons

de 10 MeV selon la réaction nucléaire ^{209}Bi (d, 3n) ^{209}Po.

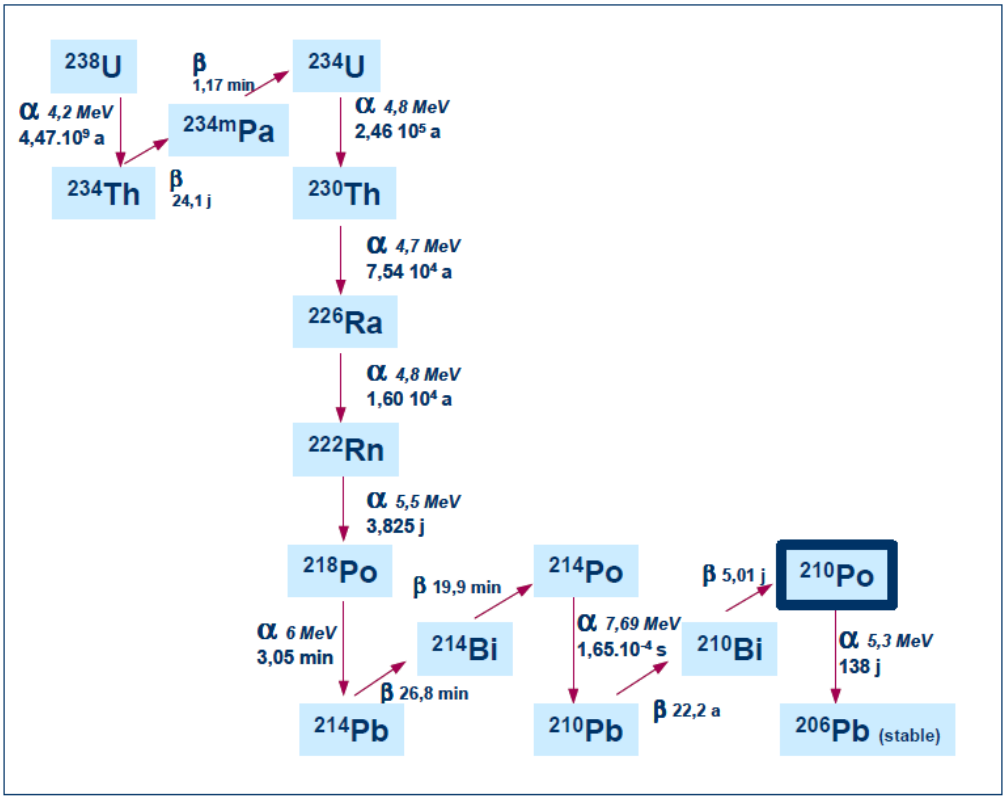


Figure VI. Schéma de décroissance de l'uranium 238

I.3. **Polonium 210**

Le ^{210}Po est l'isotope le plus abondant dans la nature, en raison de sa longue demi-vie (138.376 jours). C'est celui qui est à l'origine de la découverte du Po par Marie Curie et qui a été un moment appelé « Radium F ». Il provient essentiellement de la décroissance de ^{238}U (Figure VI). Il peut également être produit de manière artificielle par le bombardement neutronique du ^{209}Bi qui génère le ^{210}Bi qui se transforme, par décroissance bêta, en ^{210}Po.

Il présente la période la plus longue des isotopes naturellement présents ($t_{1/2}$ = 138,376 jours). ^{210}Po est un émetteur alpha ; il décroit *via* l'émission de particules alpha de 5,305 MeV (99,999%) et 4,502 MeV (0,001 %) (Figure VII). Sa décroissance s'accompagne également de l'émission d'un rayonnement gamma de 803 KeV avec cependant une faible abondance ($1{,}23.10^{-5}$).

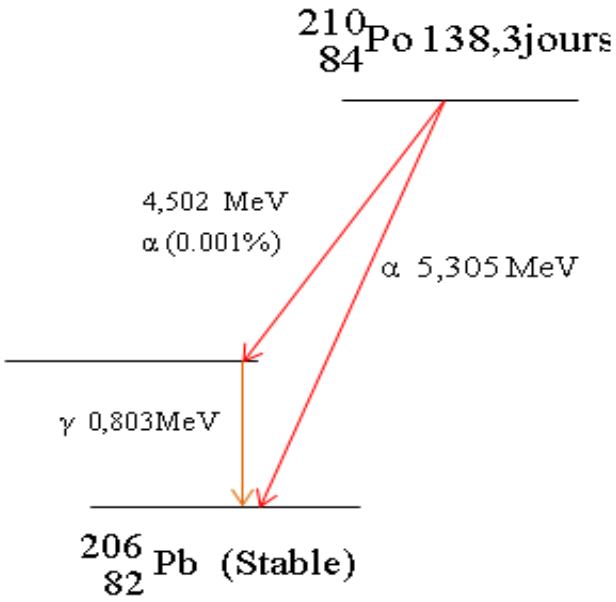


Figure VII. Schéma de décroissance du ^{210}Po

II. Problématiques autour du Polonium et contexte de l'étude

II.1. Questions de société

Le ^{210}Po est un isotope très toxique en raison de son activité spécifique très importante

(1,66 10^{14} Bq/g) [15]. Il est l'un des poisons les plus mortel en terme de DL50 (dose léthale médiane), cette valeur représentant la masse de substance à ingérer pour tuer 50% des animaux d'un lot (en général des souris ou des rats).

Substance	DL50 (mg/kg)
Alcool éthylique	10.000
Chlorure de césium	2.000
Uranium	1.500-100
As_2O_3	10
Cyanure de sodium	10
Strychnine	5
Nicotine	2
Sarine	0.1
Plutonium	0.02-0.001
Dioxine (TCDD, rongeurs seulement	0.001
Curium oxide	0.001
Toxine botulique, Polonium-210	0.00001

Tableau I. Comparaison des DL50 pour différentes substances [16].

Or, le ^{210}Po est un isotope naturellement présent et peut se retrouver en contact avec l'homme [17, 18] par l'atmosphère (apport d'aérosols stratosphériques, émissions volcaniques, émissions de fumées de feu de la biomasse, et surtout par décroissance de l'uranium 238, *via* l'exhalation de radon 222 à partir des couches superficielles de la croûte terrestre (Tableau I) ou le milieu marin (*via* le radium 226 dissous dans l'eau de mer ou *via* l'atmosphère).

Il faut bien évidemment ajouter à cela les activités anthropiques qui peuvent conduire à une surconcentration locale en ^{210}Po. Il s'agit essentiellement des activités minières d'extraction de l'uranium pour le combustible des centrales nucléaires, les activités de

forage mais également des activités liées à l'industrie des phosphates. Les rejets atmosphériques annuels de ^{210}Po seraient de 660 GBq/an dont 490 GBq/an par l'industrie des phosphates [19].

L'utilisation des engrais phosphatés en quantités relativement importantes peut augmenter le taux de polonium dans les aliments. Les régimes alimentaires composés de graines, végétaux et viandes peuvent donc être la source principale de ce radioisotope. A Nowe Miasto, il a été reporté que l'apport en ^{210}Po des fleurs (7,99 ± 2,17 mBq/an) était la seconde plus importante contribution après les poissons (15,3 ± 2,23 mBq/an) [20]. De plus, le dépôt de ^{210}Po de l'atmosphère sur les feuilles de tabac augmente les concentrations de cet élément dans les cigarettes ce qui conduit à un apport plus important de ce radioisotope chez les fumeurs par comparaison aux non-fumeurs. Par ailleurs, il est connu que les feuilles de tabac présentes une affinité à absorber les radioisotopes du sol comme le plomb 210 et le polonium 210. Ils peuvent donc être inhalés avec la fumée des cigarettes du fait de leur volatilité relativement importante à la température de combustion du tabac (600 et 800 °C) [21]. En 1964, Radford et Hunt ont reporté que les rayonnements alpha produits par volatilisation du ^{210}Po présents dans les cigarettes pourraient être la cause de l'augmentation des maladies de cancer du poumon chez les fumeurs [22]. Plus tard, ils ont montré que le ^{210}Po s'accumule particulièrement dans l'épithélium bronchique des fumeurs dans la région de bifurcation segmentaire [23]. Holtzman et Little ont trouvé que la concentration du ^{210}Po qui peut être accumulée dans la trachée, les branches lobaires et la bifurcation segmentaire peut atteindre respectivement 0,12, 0,19 et 4,5 pCi /g [24]. En 1996, Kilthau a reporté que le ^{210}Po et le

^{210}Pb sont présents dans les phases gazeuses et particulaires de la fumée et que ces deux isotopes contribuent à des risques de cancer dû à leur déposition dans les poumons [25]. Un article publié par Watson (1983) sur les niveaux de radioactivité dans les cigarettes consommées dans le monde entier, montre que les concentrations du ^{210}Po variaient entre 0,22 et 0,65 pCi/g (de 8,14 à 24,05 mBq par gramme) par échantillon sec [26]. En 1987, Batarek et Teherani ont montré que la concentration de ^{210}Po dans les cigarettes syriennes est très faible et varie entre 0,02 et 0,08 pCi/g [27]. Il a été montré ensuite que des cigarettes brésiliennes présentaient la concentration de ^{210}Po la plus élevée (entre 10,6 et 26,8 mBq/g selon [28] et entre 10,9 et 27,4 selon [29]). Certains auteurs ont mesuré la quantité de ^{210}Po saisie directement par le corps humain en utilisant des échantillons de sang prélevés sur des fumeurs [30]. Après la découverte du ^{210}Po dans la fumée des cigarettes au début de l'année 1960, la majorité des fabricants de tabac américains se sont concentrés sur le développement de méthodes pour réduire la quantité de cet élément dans les cigarettes, comme par exemple le cas de Philip Morris qui a développé le premier laboratoire capable de mesurer les doses libérées de manière fiable. Cependant, les différentes tentatives des entreprises de tabac de réduire la présence de polonium dans les plantes étaient insatisfaisantes.

Un autre sujet d'étude concerne la gestion post-exploitation des mines d'uranium et leur impact sanitaire et environnemental. Le Groupe d'Expertise Pluraliste (GEP) Limousin de 2010 recommande notamment de « développer une stratégie d'études et recherches en vue de renforcer les connaissances nécessaires à la bonne compréhension des processus en jeu et à l'acquisition d'une capacité prédictive sur leur évolution ». Cette

recommandation a été intégrée dans le cadre du Plan National de Gestion des Déchets et Matières Radioactifs (PNGDMR). Au-delà du territoire national, la problématique de l'impact des mines d'uranium et des résidus associés se pose également pour de nombreux pays concernés au titre d'une activité d'extraction passée, en cours ou en projet (pays de l'ex URSS, pays africains). Si les deux radioéléments les plus importants à étudier sont l'uranium et le radium, le polonium reste un élément d'intérêt. Du fait de la relative « courte » période du ^{210}Po (138,4 jours), son impact est attendu négligeable s'il reste confiné au niveau du site pendant environ 4 ans. Ceci reste plausible compte tenu de la forte réactivité du Po(IV) vis-à-vis du milieu environnant [31, 32]. En effet, le polonium va être fortement retenu et donc peu mobile. Cependant, cette réactivité est à double tranchant puisque le polonium peut également fortement réagir avec les colloïdes présents dans la phase liquide mobile. Dans ce cas, cette forte réactivité va conduire à faciliter son transport. L'impact des colloïdes sur le transport du polonium dans l'environnement est donc un aspect particulier à étudier.

Enfin, le polonium peut également être utilisé comme poison. Un exemple flagrant est l'empoisonnement de l'espion russe, Alexandre Litvinenko. Selon une publication récente des scientifiques suisses, ce radionucléide a été détecté dans certains objets appartenant à Yasser Arafat et ils ont avancé l'hypothèse d'une possibilité d'empoisonnement avec le polonium 210 [33, 34]. A titre d'illustration, je présente ci-dessous un résumé d'un article du Nouvel Observateur [35].

« Polonium 210: L'assassin qui dévore les globules rouges »

Le Polonium 210 est mille fois plus toxique que le plutonium et un million de fois plus que le cyanure : un seul centième de milligramme (10 microgrammes) suffit à tuer en quelques semaines un homme de poids moyen; une dose évidemment invisible à l'oeil nu, indétectable par la police ou les douanes - la radioactivité alpha est arrêtée par une simple feuille de papier, ou quelques centimètres d'air. Mais une dose à manipuler avec mille précautions quand on souhaite s'en servir pour éliminer quelqu'un comme l'ex-espion russe Alexandre Litvinenko, qui succomba à une ingestion de Polonium 210 l'an dernier.

Il faut de plus faire vite car le Polonium perd la moitié de sa radioactivité tous les 138 jours, il s'achète au rayon des poisons frais. C'est-à-dire que pour une efficacité optimale (de l'échantillon) il doit avoir été récemment fabriqué par irradiation du bismuth dans un réacteur nucléaire. Après l'ingestion du poison, il passe de l'estomac dans la circulation sanguine. Chaque atome de Polonium est alors porteur d'un projectile alpha expulsé à grande vitesse : de quoi littéralement griller toutes les cellules de l'organisme, les globules rouges en premier, et causer une mort dite "multifactorielle".

Ce redoutable métal est pourtant omniprésent dans la nature, produit en permanence par la désintégration de l'uranium et du thorium, qui abondent dans la croûte terrestre. Si bien qu'on le trouve à l'état de traces dans tout organisme humain.

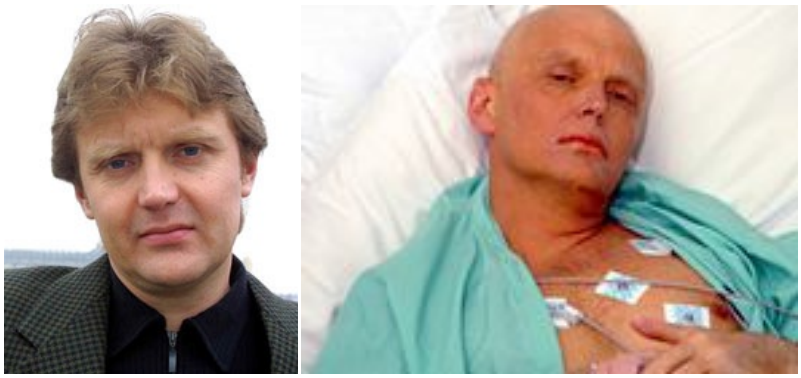


Figure VIII. Photos de l'espion Alexandre Litvinenko avant et après empoisonnement par le polonium 210

En conclusion, l'élément polonium est associé à des sujets de société avec deux questions fondamentales posées aux chimistes/biologistes: comment se comporte le polonium et quel traitement peut-on envisager en cas de contamination ? Ces deux questions demandent bien évidemment des connaissances sur la chimie du polonium et sa réactivité, et plus particulièrement en solution aqueuse. Celles-ci restent cependant limitées pour deux raisons. La première est qu'il n'existe pas d'isotope stable du polonium et sa manipulation demande des autorisations spéciales. Le deuxième concerne la quantité de matière que l'on peut manipuler au laboratoire et qui est faible, pour des raisons de disponibilité et de radioprotection. A titre d'exemple, le laboratoire Subatech dispose d'une autorisation de 1 MBq de ^{210}Po. Ceci correspond à une masse de 6,014 µg. De ce fait, les outils classiques utilisés en chimie ne sont pas accessibles, et plus particulièrement les outils de spectroscopie qui permettent d'obtenir des informations à l'échelle moléculaire. Ceci limite bien évidemment l'état des connaissances de cet élément, les informations étant généralement obtenues de manière indirecte à partir de méthodologies utilisées par les radiochimistes et adaptées à l'étude des éléments à

l'échelle des ultra-traces.

II.2. Quelques propriétés physico-chimiques du Polonium

Cette partie se limitera aux propriétés physico-chimiques du polonium qui sont considérées comme acquises. Je reviendrai plus en détail sur les données de la littérature pour lesquelles des compléments d'information semblent nécessaires et qui sont directement liées à mon travail.

La configuration électronique du polonium dans son état fondamental est [Xe] $4f^{14}$ $6s^2$ $5d^{10}$ $6p^4$. Il se trouve dans la colonne de l'oxygène et appartient à la famille des chalcogènes (O, S, Se, Te). Les propriétés chimiques du Po restent cependant différentes des éléments de cette famille, le polonium présentant un caractère métallique plus prononcé.

Ceci se traduit notamment par la réactivité du Po(IV), le degré d'oxydation le plus stable du Po en solution aqueuse, qui a tendance à fortement s'hydrolyser et se complexer [31]. En plus du degré d'oxydation 0 pour lequel le Po se trouve à l'état métallique, plusieurs degrés d'oxydation sont proposés : -II, +II et +VI. La forme –II existerait en solution aqueuse sous la forme PoH_2 dans des conditions fortement réductrices. La forme +VI existerait uniquement à l'état solide, par analogie avec l'existence du composé TeO_3. Enfin, la forme Po^{+2} serait présente en milieu aqueux, mais uniquement dans des conditions d'acidité forte et dans des conditions réductrices.

L'ensemble des expériences présentées dans ce document ayant été réalisées en présence de O_2, on considèrera que le polonium existe sous la forme + IV.

Le Polonium métallique pur peut être préparé par plusieurs méthodes. La sublimation sous vide sur du quartz ou du verre produit le métal pur alors que la sublimation sous air produisait l'oxyde. Pour de faibles quantités (de l'ordre de quelques milligrammes), le polonium métallique peut être aussi préparé par dépôt cathodique sur du platine [36, 37] ou de l'or [38]. Le dépôt spontané du polonium sur des métaux moins noble comme l'argent [39] ou le nickel est aussi réalisable mais souvent le métal obtenu est contaminé par du Bi. Une autre méthode pour préparer de faibles quantités (de l'ordre de quelques milligrammes) de polonium métallique pur est de précipiter le sulfure dans une solution diluée de HCl, suivi par un chauffage du précipité formé à 500°C pour sublimer le polonium métallique pur formé. En fonction de la température, il existe deux variétés allotropiques de polonium : L'α – polonium ayant un réseau cubique simple et existant à basse température, et le β – polonium qui existe à haute température avec un réseau rhomboédrique simple [36, 37, 40]. Le métal pur fraîchement préparé existe sous la forme β. Cette phase si convertit lentement en phase alpha sous l'effet des rayonnements émis lors de la désintégration de l'isotope radioactif du Po.

II.3. **Outils analytiques**

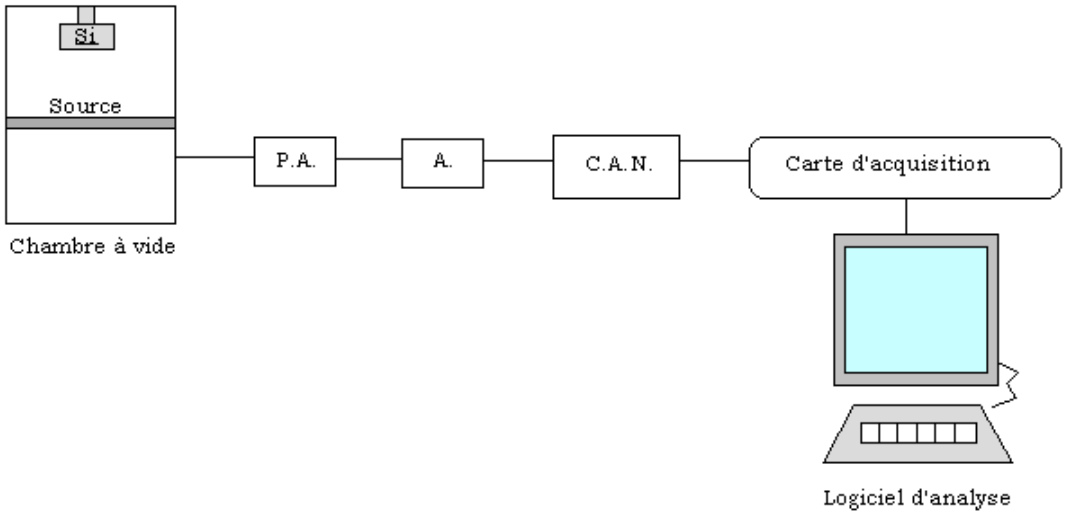
Le schéma de décroissance du polonium 210 a été présenté précédemment (Figure. VII). Le rayonnement gamma étant émis en faible quantité, l'analyse de ^{210}Po est réalisée au travers de la détection des particules alpha. Trois techniques de mesure ont été utilisées dans ce travail: la spectrométrie alpha, la scintillation liquide et le compteur

proportionnel à gaz [41]. Ces techniques sont brièvement décrites ci-dessous. Des détails supplémentaires/complémentaires pourront être trouvés dans la deuxième partie de la thèse.

a) La spectrométrie alpha

Une particule alpha est une forme de rayonnements émis par certains isotopes radioactifs. Le rayonnement alpha est constitué par le noyaux d'hélium (2 protons + 2 neutrons) et possède un libre parcours moyen très faible. L'énergie d'une particule alpha est très variable et pour la plupart des émetteurs alpha, cette énergie varie entre 3 et 10 MeV. Malgré son énergie importante, une particule alpha a un faible parcours dans les solides et quelques centimètres dans l'air à cause de sa masse relativement élevée, contrairement aux autres types de rayonnements (bêta, gamma ou neutronique). Elle est donc peu pénétrante et fortement ionisante d'où la nécessité d'utiliser des détecteurs minces ayant une très bonne résolution.

Une chaîne de spectrométrie alpha est constitué essentiellement des éléments suivants (Figure IX): d'une chambre à vide (chambra alpha) contenant un détecteur (semi-conducteur à base de Ge ou Si) et un porte-échantillon, une pompe à vide, un préamplificateur et un amplificateur, un convertisseur analogique-numérique (ADC), un analyseur multicanaux (MCA), un logiciel de représentation du spectre sur l'écran (nombre des coups cumulés pendant le comptage en fonction de l'énergie) et de traitement complet du spectre, et une imprimante.



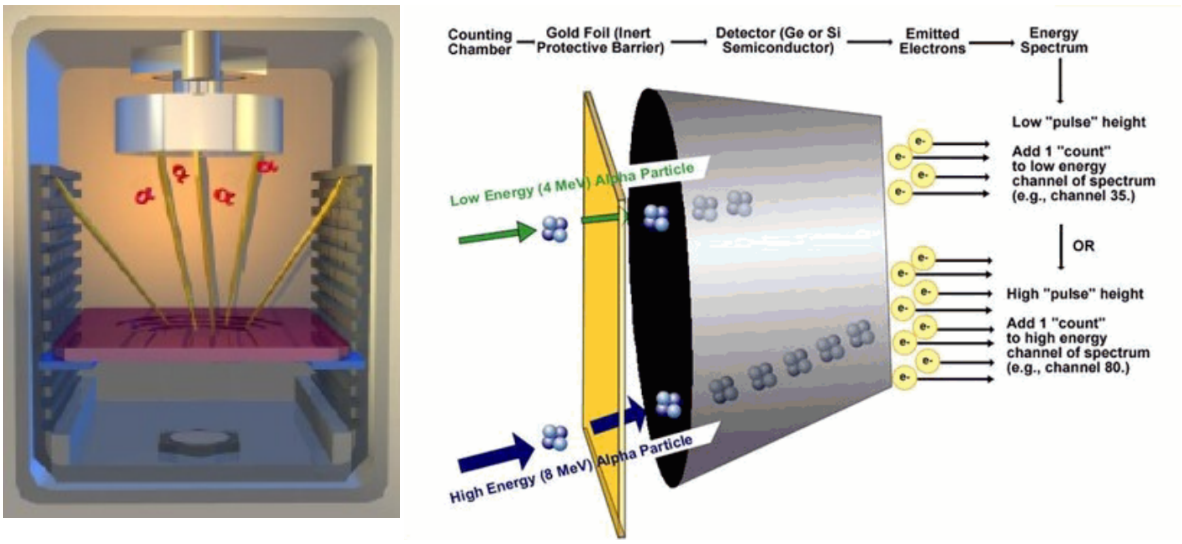


Figure IX. Schéma d'une chaîne et de principe d'un détecteur de spectrométrie alpha.

Une couche mince contenant les émetteurs alpha est déposée sur un disque métallique en acier inoxydable ou autre métal (Ag, Ni,…). Le disque est ensuite mis sur un porte-échantillon à l'intérieur de la chambre du spectromètre alpha à une distance donnée du détecteur. Le vide est ensuite réalisé à l'intérieur de la chambre alpha en utilisant une pompe afin de faciliter la transmission des particules alpha vers le détecteur (Figure IX). Le détecteur est couvert par une feuille d'or très fine. Lorsqu'une particule alpha arrive au détecteur, elle heurte tout d'abord la feuille d'or utilisée pour protéger le cristal et fournir un contact électrique pour le système de détection. La feuille d'or est très fine (épaisseur <1μm) de sorte qu'elle ne puisse pas arrêter les particules alpha. L'énergie des particules alpha est dissipée en produisant des électrons dans le cristal du détecteur. Le nombre d'électrons produits est proportionnel à l'énergie de la particule alpha. Les signaux électroniques sont analysés par l'analyseur multicanaux qui les convertit en un signal digital équivalent à l'énergie des particules alpha. Le circuit électronique de l'instrument peut mesurer l'énergie d'une particule alpha incidente et distinguer entre les réponses de plusieurs particules alpha ayant des énergies différentes. Le spectromètre alpha possède 4096 canaux où les informations peuvent être stockées en fonction de l'intensité de leur impulsion. L'intensité d'impulsion est directement proportionnelle à l'énergie des particules alpha. Ces positions sont appelées canaux et le numéro du canal est directement lié à l'énergie. Finalement, le spectre est analysé par un logiciel informatique spécial qui identifie et quantifie les énergies alpha.

La chaîne de spectrométrie alpha équipée d'un détecteur au silicium PIPS et du logiciel Génie 2K de traitement des spectres de chez Canberra ont été utilisée pour effectuer les

mesures d'activité de polonium 210.

La préparation de l'échantillon est très importante puisqu'elle doit permettre aux particules alpha de « sortir » de l'échantillon pour être analysées. Des couches minces doivent donc être préparées. Ce dernier point a été particulièrement étudié dans la littérature [42-44]. Plusieurs supports ont été étudiés (Ag, Ni et Cu) et également plusieurs milieux (Figure X et XI).

Figure X. Le schéma du dépôt spontané de polonium sur disque d'acier inoxydable

Sur la base des résultats publiés, nous avons choisi de travailler en milieu HCl 1M en présence d'un disque d'argent en tant que support. Pour permettre d'optimiser les conditions de préparation, le dépôt est réalisé à 80°C dans un bain d'eau. Cette méthode a été utilisée pour vérifier la pureté radioisotopique des solutions de ^{210}Po produites à ARRONAX.

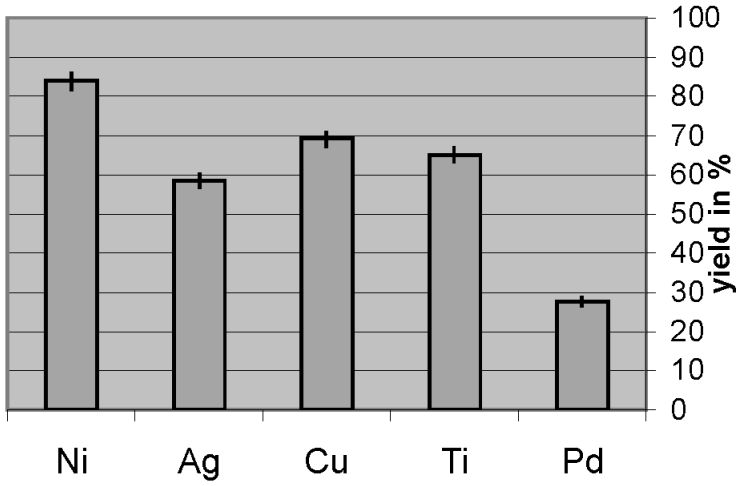


Figure XI. Rendements de dépôt de ^{210}Po sur des feuilles métalliques [45]

b) La scintillation liquide

Cette technique de mesure consiste à transformer le rayonnement ionisant émis par un noyau radioactif en un rayonnement lumineux détectable et quantifiable. Les principaux avantages de la scintillation liquide sont la facilité de préparation des sources radioactives, l'efficacité géométrique de détection de 4π et l'absence de barrière physique entre le radionucléide à mesurer et le détecteur, autorisant ainsi la détection de rayonnement peu pénétrants. Le principe de cette technique repose sur l'excitation d'un liquide scintillant par les particules α lors de la mise en contact avec la solution contenant le polonium et l'émission de photons lumineux après désexcitation du système scintillant (Figure XII). Lorsqu'une particule alpha excite les molécules fluorescentes qui constituent le scintillant, le retour à l'état fondamental de celle-ci est accompagné par l'émission de photons de fluorescence qu'il suffira de détecter au moyen d'une photocathode. Ces photons lumineux, en nombre proportionnel à l'énergie de l'alpha particule, sont collectés à l'aide de photomultiplicateurs et une électronique d'analyse.

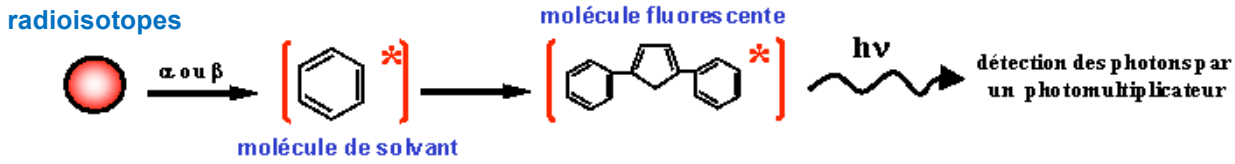


Figure XII. Schéma d'excitation des molécules scintillantes par le rayonnement ionisant

Le liquide scintillant utilisé dans ce travail est le cocktail Ultima Gold LLT ou AB. Il est composé d'un solvant organique de types aromatique (pseudocumène, di-isopropylnaphtalène) et de deux solutés : un soluté primaire (PPO 2,5-diphenyloxazole) qui possède une bonne solubilité dans les solvants et un soluté secondaire (Bis-MSB (p-bis-(O-méthylstyryl)-benzène, diméthyl-POPOP, (1,4[bis-2-(4-methyl-5-phényloxazoyl)]-benzène). C'est le soluté secondaire qui produit les photons lumineux de fluorescence détectable par les photomultiplicateurs.

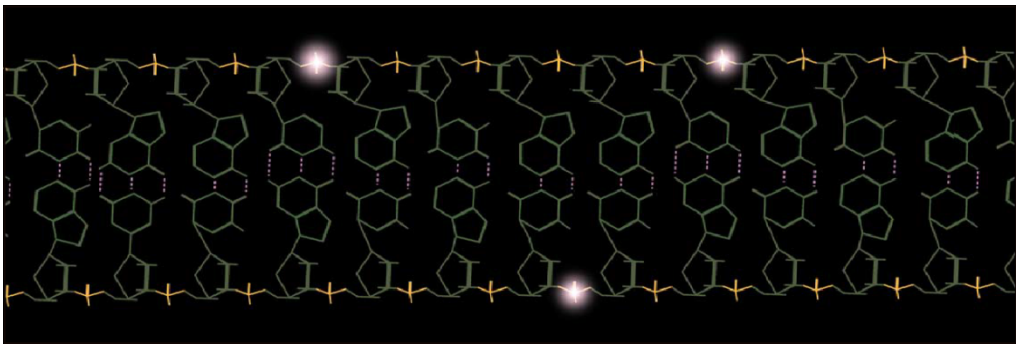


Figure XIII. Illustration du phénomène qui se produit dans le liquide scintillant suite à l'excitation par une particule alpha ; les petites étincelles montrent le processus de scintillation de la matière

Dans certaines conditions, des effets parasites comme l'affaiblissement lumineux (ou phénomène de quenching) peuvent perturber la mesure entraînant une sous évaluation de l'activité des échantillons mesurés. Aussi, des échantillons du même radionucléide mesurés dans les mêmes conditions, mais avec un quenching variable, ne sont pas

détectés avec la même efficacité de comptage (ou rendement de détection). C'est dans le liquide scintillant que le phénomène de quenching se produit.

Pour évaluer le quenching, la correction par standardisation externe a été utilisée. Le degré de quenching est évalué grâce à un standard externe appelé tSIE (« Transformed Spectral Index of External Standard »), paramètre utilisé par les appareils type Packard et et SQP(E) (Spectral Quench Parameter of the External standard) pour les appareils type Quantilus.

Une courbe de quenching permettant de représenter l'efficacité de comptage d'un échantillon contenant du polonium 210 en fonction du tSIE a éte mesurée et nous avon obtenu les relations suivant:

pour la solution de TBP dissout dans le *para*-xylène *:*

$A = A_m\,(-9.10^{-5}\times TSIE^3 + 673\ 10^{-4}\times TSIE^2 - 17.26\times TSIE + 1565.8)$

pour la solution de dithizone dissoute dans le chloroforme *:*

$A = A_m\,(5.10^{-12}\times TSIE^5 - 10^{-8}\times TSIE^4 + 10^{-5}\times TSIE^3 - 4.310^{-3}\times TSIE^2 + 0.9587\times TSIE + 10.988)$

avec A_m l'activité de polonium 210 d'un échantillon mesuré par le scintillateur liquide. Cette expression a été obtenue en faisant varier la concentration de TBP(dissout dans le *para*-xylène) et de dithizone (dissout dans le chloroformé) dans des échantillons contenant une activité connue du polonium.

Cette technique a été utilisée pour toutes les expériences de type « batch » réalisées en présence de polonium 210.

c) Comptage α global et β global

Le principe de cet appareil est fondé sur la distance parcoure dans la matière par les particules issues de la désintégration qui est liée à leurs charges, leurs masses et la densité de la matière traversée. Les rayonnements alpha, bêta ou gamma interagissent donc de manière différente avec la matière.

Dans le cas de compteur proportionnel à gaz, les particules alpha et/ou bêta ionisent un gaz circulant dans le détecteur polarisé par une alimentation haute-tension et fonctionnant en régime proportionnel. Le comptage consiste à mesurer le nombre de particules pénétrant dans le détecteur pendant le temps de comptage. Pour chaque particule détecté, l'appareil délivre alors un signal dont l'amplitude doit être supérieure à la fluctuation du bruit de fond engendré dans le détecteur pour être compté. L'activité de l'échantillon est ainsi déterminée quantitativement en régime impulsionnel.

Pour un matériau donné, plus l'énergie de la particule est importante, et plus important sera le nombre d'ionisations crée sur le trajet de la particule. En utilisant un gaz comme l'argon dans le détecteur (Figure XIV), une très bonne sensibilité est obtenue avec les particules alpha et bêta, alors que la réponse avec les rayonnements gamma est faible (peu d'ionisations engendrées). L'idée est donc de discriminer entre les particules en jouant sur la densité du matériau et sur la capacité d'ionisation.

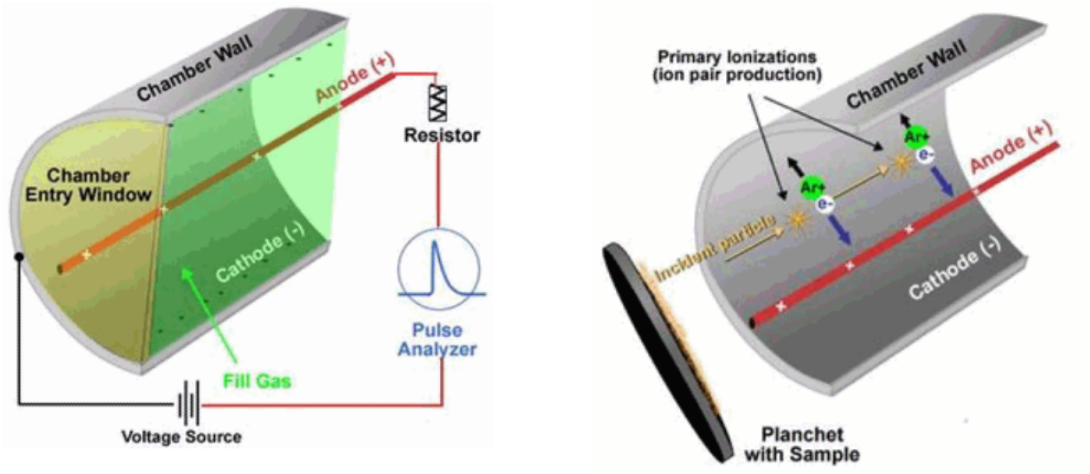


Figure XIV. Le schéma de principe d'un détecteur ***Compteur alpha / bêta à circulation de gaz***

Les mesures sélectives peuvent être réalisées en appliquant une tension de polarisation différente pour chaque type de particules.

Un compteur proportionnel à gaz alpha-bêta d'ITECH (Figure XV.) était utilisé pour la mesure de l'activité du polonium 210. Le gaz utilisé dans le compteur proportionnel à gaz était le mélange argon 90% + CO_2 10 % et la tension de polarisation appliquée aux électrodes était de 1,25 kV. Un volume d'échantillon de phase organique ou aqueuse est déposé sur un disque acier inoxydable puis séché doucement sur une plaque chauffante.

Cette technique a été utilisé par vérifier la pureté du radioisotopique du Po-210 et pour les expériences de type " batch".

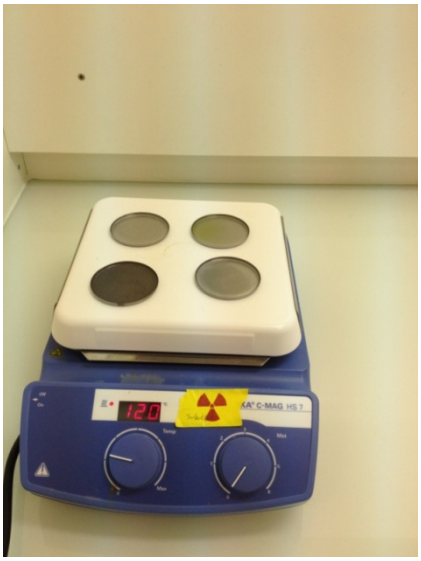


Figure XV. Le schéma de la préparation des échantillons du ^{210}Po pour *compteur alpha / beta.*

II.4. Objectifs du travail

Compte tenu des enjeux sociétaux et de l'intérêt scientifique de travailler avec le polonium, le groupe de radiochimie, dans la continuité des activités de recherche qu'il mène autour des radioéléments « rares » comme l'astate ou le technétium, a décidé de démarrer un programme de recherche dans le but de mieux comprendre la chimie du polonium, et plus particulièrement les propriétés du polonium à l'état d'oxydation IV en solution aqueuse. C'est dans le cadre de ce programme que mon sujet de thèse s'inscrit.

Deux objectifs étaient fixés :

- Le polonium-210 étant un isotope radioactif présent en faible quantité dans la croute terrestre (~10Bq/kg), sa période étant relativement courte et son intérêt commercial limité, il est bien évidemment illusoire de développer des méthodes d'extraction du

polonium-210 de la croute terrestre, comme cela a pu être fait par Marie Curie. Il est donc nécessaire de le produire de manière artificielle afin de l'étudier dans le cadre d'études scientifiques. Le polonium 210 peut être acheté auprès des fournisseurs spécialisés; par exemple dans le cadre de la thèse, des solutions de polonium 210 ont été achetées chez Eckert & Ziegler, (1,3 k€ pour 1MBq). Pour permettre une étude pérenne dans le temps, il a été décidé de développer une méthode de production du polonium 210 au cyclotron ARRONAX. Le premier objectif de mon travail consistait donc à produire ce radioisotopes et l'obtenir dans une solution finale purifiée.

- L'équipe CORAIL du laboratoire CEISAM développe et synthétise des molécules pour la complexation des métaux. Parmi les molécules développées, certaines apparaissent intéressantes pour complexer fortement le polonium. Le deuxième objectif de ma thèse était donc de synthétiser une ou plusieurs molécule(s) complexante(s) et d'étudier la complexation du polonium (IV) avec ces molécules afin d'estimer leur intérêt potentiel pour des applications en tant qu'agents décorporants.

La suite de cette partie a pour but de présenter, pour les deux objectifs de mon travail, le contexte de l'étude, les approches suivies ainsi qu'un résumé succinct des résultats. Ces derniers seront détaillés dans la partie II de ce manuscrit.

III. Production du Polonium 210

III.1. Approches proposées dans la littérature

Le polonium peut être obtenu naturellement et artificiellement. Naturellement, il est possible de l'extraire à partir de sels de radium naturel qui contiennent environ 0,2 mg de polonium par gramme de radium à l'équilibre, Cependant, les risques d'irradiation associés à la manipulation et le coût de l'extraction sont deux paramètres qui limitent l'utilisation du radium comme une source de polonium [46]. Artificiellement, il peut être produit dans un réacteur nucléaire *via* le bombardement de bismuth-209 stable par un faisceau de neutrons, protons ou de deutérium [47]. La méthode la plus largement utilisée, est la production par irradiation d'une cible de ^{209}Bi par des neutrons produisant ainsi du bismuth 210. ^{210}Bi peut exister dans l'état fondamental ^{210g}Bi (5,013 jours) et dans un état isomère ^{210m}Bi (3,04 10^6 années). ^{210g}Bi décroit par émission β en ^{210}Po alors que ^{210m}Bi émet une particule α pour produire ^{206}Ti (Figure XVI.).

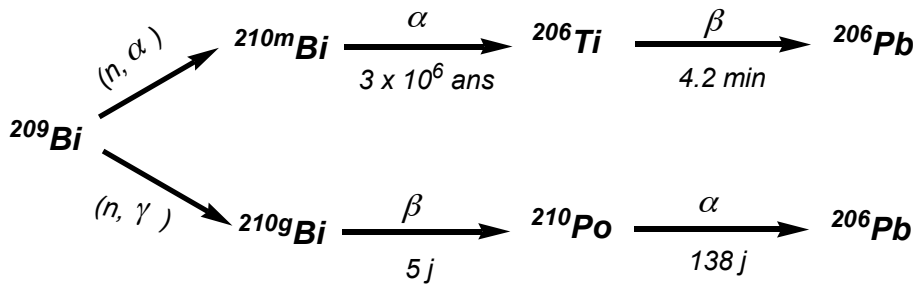


Figure XVI. Méthodes de production du ^{210}Po par irradiation du ^{209}Bi.

L'objectif était d'utiliser l'outil présent à Nantes, le cyclotron ARRONAX. Son nom signifie « Accélérateur pour la Recherche en Radiochimie et Oncologie à Nantes

Atlantique ». Il s'agit d'un accélérateur de particules (cyclotron). ARRONAX est également un clin d'œil au Professeur Arronax, personnage du roman de Jules Verne (né à Nantes en 1828) intitulé « 20.000 lieux sous les mers ». Il est composé d'un aimant circulaire en deux parties. Entre ces deux pièces règnent un champ magnétique qui fait tourner les particules et un champ électrique alternatif qui accélère leur mouvement à chaque tour : les particules décrivent des cercles de plus en plus grands. Lorsqu'elles sont aux limites extérieures de l'aimant, elles sont éjectées et dirigées dans une ligne jusqu'à une cible où elles produisent par réaction des isotopes radioactifs. ARRONAX est équipé de six lignes, offrant ainsi une grande variété de cibles et de radioisotopes créés. C'est une machine d'environ quatre mètres de diamètre dans un bâtiment de 3000 m^2. Les particules, accélérées jusqu'à 70 MeV, sont des noyaux d'hydrogène (protons) ou des noyaux d'hélium (particules alpha).

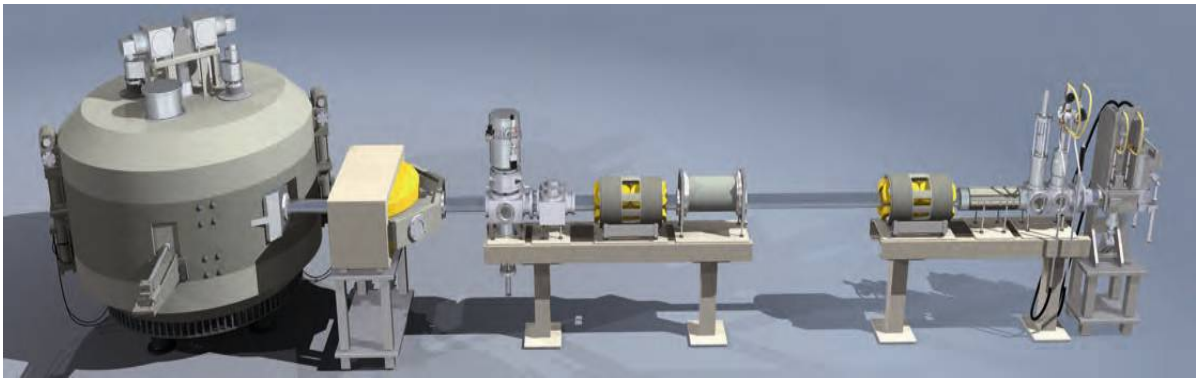
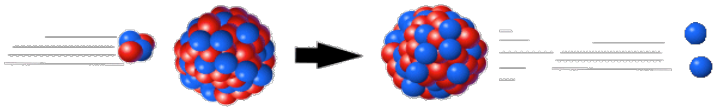


Figure XVII. Représentation en 3D d'une ligne du cyclotron ARRONAX.

Sur la base des données disponibles dans la littérature, il a été choisi de produire le ^{210}Po par décroissance de l'astate 210 qui est produit suivant la réaction ^{209}Bi(α, 3n)^{210}At.

$$E\ (\alpha) = 28\ \text{MeV} : \ {}^{4}He \ + \ {}^{209}Bi \ \rightarrow \ {}^{211}At \ + \ 2n$$



$$E(\alpha) = 35\ \text{MeV} : \quad {}^{4}He + {}^{209}Bi \rightarrow {}^{210}At + 3n$$

$$E(\alpha) = 60\ \text{MeV} : \quad {}^{4}He + {}^{209}Bi \rightarrow {}^{209}At + 4n$$

III.2. Contexte de ce travail

Les données nucléaires étant bien connues, la cible a été adaptée pour permettre une production optimale d'astate 210. Le challenge du travail réside plutôt dans la délicate séparation Po/Bi afin d'obtenir une solution pure de polonium que ce soit d'un point de vue isotopique (pas d'autres isotopes radioactifs), chimique (pas d'éléments chimiques présents) et radiochimique (milieu final permettant de bien contrôler la forme chimique du Po avant les expériences). En effet, cette séparation demeure un challenge pour deux raisons :

- les deux éléments sont côte à côte dans le tableau périodique et présentent tous deux un caractère métallique très prononcé.
- le bismuth est présent en quantité importante par rapport aux traces de polonium produites.

Une première possibilité qui s'offrait à nous était d'isoler au préalable l'astate de la cible et de le laisser tranquillement décroitre afin d'obtenir une solution de polonium pure. Cette approche est d'autant plus alléchante qu'une méthode par voie sèche est développée

pour la production de ^{210}At dans le but de l'utiliser en médecine nucléaire. Le principe de la médecine nucléaire est d'injecter des isotopes radioactifs au patient et de cibler un organe ou une zone du corps dans le but de diagnostiquer ou de traiter des maladies (Figure XVIII.). Le ciblage peut être associé aux propriétés intrinsèques du radionucléide utilisés (I^- pour la thyroïde ou Ra^{2+} pour les os,…) ou être réalisé *via* une molécule vectrice (anticorps, peptide, sucre,…) [48].

Le problème de la méthode est la contamination éventuelle du four par du ^{210}Po. En effet, il apparaît délicat de produite de l'astate 211, un potentiel médicament pour des applications en thérapie alpha ciblée, dans un appareil qui sert également à produire un poison !

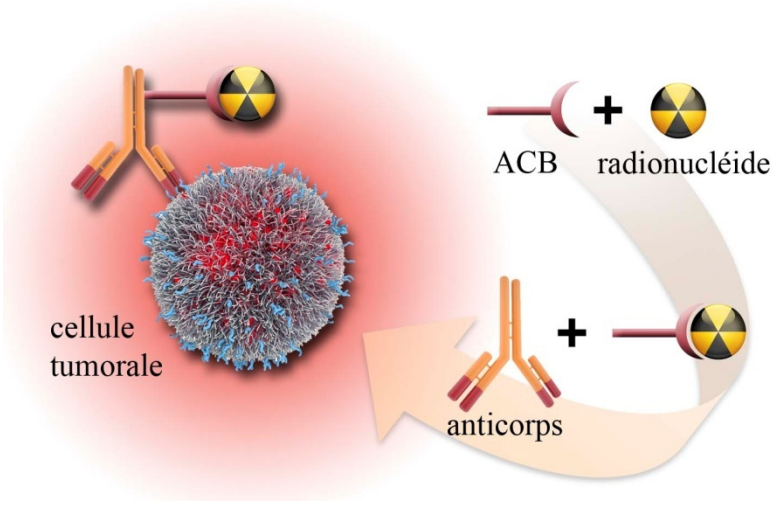


Figure XVIII. ***Principe de la radiothérapie ciblée (médecine nucléaire).***

Il a donc été décidé de s'orienter vers une voie humide, après décroissance de ^{210}At, où la séparation Bi/Po est réalisée dans un système biphasique après dissolution de la cible de Bi. Une liste de méthode tirée de la littérature est donnée dans le (Tableau I.)

Extractant	Phase organique	Phase aqueuse	Extraction du polonium (%)	Référence
Diéthyldithiocarbamate de cuivre Cu(DDTC)$_2$	Cu(DDTC)$_2$ - CCl_3	1,06 M HNO_3	55	49
		0,07-0,72 M HNO_3	65 -86	49
		0,03 M HNO_3	60	49
Phosphate de tributyle (TBP)	TBP(20%)-Dibutylether	6 M HNO_3	99	50
	TBP(10%)-DEKALIN	7-9 M HCl	98	51
	TBP (10%)-ortho xylene	7 M HCl	85%	52
Triuylamine	Triuylamine -xylene	7 M HCl	91%	53
Dibutyl carbitol (DBC)	DBC	3 M HNO_3	91%	54

Tableau II. Méthodes proposées dans la littérature pour la séparation Po/Bi par liquide- liquide extraction.

Il a été décidé de travailler avec le ligand couramment utilisé dans le domaine du nucléaire, le phosphate de tributyle, couramment appelé TBP.

III.3. Résultats

Les résultats obtenus indiquent que le polonium 210 peut être produit par le bombardement de bismuth 209 avec un faisceau de particules alpha de 37,019 MeV *via* la réaction nucléaire ^{209}Bi (α, 3n) ^{210}At (8,1 heures) qui décroit en polonium 210. Afin d'optimiser les conditions de séparation Bi/Po, après dissolution de la cible de Bi, plusieurs paramètres ont été testés (nature de la phase organique, concentration en TBP, milieu d'extraction et de dés-extraction). Les traces de ^{210}Po produites (2,6 10^{-13} mol, soit 10 KBq) sont isolées des quantités macroscopiques de bismuth (0,028 mol) provenant de la cible selon une méthodologie d'extraction liquide-liquide. Le polonium peut être totalement récupéré (96,0 ± 4,7%) par extraction avec le TBP (10% en solution dans le *p*-

xylène) à partir d'une solution de HCl 7 M tout en laissant la plus grande partie du Bi dans la phase aqueuse. La faible quantité de Bi extraite est éliminée par lavage avec une solution de HCl 7M. ^{210}Po est finalement récupéré en phase aqueuse en milieu HNO_3 9 M avec un rendement de 95,9 ± 4,1 %. Le rendement global de récupération du ^{210}Po est de 85,2 ± 4,5 % et les analyses réalisées montrent des bonnes puretés isotopique et chimique.

Une partie des données obtenues en milieu HCl pour permettre de définir les conditions optimales de séparation ont été exploitées afin d'identifier les espèces présentes en phases aqueuses, ainsi que celles extraites en phases organiques. Le tableau III. ci-dessous reprend l'ensemble de résultats obtenus.

Réaction	**logarithme des constantes thermodynamiques**
Équilibre aqueuse:	
$PoCl_4 + 2Cl^- \rightleftharpoons PoCl_6^{2-}$	4,6±0,2
$Po(OH)_2Cl_2 + 4Cl^- + 2H^+ \rightleftharpoons PoCl_6^{2-} + 2H_2O$	2,7±0,8
Équilibre d'extraction:	
$PoCl_6^{2-} + H^+ + 2\overline{TBP} \rightleftharpoons \overline{HPoCl_5(TBP)_2} + Cl^-$	2,14± 0,07
$PoCl_6^{2-} + 4\overline{TBP} \rightleftharpoons \overline{PoCl_4(TBP)_4} + 2Cl^-$	3,35± 0,08
$PoCl_6^{2-} + 2H_2O + 2\overline{TBP} \rightleftharpoons \overline{Po(OH)_2Cl_2(TBP)_2} + 2H^+ + 4Cl^-$	2,18± 0,06

Tableau III. Résultats des données thermodynamiques de spéciation polonium (SIT paramètre pour $PoCl_6^{2-}$: ε = -0,01 ± 0,008)

IV. Les agents de décorporation du Polonium

IV.1. Approches proposées dans la littérature

Il n'existe pas de ligand spécifique pour le polonium. Les travaux réalisés dans le domaine ont tous la même approche : « on teste sur des souris et on voit ». En effet, la chimie de coordination du polonium n'est pas bien définie et les études publiées sont plus intéressées par le test d'agents chélatants déjà connus pour être efficaces pour d'autres ions métalliques [31].

Chimiquement, le polonium a tendance à se lier le plus favorablement avec des atomes de soufre ou d'azote. Suivant la classification HSAB de Pearson [55], il s'agit d'un acide « mou ». C'est pourquoi, les ligands classiques de type EDTA (acide éthylène diamine tétraacétique) et DTPA (acide diéthylene triamine pentaacétique) ne sont pas adaptés pour la décorporation du Po-210.

Aujourd'hui, le chélate recommandé est le dimercaprol (2,3-dimercaptopropanol) ou British Anti Lewisite (BAL). Il est en usage dans la communauté médicale depuis 1941 soit depuis plus de 70 ans. Il est utilisé majoritairement comme chélateur pour traiter des intoxications à l'arsenic, au mercure, à l'étain ainsi qu'à l'or et au plomb (saturnisme). Le dimercaprol s'administre par voie intramusculaire. Le traitement par le dimercaprol entraîne divers effets indésirables : élévation de la pression artérielle, tachycardie, nausées, vomissements, céphalées, sensations de brûlures de la bouche, de la gorge, du pénis, salivation, lacrymation, rhinorrhée et douleurs diverses.

Trois autres dérivés chimique du BAL, également développés pour la décorporation des métaux, l'acide 2,3-dimercapto-1-propanèsulfonique (DMPS), l'acide N-(2,3-dimercaptopropyl)phthalamidique (DMPA) et l'acide méso dimercaptosuccinique (DMSA), apparaissent également intéressant pour éliminer le Po-210 suite à une contamination effectuée sur des rats [31].

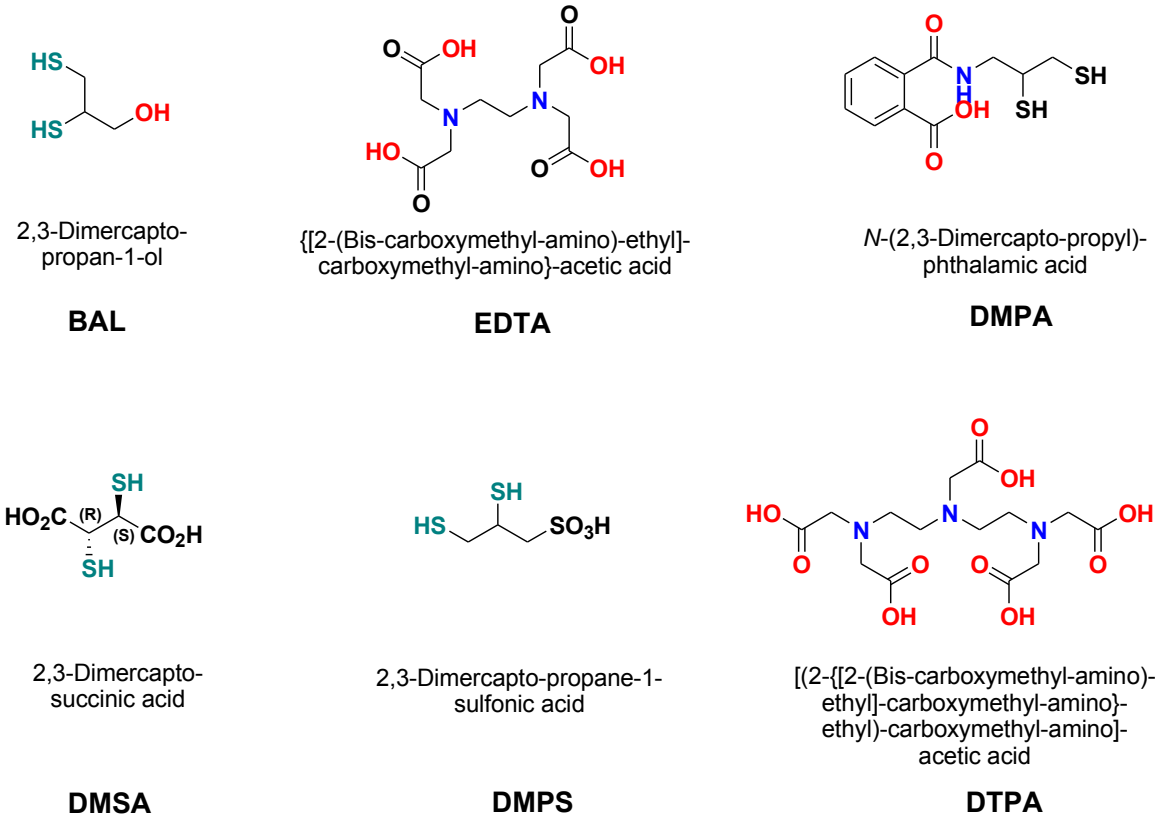


Figure XIX. ***Structure des ligands mentionnés dans le texte***

IV.2. Contexte de ce travail

Dans ce travail, on propose une approche plus rationnelle, de type "bottom-up" en réfléchissant aux critères que devraient présenter le ligand pour être un bon agent

décorporant :

- Il doit présenter une forte constante de stabilité vis-à-vis du polonium et donc présenter les hétéroatomes avec lesquels le polonium présente une forte affinité. Comme cela a été dit au point précédent, il faut privilégier le soufre et l'azote.

- Il doit présenter une structure adaptée pour complexer le polonium ; le cas idéal est que la molécule soit pré-organisée pour permettre une stabilisation optimale du polonium par rapport aux autres métaux présents dans l'organisme. Il n'existe pas de structure de complexes du polonium. Par contre, des études théoriques indiquent que le nombre de coordination du polonium est de 6 [31]. Ceci est cohérent avec l'existence présumé de complexe de type $Po(X)_6^{2-}$ en milieu fortement acide ($X=Cl^-$, Br^- et I^-) [5].

- La cinétique de complexation doit être rapide ce qui élimine les structures macrocycliques trop rigides.

- Dans le cas particulier de la décorporation, les caractéristiques du ligand sont importantes pour permette de cibler les endroits du corps où est localisé le polonium. Si le traitement est administré rapidement, il est nécessaire d'utiliser un ligand hydrosoluble qui puisse aller « chercher le polonium » dans le sang. Avec le temps, le polonium peut devenir moins disponible et il faudra que la molécule puisse accéder aux endroits cibles du polonium (comme le foie par exemple). Le caractère lipophile du ligand devient à ce moment-là important. D'autre part, une vectorisation

possible du ligand pourrait être intéressante.

Sur la base de ces points, nous proposons les molécules du type « N_2S_2/N_4 ». Elles présentent une plateforme dans laquelle le métal trouve quatre sites de coordination dans un environnement plan carré. La liberté conformationnelle de la molécule permet un arrangement de type N_4 ou N_2S_2 au niveau des thiazoles par libre rotation autour de la liaison « hétérocycle-N ». L'ajout de deux bras chélatants sur la structure « N_2S_2/N_4 » permettrait de compléter la sphère de coordination à 6 et ainsi d'augmenter la constante de complexation du ligand pour le métal. D'autre part, la présence de fonctions acides carboxyliques rendrait la molécule hydrosoluble sachant qu'une grande partie de la molécule peut être considérée comme hydrophobe. Enfin, la chaine aminoéthyle sur le noyau benzénique permettrait une vectorisation ultérieure des molécules après couplage sur un vecteur biologique (Figure XX.).

Les objectifs du travail étaient donc d'une part de synthétiser les molécules $N_2S_2O_2$ et N_2S_2 et d'autre part d'estimer leur intérêt par rapport aux molécules de référence que sont le BAL et le DTPA. Même si le DTPA n'est pas, *a priori*, une molécule intéressante pour la décorporation du Po, il s'agit d'une molécule de choix pour laquelle de nombreuses données sont disponibles dans la littérature (constantes de stabilité, structure), et plus particulièrement pour les éléments tétravalents [56].

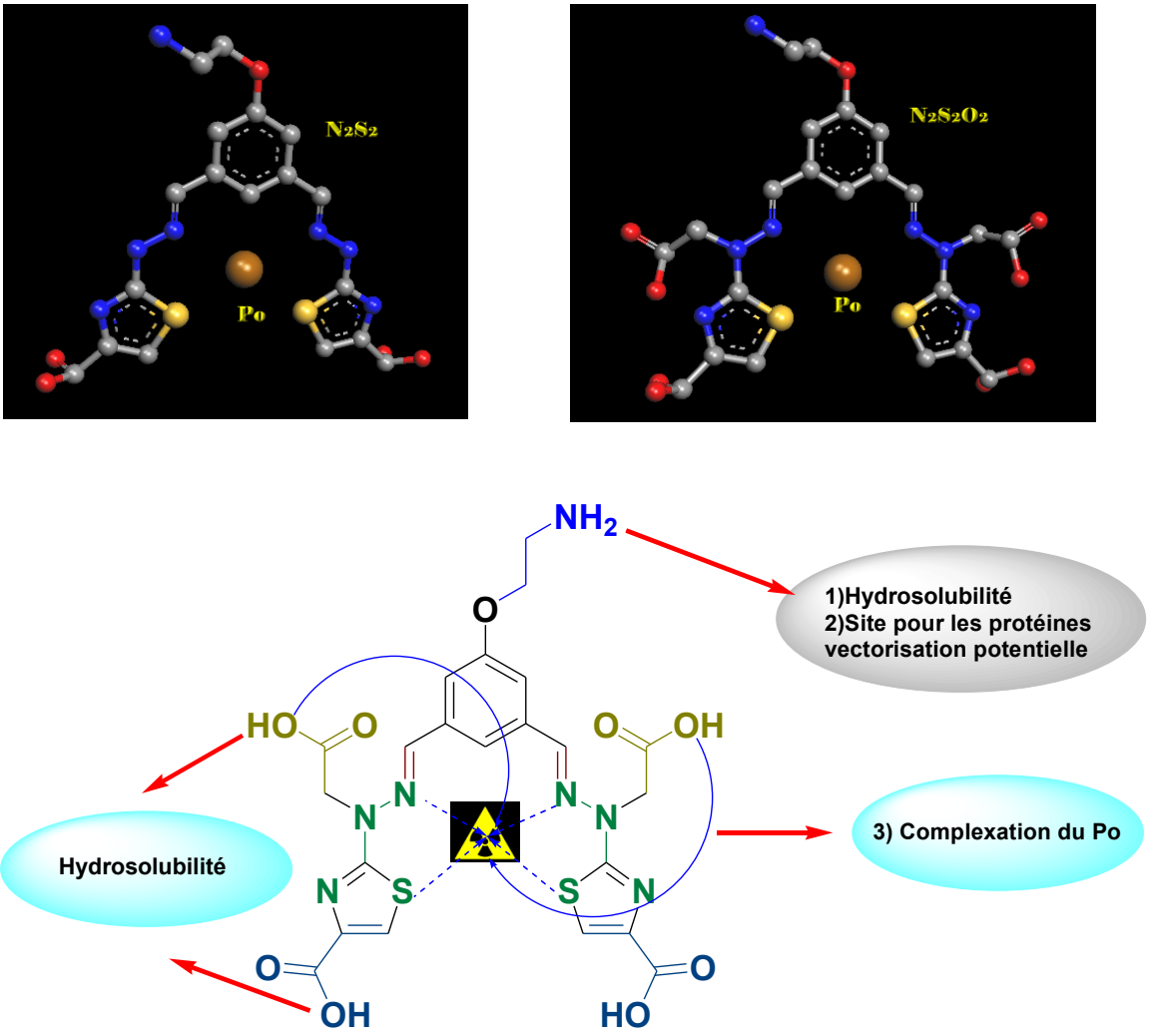


Figure XX. Structure et la propriétés des ligands proposés $N_2S_2O_2$ et N_2S_2

Dans le cadre de travail, nous nous sommes limité à comparer les propriétés de complexation des divers agents chélatants vis-à-vis du Po(IV) au pH physiologique, c'est-à-dire à pH=7,4 dans un système biphasique liquide-liquide. Ces données permettent d'estimer la force de complexation et le caractère lipophile du complexe formé.

La méthode utilisée pour la détermination de la force du complexation est basée les travaux de Schubert dans les années 1950 [57-59]. Il a développé son concept autour du partage liquide/liquide et l'utilisation de résines échangeuses d'ions pour déterminer les propriétés d'interaction entre un radiométal à l'échelle des traces et un agent complexant. Cette méthode se base sur le partage de l'isotope entre deux phases (aqueuse et organique) non miscibles. Dans ce travail, nous avons utilisé une molécule extractante, la dithizone. La dithizone est un composé organique contenant du soufre. C'est un bon ligand, qui forme des complexes avec de nombreux métaux tels que polonium, plomb et le mercure [60].

Afin de déterminer la constante d'interaction du polonium, la méthode s'organise en deux étapes. Dans un premier, il faut étudier le comportement du polonium entre deux solvants non miscible en fonction de différents paramètres (pH, temps d'agitation….) en présence de la molécule extractante qui permet d'extraire le polonium de la phase aqueuse en l'entrainant dans la phase organique. Ces paramètres sont choisis de façon à avoir un coefficient de partage supérieur à 1.

$$D = \frac{\overline{[Po]}}{[Po]} \quad (1)$$

D le coefficient de partage ; $\overline{[Po]}$ la concentration du polonium dans la phase organique et $[Po]$ sa concentration dans la phase aqueuse.

Le coefficient de partage reflète l'affinité de la molécule pour la phase organique par rapport à son affinité pour la phase aqueuse. Une variation de ce coefficient traduit un changement de spéciation dont l'origine peut être déduite d'une approche par modélisation basée sur le loi d'action de masse.

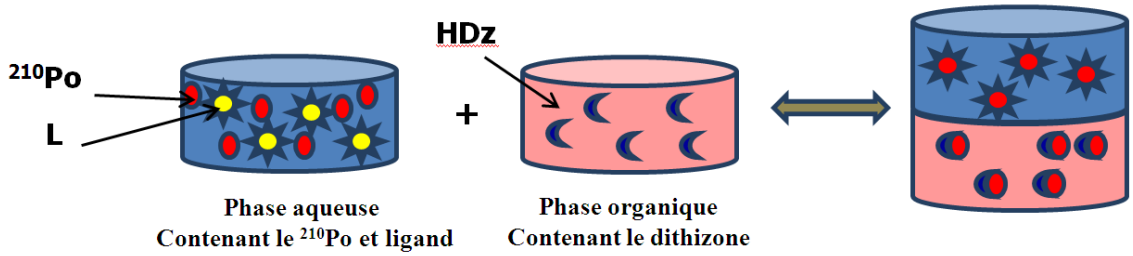


Figure XXI. ***Schéma de partage du polonium entre dexs phases non miscibles***

IV.3. **Résultats**

En ce qui concerne la partie synthèse organique nous avons mis au point une stratégie efficace et générale de ligands qui nous a permis d'accéder aux deux molécules cibles N_2S_2 et $N_2S_2O_2$. Le ligand tétradentate N_4/N_2S_2 est accessible en 7 étapes avec un rendement global de 16% à partir de l'acide 5-hydroxyisophthalique commercial. Les étapes clés sont d'une part la réduction sélective des fonctions esters en aldéhydes correspondants et la cyclisation de la *bis*-thiosemicarbazone en *bis*-thiazole par action de bromopyruvate d'éthyle. Afin de disposer du ligand hydrosoluble $N_2S_2O_2$ nous sommes repartis du ligand protégé N_4/N_2S_2 et nous avons ajouté deux fonctions méthylesters par alkylation des amines secondaires. La réaction à lieu en milieu basique, en présence de

tertio-butanolate de potassium, avec un rendement modeste de 12% mais non optimisé. La raison est due (i) à la difficulté de purification du produit final qui est pollué par du produit de départ et du composé mono alkylé, les trois molécules étant très difficiles à séparer par chromatographie sur silice, (ii) à la présence des différents groupements protecteurs susceptibles de réagir avec la base lors de la réaction de couplage. Quoiqu'il en soit, après déprotections des esters et de l'amine, la molécule hexadentate hydrosoluble cible $N_2S_2O_2$ est obtenue avec un rendement de 75%.

La deuxième partie de ce travail était de déterminer la capacité des ligands N_2S_2 et $N_2S_2O_2$ à complexer le Po (IV) à pH = 7,4. Les résultats de l'étude comparative indique l'ordre de stabilité suivant: N_2S_2 (N_4) <DTPA << BAL <$N_2S_2O_2$ (N_4O_2). L'importance des deux bras de liaison pour $N_2S_2O_2$ est donc vérifiée. De plus, l'interaction Po/ $N_2S_2O_2$ apparaît plus forte que pour le system Po/ BAL. Ce résultat indique que la molécule $N_2S_2O_2$ l'interaction est plus forte que pour les BAL, cela rend $N_2S_2O_2$ est un intéressant agent chélatant potentiellement intéressant comme agent décorporant.

Bibliographie du Partie I

1. Curie, P.; Sklodowska-Curie, M., Sur une substance nouvelle radioactive contenue dans la pechblende. *Compte Rendus Acad. Sciences* **1898,** 127, 175-178.
2. Goriely, S.; Samyn, M.; Pearson, J. M., Further explorations of Skyrme-Hartree-Fock-Bogoliubov mass formulas. VII. Simultaneous fits to masses and fission barriers. *Physical Review C* **2007,** 75, (6), 064312.
3. Hawkes, S. J., Polonium and Astatine Are Not Semimetals. *Journal of Chemical Education* 87, (8), 783.
4. Sklodowska-Curie, M., *Compte Rendus Acad. Sciences* **1898,** 126, 1101.
5. Figgins, P. E., *The radiochemistry of polonium*. Subcommittee on Radiochemistry, National Academy of Sciences-National Research Council; available from the Office of Technical Services, Dept. of Commerce: Washington, **1961**.
6. Curie, M., *Revue Generale des Sciences* **1898,** 10, 41.
7. Marckwald, W., Ueber den radioactiven Bestandtheil des Wismuths aus Joachimsthaler Pechblende. *Berichte der deutschen chemischen Gesellschaft* **1902,** 35, (4), 4239.
8. Marckwald, W., *Phys. Z.* **1902,** 4, 51.
9. Rutherford, E., *Philosophical Magazine* **1904,** 8, 636.
10. Rutherford, E., *Nature* **1905,** 71, 341.
11. Rutherford, E., *Philosophical Magazine* **1905,** 10, 290.
12. Meyer , S.; Schweidler, E., Abt. IIa ed.; **1906**; Vol. 115.
13. Collé, R.; Laureano-Perez, L.; Outola, I., A note on the half-life of ^{209}Po. *Applied Radiation and Isotopes* **2007,** 65, (6), 728-730.
14. Kelly, E. L.; Segré¨, E., Some Excitation Functions of Bismuth. *Physical Review* **1949,** 75, (7), 999.
15. Scott, B. R., Health risks evaluations for ingestion exposure of humans to polonium-210. *Dose-Response* **2007,** 5, 94-122.
16. Ansoborlo, E., Le Polonium. In Nantes, 2010.
17. Nho-Kim, E. Le polonium-210 dans les aérosols : contribution à l'étude des feux de savanes et des émissions volcaniques. Paris, **1996**.
18. Sabroux, J.C. *Le radon, traceur de phénomènes naturels. In : Mettivier H et Robé MC, Le radon de l'environnement à l'homme.*; Collection IRSN , EDP Sciences: Les Ulis (France), **1998**; pp 31-49.
19. UNSCEAR, *Sources and Effects of Ionizing Radiation.* Annex B :Exposures from natural radiation source ed.; United Nations Scientific Committee on the Effects of Atomic Radiation: Vienne, **2000**; Vol. I : Sources, p 84-141.
20. Pietrzak-Fils; Z., *The Science of the Total Environment* **1997,** 203, 157-165.

21. Singh, D. R.; Ninikelani, S. R., Measurement of polonium activity in Indian tobacco. *Health Phys.* **1976,** 31, 393-394.
22. Radford, E. P.; Hunt, V. R., Polonium-210: A Volatile Radioelement in Cigarettes. *Science* **1964,** 143, (3603), 247-249.
23. Little, J. B.; Radford, E. P.; McCombs, H. L.; Hunt, V. R., Distribution of Polonium210 in Pulmonary Tissues of Cigarette Smokers. *New England Journal of Medicine* **1965,** 273, (25), 1343-1351.
24. Little, J. B.; Radford, E. P.; Holtzman, R. B., Polonium-210 in Bronchial Epithelium of Cigarette Smokers. *Science* **1967,** 155, (3762), 606-607.
25. Kilthau, G. F., Cancer risk in relation to radioactivity in tobacco. *Radiologic technology* **1996,** 67, (3), 217-222.
26. Watson, A. P. *Polonium-210 and lead-210 in food and tobacco products: a review of parameters and an estimate of potential exposure and dose*; ORNL/TM-8831; Other: ON: DE83016395; TRN: 83-021686 United StatesOther: ON: DE83016395; TRN: 83-021686Thu Feb 07 01:32:12 EST 2008NTIS, PC A03/MF A01; 1.ORNL; ERA-08-049862; NTS-83-022125; EDB-83-188183English; 1983; p Medium: X; Size: Pages: 45.
27. Batarekh, K.; Teherani, D. K., Determination of polonium-210 in cigarettes from Syria. *Journal of Radioanalytical and Nuclear Chemistry* **1987,** 117, (2), 75-80.
28. Godoy, J. M.; Gouvea, V. A.; Mello, D. R.; Azeredo, A. M. G., $^{226}Ra/^{210}Pb/^{210}Po$ equilibrium in tobacco leaves. *Radiation Protection Dosimetry* **1992,** 117, 75-80.
29. Peres, A. C.; Hiromoto, G., Evaluation of 210Pb and 210Po in cigarette tobacco produced in Brazil. *Journal of Environmental Radioactivity* **2002,** 62, (1), 115-119.
30. Shabana, E. I.; Abd Elaziz, M. A.; Al-Arifi, M. N.; Al-Dhawailie, A. A.; Al-Bokari, M. M., Evaluation of the contribution of smoking to total blood polonium-210 in Saudi population. *Applied radiation and isotopes : including data, instrumentation and methods for use in agriculture, industry and medicine* **2000,** 52, (1), 23-26.
31. Ansoborlo, E.; Berard, P.; Den Auwer, C.; Leggett, R.; Menetrier, F.; Younes, A.; Montavon, G.; Moisy, P., Review of Chemical and Radiotoxicological Properties of Polonium for Internal Contamination Purposes. *Chemical Research in Toxicology* **2012**, 25, (8), 1551.
32. Coppin, F.; Roussel-Debet, S. *Polonium 210 et environnement*; Institut de radioprotection et de sureté nucléaire (IRSN): France, **2004**.
33. Froidevaux, P.; Baechler, S.; Bailat, C. J., Improving forensic investigation for polonium poisoning. *The Lancet* **2013,** 382, (9900), 1308.
34. Froidevaux, P.; Baechler, S.; Bailat, C. J., Supplementary appendix of Improving forensic investigation for polonium poisoning. *The Lancet* **2013,** 382, (9900), 1308.
35. Gruhier, F. *Polonium 210: L'assassin qui dévore les globules rouges Nouvel Observateur.*; 11-17 Janvier **2007**.

36. Beamer, W. H.; Maxwell, C. R., The Crystal Structure of Polonium. *The Journal of Chemical Physics* **1946,** 14, (9), 569-569.
37. Beamer, W. H.; Maxwell, C. R., Physical Properties of Polonium. II. X-Ray Studies and Crystal Structure. *The Journal of Chemical Physics* **1949,** 17, (12), 1293-1298.
38. Bagnall, K. W.; D'Eye, R. W. M.; Freeman, J. H., The polonium halides. Part I. Polonium chlorides. *Journal of the Chemical Society (Resumed)* **1955**, 2320-2326.
39. Curie; Debierne, *Compte Rendus Acad. Sciences* **1910,** 150, 389.
40. Lawson; E.g., *Sitzungsber.Akad. Wiss. Wien, Abt. IIa* **1915,** 124, 509-637.
41. Ansoborlo, E.; Aupiais, J.; Baglan, N.; Bertaux, M.; Biscarrat, C., ,; Bonniec, F.; Chevallier, P.; Curnier, J.-P.; Decosse, H.; Duda, J.; Floris-Fleury, S.; Grangeon, T.; Henry, A.; Liozon, G.; Losset, Y.; Maillard, C.; Maloubier, D.; Mokili, B. M.; Pierre, S.; Cassette, P.; Loidl, M.; Poilane, F.; Ramadier, J.-J.; Tauvel, Y.; Tenailleau, L., *Mesure du Rayonnement alpha*. Edition TEC & DOC Lavoisier: **2012**.
42. Godoy, J. M.; Schuttelkopf, H. *Eine radiochemische Methode zur Bestimmung von Po-210 in Umweltmaterialien*; KfK 2987; Kernforschungszentrum Karlsruhe.: 1980.
43. Ordonez-Regil, E.; Iturbe, J. L., Isolation and electroplating of ^{210}Po. *J. Radioanal. Nucl. Chem.* **1993,** 175, 47–53.
44. Smith, J. D.; Hamilton, T. F., Improved technique for recovery and measurement of polonium-210 from environmental materials. *Anal. Chim. Acta* **1984,** 160, 69–77.
45. Rieth, U.; Hummrich, H.; Kratz, J. V. electrodeposition of Po-210 on various electrode materials. D-55099, **2002**.
46. Bagnall, K. W., *Chemistry of the Rare Radioelements*. A.E.R.E: Harwell. London, **1957**.
47. Chung, Y. H.; Lee, C. S.; Nahm, K. Y.; JooJ, K. S.; Chai, S., Chun, ; K.S., Cross Sections of Bismuth and Polonium Isotopes in the Reaction of 209Bi with Protons. *Journal of the Korean Physical Society* **2011,** 59, (2), 1007-1010.
48. Zimmermann; R., La radioactivité au service du diagnostic et de la thérapie EDP Sciences. *La médecine nucléaire* **2006**
49. Wai; C.M.; Lo; J.M., Extraction and separation of ^{210}Pb, ^{210}Bi and ^{210}Po by diethyldithiocarbamate. *Radiochem. Radioanal. Lett.* **1982,** 50, 293-298
50. Karraker, D. G.; Templeton, D. H., Polonium Isotopes Produced with High Energy Particles. *Physical Review* **1951,** 81, (4), 510.
51. Bagnall, K. W.; Robertson, D. S., Solvent extraction studies with polonium. *Journal of the Chemical Society (Resumed)* **1957**, 509-512.
52. Chen.; YM.; Shu; RY., *J. Chin. Chem. Soc.* **1966,** 13, 82-89.
53. Sheppard, J. C.; Warnock, R., The distribution of bismuth (III) and polonium (IV) between trilaurylamine solutions of xylene and hydrochloric and hydrobromic acid

solutions. *Journal of Inorganic and Nuclear Chemistry* **1964,** 26, (8), 1421.
54. Schulz, W. W.; Richardson, G. L., Dibutyl Carbitol Solvent Extraction of Polonium-210 from Nitric Acid Solutions of Irradiated Bismuth. *Industrial & Engineering Chemistry Process Design and Development* **1968,** 7, (1), 149.
55. Pearson, R. G., Hard and Soft Acids and Bases. *Journal of the American Chemical Society* **1963,** 85, (22), 3533.
56. Brown, M. A.; Paulenova, A.; Gelis, A. V., Aqueous Complexation of Thorium(IV), Uranium(IV), Neptunium(IV), Plutonium(III/IV), and Cerium(III/IV) with DTPA. *Inorganic Chemistry* **2012***,* 51, (14), 7741.
57. Schubert, J., The Use of Ion Exchangers of the Determination of Physical-Chemical Properties of Substances, Particularly Radiotracers, in Solution. I. Theoretical. *The Journal of Physical and Colloid Chemistry* **1948,** 52, (2), 340.
58. Schubert, J.; Richter, J. W., The Use of Ion Exchangers for the Determination of Physical-Chemical Properties of Substances, Particularly Radiotracers, in Solution. II. The Dissociation Constants of Strontium Citrate and Strontium Tartrate. *The Journal of Physical and Colloid Chemistry* **1948,** 52, (2), 350.
59. Schubert, J.; Richter, J. W., *J. Am. Chem. Soc.* **1951,** 71, 4488-4489.
60. Suganuma, H., Solvent extraction study on the hydrolysis of tracer concentration of polonium(IV) in perchlorate solution. *Journal of Inorganic and Nuclear chemistry* **1981,** 43, 2101-2104.

Partie II: Résultats

Les principaux résultats de ma thèse sont détaillés dans cette partie. Nous avons choisi de présenter ces derniers au travers des quatre publications qui sont issues de mon travail.

Le but de la première publication est de faire une revue liée à la toxicité du Po. Ce travail a été initié par E. Ansoborlo du CEA. Je suis intervenu uniquement pour la partie bibliographique liée aux propriétés physico-chimiques du Po. Bien que mon implication ait été limitée, la présentation de l'article dans le document me paraissait intéressante en complément du travail bibliographique qui a été fait dans la partie I du manuscrit.

Les trois autres articles sont issus en totalité de mon travail. Deux sont liés à mon premier objectif qui était la production du Po-210: un article présente les résultats de l'étude de production du Po-210 alors que l'autre a pour objectif d'interpréter une partie des données qui ont permis d'optimiser les conditions de séparation Bi/Po-210. Le quatrième article est directement lié au deuxième objectif de ma thèse.

Article 1 : Review of chemical and radiotoxicological properties of polonium for internal contamination purposes

Review of chemical and radiotoxicological properties of polonium for internal contamination purposes

I. Abstract

The discovery of polonium (Po) was first published in July 1898 by P. and M. Curie. It was the first element to be discovered by the radiochemical method. Polonium can be considered as a famous but neglected element: only a few studies of polonium chemistry have been published, mostly between 1950 and 1990. The recent (2006) event in which ^{210}Po evidently was used as a poison to kill A. Litvinenko has raised new interest in polonium. 2011 being the 100th anniversary of Marie Curie Nobel Prize in Chemistry, the aim of this paper is to review several aspect of polonium linked to its chemical properties and its radiotoxicity, including : i) its radiochemistry and interaction with matter; ii) its main sources and uses; iii) its physico-chemical properties; iv) its main analytical methods; v) its background exposure risk in water, food, and other environmental media; vi) its biokinetics and distribution following inhalation, ingestion and wound contamination; vii) its dosimetry and viii) treatments available (decorporation) in case of internal contamination.

Keywords: Polonium, radiochemistry, radiotoxicity, biokinetics, decorporation

I.1 Introduction and history of polonium discovery

At the turning point of the XIXth century, the discovery of Röntgen rays (X-rays) attracted most of the physicists. By the end of 1897 a chemist, Marie Curie born Sklodowska, undertook her experiments on Becquerel's rays, also named the mysterious "uranium rays", a subject which seemed suitable for a thesis.

Having found that the radioactivity of uranium and thorium minerals was much greater than could be predicted by the content of isolated uranium and thorium, Pierre and Marie Curie undertook the extraction of the substance responsible for this anomaly from different uranium minerals such as chalcolite, uranite, and the famous Joachimsthal pitchblende.

Their note to the French Academy of Sciences in April 1898[1] put forward a possible explanation: ". . . the minerals may contain an element much more active than uranium".

Since the only known property of this hypothetical substance was its radioactivity, P. and M. Curie developed a new experimental radiochemical method which has remained the basis of all radioactive chemistry. They had an excellent aid at their disposal: an electrometer for the measurement of weak electrical currents, which was constructed by P. Curie and based on the piezoelectric effect. Each isolated product was placed on one of the plates of a condenser and the conductivity acquired by the air was measured with the aid of this electrometer. They had not only an indication but quantitative information about the strength of the product in the active substance

They carried out separations of the various substances in the mineral and measured the radioactivity of each portion. They quickly found that the activity became concentrated,

partly with the alkaline earths and partly with the sulphides precipitated from acid solution.

At the end, they obtained a substance 400 times more active than uranium by applying the sublimation method (a method studied mostly by P. Curie) to the sulphide fraction that was concentrated by Marie as a result of different chemical steps.

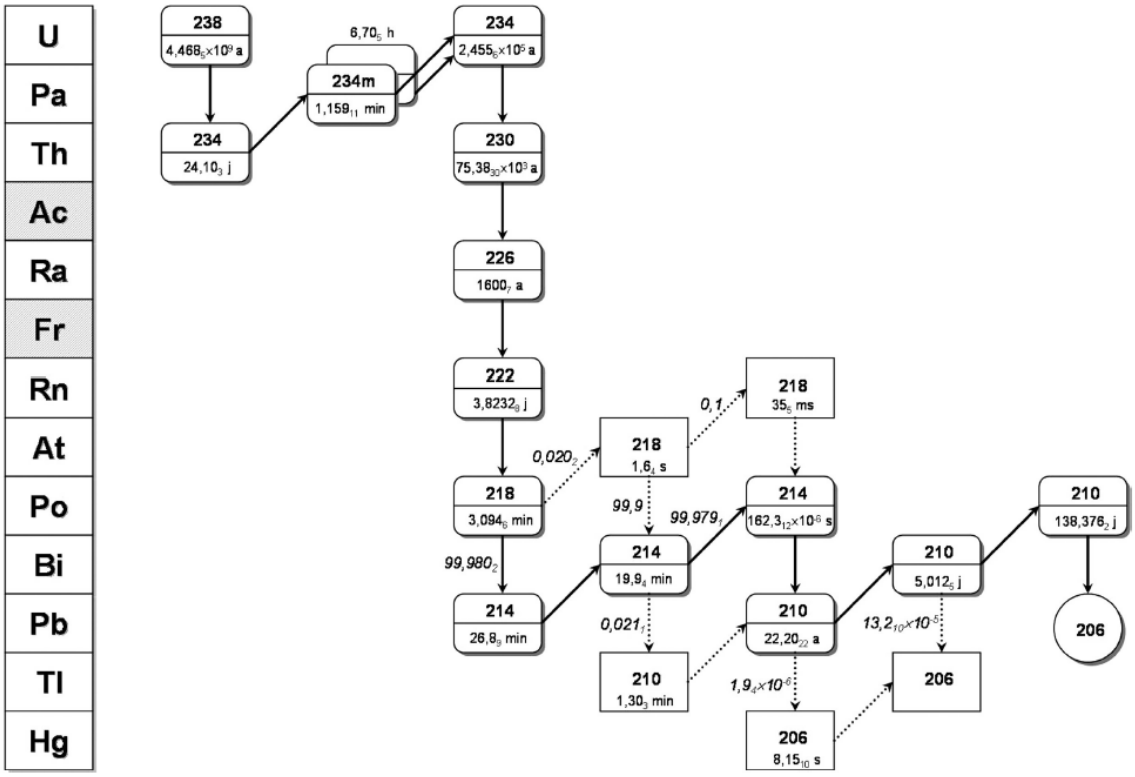


Figure 1: Natural uranium ^{238}U decay series leading to ^{210}Po.

The discovery of polonium (Po) was first published by P. and M. Curie in a Science Academy report (July 1898) with the following title: “On a new radioactive substance contained in pitchblende”. The Curies announced: “We believe therefore that the

substance which we have removed from pitchblende contains a metal not yet reported close to bismuth in its analytical properties. If the existence of this new metal is confirmed, we propose to call it polonium from the name of the country of origin of one of us". It was the first element to be discovered by the radiochemical method.

M. Demarcay examined the optical spectrum of the isolated element but was not able to distinguish any characteristic line apart from those ascribable to impurities.

This discovery led to the first controversy in radiochemistry between the Curies and German competitors (*e.g.* J. Elster , H. Geitel, F. Giesel and W. Marckwald)[2].

It was only a few years later, in 1910, that M. Curie and A. Debierne separated 2 milligrams of a final product from several tons of uranium ore residues. This product containing about 0.1 mg of polonium was characterised by a spark spectrum technique.

In 1911 M. Curie received the Nobel Prize in chemistry for the discovery of polonium and radium.

Polonium can be considered as a famous but neglected element. Only a few studies of polonium chemistry have been published, mostly between 1950 and 1990. Among these studies, one of the most complete document is the Gmelin Hanbook[3] on inorganic chemistry, with detailed chapters on: history; occurrence; nuclear and decay properties; production, isolation and purification; general properties of atoms and ions; analytical chemistry and methods; different sources and uses; radiological problems on handling; metabolism, toxicology and removal from the body; compounds; solution chemistry; and solvent extraction.

The recent (2006) event in which ^{210}Po evidently was used as a poison to kill the former

Russian spy Alexander Litvinenko has raised new interest in polonium, but most of the subsequently published papers dealing with radiotoxicological purposes are reviews rather than research studies, except in the field of decorporation studies. Nevertheless we should mention that there has been recently a growing number of research oriented papers focusing on polonium concentration and dose assessment in the environment.

2011 being the 100th anniversary of Marie Curie Nobel Prize in Chemistry[4], the aim of this paper is to review several aspect of polonium linked to its radiotoxicity, including:

i) its radiochemistry and interaction with matter;

ii) its main sources (natural and industrial) and main uses;

iii) its physico-chemical properties;

iv) its main analytical methods;

v) its background exposure risk in water, food, and other environmental media;

vi) its biokinetics and distribution following inhalation, ingestion and wound contamination;

vii) its radiotoxicity;

viii) Treatments available (decorporation) in case of internal contamination.

II. Radiochemistry

Polonium is the first element of the periodic table for which all of its isotopes (33 isotopes with masses ranging from 188 to 220) are radioactive. Polonium-210 is the predominant naturally occurring and most widely available isotope of polonium. It is a radioactive decay product in the natural uranium-238 decay series (Figure 1); along with

lead-210 it is one of two relatively long-lived decay products of radon- 222. Polonium-210 is an alpha emitter (5.29 MeV) that has a half-life of 138.4 days, and a single gamma ray (0.803 MeV) with a very low abundance (1.23×10^{-5}).

Its high specific activity (1.66×10^{14} Bq/g) is a very important aspect of its radiochemistry as well as its toxicity. For example, 1 µg of ^{210}Po emits as many alpha particles per second as 4.5 mg of ^{226}Ra, 262 mg of ^{238}Pu, or 446 kg of ^{238}U. Polonium-210 decays directly to its stable daughter isotope ^{206}Pb. The two other most stable isotopes are ^{209}Po ($T_{1/2}$=102 y) and ^{208}Po ($T_{1/2}$=2.898 y). Recently, large amounts (approximately 2 g) of those isotopes have been produced during the MEGAPI experiment[5] with the irradiation of a lead-bismuth target by a proton beam. Under these conditions, polonium is formed as a product of proton capture (p, n) and neutron capture (n, γ) reactions of ^{209}Bi. During the experiments, a similarity in the behaviour of polonium concerning evaporation was also observed with the chalcogen series (S, Se and Te)[6]. It should also be noted that Po was purified from the lead-bismuth eutectic by alkaline extraction[7] following the traditional separation process for ^{210}Po production[8].

The energy released by the decay of ^{210}Po is so large (140 watts/g) that a capsule containing about half a gram reaches a temperature above 500°C. The linear energy transfer (LET) of ^{210}Po is approximately 100 keV/µm in water, which is similar to biological tissues. Its average distance in such media is ~ 50 µm. This means it can penetrate cells of the human body, which are typically between 10 and 30 µm in diameter.

III. Extraction of polonium from pitchblende by the Curies

The Joachimsthal pitchblende was dissolved with acids, and the solution was treated with hydrogen sulphide. Uranium and thorium remained in solution, and the precipitated sulphides appeared to contain a highly active substance together with lead, bismuth, copper, arsenic, and antimony. This substance was completely insoluble in the ammonium sulphide which separated it from arsenic and antimony. The sulphides that were insoluble in ammonium sulphide were dissolved in nitric acid, and the active substance was partially separated from lead by selective dissolution with dilute sulphuric acid of the active substance co-precipitated with lead sulphate

The active substance present in solution with bismuth and copper was then precipitated completely by ammonia, allowing an efficient separation from copper. Only bismuth remained with the active substance.

The Curies observed that on heating pitchblende one obtains some highly active products by sublimation. This led them to a final separation process Po/Bi based on the difference in volatility between the active sulphide and bismuth sulphide. The sulphides were heated in a vacuum to about 700° C in a tube of Bohemian glass. The active sulphide deposited in the form of a black coating in those regions of the tube which were at 250° to 300° C, while the bismuth sulphide stayed in the hotter parts. More and more active products were obtained by repetition of these different operations.

Finally they obtained the purified substance, the so called “polonium”, whose activity was about four hundred times greater than that of uranium.

IV. Sources

Polonium is a rare natural element, existing in uranium ores in amounts of about 100 μg per ton of ore; however it is impractical to obtain commercial quantities by extraction. Polonium-210 could also be obtained by purifying aged radium sources, but handling large-activity radium sources is not simple and requires local shielding.

Consequently, the principal commercial method for producing significant quantities of ^{210}Po (a few grams) is to neutron-irradiate a bismuth-209 (stable isotope) target in a nuclear reactor. This forms radioactive bismuth-210, which has a half-life of 5 days. ^{210}Bi decays to ^{210}Po through beta decay.

V. Uses of polonium

Polonium-210 is mainly used in static eliminators (~ 100 mCi) in some specific processes such as paper rolling, manufacturing sheet into brush, and spinning synthetic fibres. It is electroplated onto a backing foil and inserted into a brush, tube or other holder. These devices generally need to be replaced every year because of the short half-life of this radioisotope.

Polonium-210 can also be combined with beryllium to produce neutron sources and was used as neutron-producing initiators of the first generation of atomic weapons. It has been investigated as a heat source for thermoelectric power devices for space applications.

VI. Physico-chemical properties

Because polonium has no stable nuclides, all fundamental investigations are generally derived from radiochemical studies at trace concentrations. Therefore, classical

spectroscopic tools cannot be applied to evaluate polonium chemistry at the molecular level. Due to those difficulties, the chemistry of Po remains poorly known, most of the basic chemical properties of polonium being published in the 1960ies.

Polonium metal is silvery grey in colour and interacts to varying extent with O_2, depending on temperature. The electron configuration of neutral polonium ([Xe] $4f^{14}$ $6s^2$ $5d^{10}$ $6p^4$) resembles those of selenium and tellurium. These three elements belong to the oxygen and sulphide family and are called chalcogenides. Belonging to this family of elements, it may be considered analogous to tellurium although the later behaves as a metalloid element and has a tendency to form oxoanions. On the other hand polonium may also be compared to bismuth, his left neighbour in the periodic table. The hydration form of bismuth in its +III oxidation state has also been studied and shows some similarities with that of the lanthanide cations in the +III oxidation state[9].

Polonium has several oxidation states (-II, +II, +IV and +VI), of which the tetravalent state Po (IV) is the most stable in aqueous solution[10]. Polonium can be classified as a soft element in the hard-soft acid-base (HSAB) classification of Pearson[11]. Polonium forms soluble salts with chlorides, bromides, acetates, nitrates and other inorganic anions.

One of the most important chemical properties from the biological perspective is its tendency, like most tetravalent elements, to hydrolyze and form colloids when there is sufficient mass available (K_s ($Po(OH)_4$) = 10^{-37})[12]. Its solubility product with sulphurs (K_s (PoS_2) = $10^{-28.3}$)[13] is important, and this property was used by the Curies to isolate polonium.

Table 1: Thermodynamic stability constant of polonium (Po(IV)) selected from IUPAC[14] .

Ligand	Medium	T	I	Log K	Reference
Chloride (Cl^-)	$NaClO_4$	24°C	1 M	$K_1 = 2.34$	(15)
Nitrate (NO_3^-)	$NaClO_4$	0°C	1 M	$K_1 = 0.56$	(16)
Hydroxide (OH^-)	$NaClO_4$	25°C	1 M	$K_1 = -048$	(17)
	$NaClO_4$	20°C	0.1M	$K_1 = -1.1$	(18)
	/	/	/	Ks = -37.0	(12)
Sulfide ($S^=$)	/	25°C	/	Ks = -28.3	(13)

Selected IUPAC thermodynamic stability constants[14-18] are given in table 1 for available ligands. Values for standard potentials of polonium in acid and basic solution are shown in Figures 2A and 2B[19]. Figure 3 shows the redox potential diagram, also called the Pourbaix diagram[20], giving the redox potential E(V) as a function of pH.

On the basis of these results, polonium speciation remains a challenge. To date, no complexation data is available for the –II, +II and +VI degrees of oxidation. For Po(IV), the data is dissimilar and sometimes even contradictory.

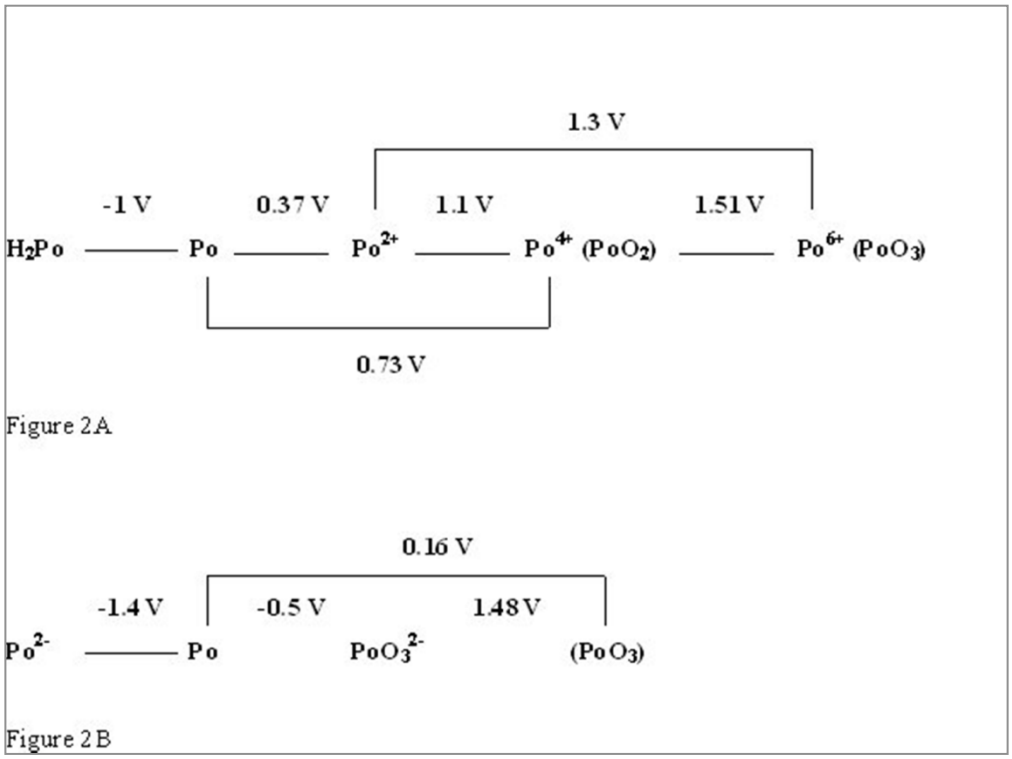


Figure 2: (2A) Standard electrode potential of Po in acid solution in V/ENH.
(2B) Standard electrode potential of Po in basic solution in V/ENH.

Concerning Po(-II), the only form admitted in aqueous solution corresponds to a dihydride (H_2Po), as Pourbaix suggested in the potential-pH diagram[20] (Figure 3). It is known that H_2Po is volatile and that the potential varies depending on the partial pressure of H_2Po.

At zero oxidation degree, polonium is a noble metal, thermodynamically stable in aqueous solution in the absence of oxidant. Nevertheless, it should be noted that the high specific activity of ^{210}Po, which is the most-studied isotope, modifies the behavior of this element, for example by oxidizing the air oxygen into ozone which then becomes a powerful polonium metal oxidant.

For Po(II), only the Po^{2+} species is admitted in highly acidic aqueous solution (pH<1).

Turning to the +VI degree of oxidation, only the solid form PoO_3 is admitted on the basis of the analogy with TeO_3, even though this form is probably not thermodynamically stable (Figure 3).

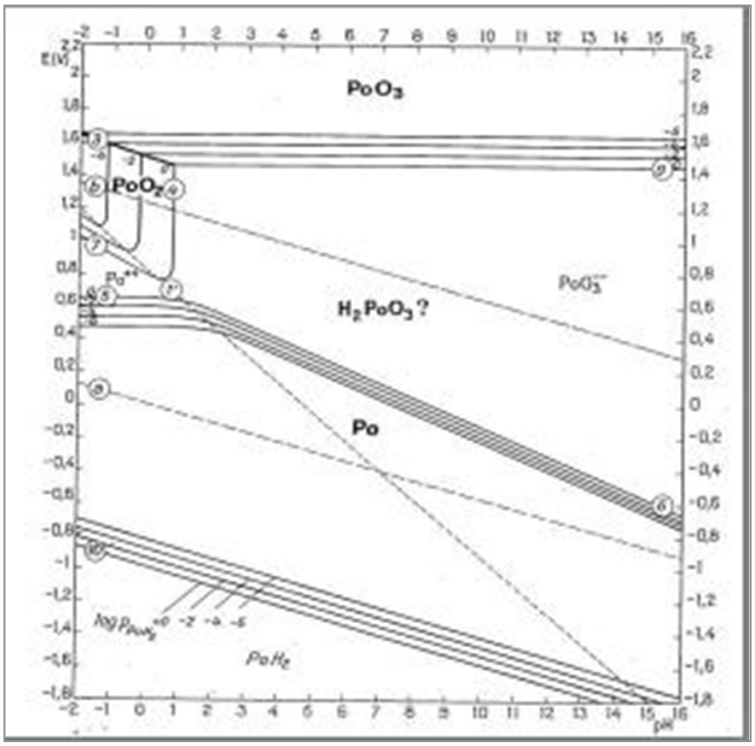


Figure 3: Polonium redox potential (E(V)) as a function of pH (Pourbaix diagram[20]).

For the +IV degree of oxidation, we find more data in the literature (table 1) which demonstrates soluble species and a solid form, PoO_2. In a non-complexing medium, the stability domains of Po(IV) hydroxides are shown in table 2. It should be noted that even for very low concentrations of Po, the formation of colloid and adsorption onto surfaces greatly complicate Po(IV) speciation in a non-complexing medium.

Recently, theoretical chemistry studies on the hydration of Po(IV) in solution, using

molecular dynamics and quantum mechanical computations, have enabled a better description of the hydration sphere[21-23]. Starting from the octahydrate aqua ion, molecular dynamics indicates a stabilization of coordination number at six. According to these theoretical approaches, a systematic study of the different hydrolyzed species derived from the hydrated Po(IV) in water, $(Po(H_2O)_n(OH)_m)^{(4-m)+}$ for $1 \leq m \leq 4$ and $4 \leq m+n \leq 9$, has been carried out. These theoretical computations[21-23] in solution conducted either by quantum chemical studies[21-22] or by *ab initio* molecular dynamics studies[23] seem to suggest that the most likely clusters are $[Po(H_2O)_5(OH)_2]^{2+}$, $[Po(H_2O)_4(OH)_2]^{2+}$ and $[Po(H_2O)_3(OH)_3]^{+}$.

Table 2: Speciation of Po(IV) depending on the pH in a non-complexing medium (I=1M, ambient temperature)[17]

pH<0.5	0.5<pH<2	2<pH<2.8	2.8<pH<3.5	3.5<pH	pH~12
Po^{4+}	$Po(OH)^{3+}$	$Po(OH)_2^{2+}$	$Po(OH)_3^{+}$	$Po(OH)_4$	$H_xPoO_3^{(2-x)-}$

In a complexing medium, the speciation of Po(IV) depends on the complexing capacity of the ligand. Moreover, given the high hydrolysis of Po(IV), complexation by numerous inorganic ligands like the ions chloride, perchlorate[24] , nitrate[25] or sulfate leads to mixed complexes in aqueous solution of the $Po(OH)_xL_y^{(4-x-y)+}$ or $Po(OH)_t(SO_4)_s^{(4-2s-t)+}$ type, depending on the acidity and the ligand concentration [15-16, 26-28]. Figgins[10] highlights the existence of hexachloride, hexabromide and hexaiodide complexes of Po(IV), their presence being controlled by the concentration of the corresponding inorganic ligand.

For organic ligands (Y) like the oxalate, acetate, citrate, tartrate, NTA and EDTA anions[10,28-29], the complexation of Po(IV) is much more complicated and the molecular form of the mixed complex has not yet been clearly defined: $PoY_y^{(4-ny)+}$, $Po(OH)_xY_y^{(4-x-ny)+}$, $POOY_y^{(2-ny)+}$. Given these different molecular forms, it is perhaps unwise to use the complexation constants without accurate information as to the stoichiometry of the complexes formed.

Polonium speciation in natural or environmental waters and in sediments.

Polonium-210 is generally present in natural waters in the form of Po(IV), which is slightly soluble due to the hydrolysis of Po(IV) and the formation of colloids, and has a high affinity for the particulate phase.

Polonium-210 is generally adsorbed on particles and colloids (mineral or organic). The major parameter controlling sorption / desorption is the redox state, with a strong decrease of ^{210}Po (in the particle form) when moving from oxic surface water to anoxic deep water[30]. This can be explained by the fact that ^{210}Po adsorbs to manganese oxides and is released when Mn(IV) is reduced to Mn(II). In addition, Benoit and Hemond[31] estimate that Po(IV) (in insoluble form) can be reduced to the state of Po(II) (in soluble form) at about the same time as the manganese[30] and in these anoxic conditions, it can precipitate as sulfide polonium[32].

Moreover, given the behavioral analogy of Po elements in the VI group (S, Se and Te), the bio-volatilization of Po in the form of an alkyl derivative has also been studied[33]. It must be remembered that the volatility of Po may well be due to the most reduced form, H_2Po, which is also volatile[3].

A review concerning the sorption of radio-elements on geological materials led to a proposition on the speciation of Po in underground water environmental conditions[34]. The major conclusion for Po in this work is that it is difficult to predict its solubility. Whilst solubility is imposed by the equilibrium of Po(s)/Po(II), it remains difficult to calculate Po solubility as little data exists on the complexation of Po(II), apart from the solubility of PoS(s). If such solubility is imposed by the solubility of Po(IV), it is necessary to consider not only the solubility of PoO_2, but also the evolution of the physicochemical form of the Po(IV) hydroxides under a colloidal form and sorbed onto surfaces[35-36].

Polonium speciation in biological medium

There is little information available on the interaction of polonium with ligands of biological media, including its broad similarities to sulfur (as a soft HSAB acid) and consequently its affinity for certain amino acids and proteins[37-38].

The interaction between ^{210}Po and metallothionein *in vivo* and *in vitro* was investigated using male Sprague-Dawley-rats. This type of interaction confirms his soft caracter in the Pearson classification (depending on its oxidation state). Polonium-210 was administered subcutaneously to rats as $^{210}PoCl_4$ at a dose of 9.2 x 10^5 Bq/kg/day for 3 days. Polonium-210 was observed to be incorporated into liver metallothionein. The isolation and identification of the metallothionein/^{210}Po peak was accomplished by chromatography on Sephadex G-75. *In vitro* studies confirmed this binding. The binding of ^{210}Po to metallothionein has implications that may help to explain some of the radiation damage caused by ^{210}Po intracellularly. This binding may be one of the factors slowing the

excretion of ^{210}Po from the body and therefore extending and increasing the radiation exposure and injury caused by it[38].

VII. Analytical methods for polonium

This short paragraph is not meant to be an exhaustive review of the literature covering all aspects of polonium analysis but merely a short description of main techniques available. A very complete review paper of analytical methodology has been recently published in 2007 by Matthews *et al.*[39] which includes more than hundred references.

Polonium has a tendency to volatilize at low temperature (~50° C) and to stick rather avidly to glass, which is a significant constraint for the sample preparation techniques and the assessment of analysis yield. Dry ashing cannot be used, and the use of wet-ashing procedure also shows some losses. Henricsson *et al.*[40] recently evaluated the polonium loss during wet-ashing by the use of a double-tracer technique and found values of about 17% when the samples were digested in microwave oven and about 30% when open glass beakers were used.

Numerous solvent extractants such as methyl isobutyl ketone (MIBK), isopropyl ether, and tributyl phosphate (TBP) are available for extraction or separation of polonium. Among the different extraction techniques for polonium, liquid-liquid extraction is currently used with solvents such as diethylammonium diethyldithiocarbamate DDTC[41], dithizone or copper dithizone in CCl_4 [42], carbon tetrachloride (CCl_4) or Tri-n-

octylphosphine oxide (TOPO), as well as a technique using extraction chromatography with strontium resin[43].

Remark: due to its high specific activity of 1.66 x 10^{14} Bq/g, the intense radiation of polonium samples can quickly decompose most organic complexing agents and even the solvents, and crystal structures of solids can be quickly altered or destroyed.

Figure 4: A thin film of polonium on a stainless-steel disk, the form in which it is sold as an alpha-particle source for scientific use.

Nowadays one of the main performing analytical techniques for ^{210}Po measurement is alpha spectrometry after deposition of a drop of the test solution on a metal disk followed by drying to give a uniform coating on the disk (Figure 4). Polonium has also the ability to deposit spontaneously on various metals, like silver, nickel or cupper[40]: this is a common method for preparing samples from environmental matrix for alpha spectrometry and it offers the possibility to separate Po from other metal ions without previous radiochemical separations. For the analysis of ^{210}Po in variety of food products

and bioassay samples a rapid and reliable radiochemical method coupled with a simple and compact plating apparatus was developed, validated, and applied[44]. Detectors generally used are semi-conductors (*e.g.* polysilicon-insulator-polysilicon (PIPS)) or scintillation (ZnS). Detection limits in waters are on the order of mBq/L or mBq/g[45]. Liquid scintillation counting is also available and offers the advantage of nearly 100% counting efficiency, combined with low background counting rates. For example, energy discrimination by window setting has been used to determine the concentration of each component of Pb-Bi-^{210}Po mixture without separation[46].

Polonium tracers (*e.g.* ^{208}Po or ^{209}Po) can also be used if any separation chemistry is to be carried out.

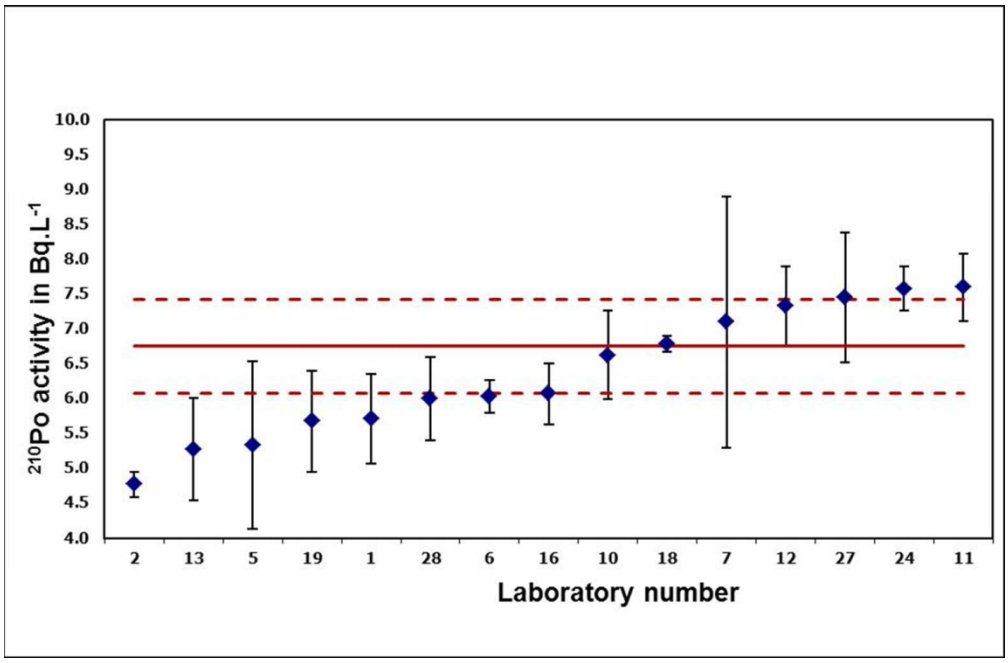


Figure 5: Results of an analytical intercomparison of ^{210}Po present in underground natural water. (Full line = certified value; dotted line = certified value ± uncertainty).

An intercomparison analysis of ^{210}Po and ^{210}Pb present in underground natural water (from Nigeria and Finland) was organized by CETAMA in 2008, gathering 15 laboratories using different analytical techniques. The aim of this round robin test was to estimate the ability of each laboratory to measure ^{210}Po applying ISO13161[45].

The results given in Figure 5 for ^{210}Po show the significant scattering of measurements around the reference value (6.7 Bq/L). This is mainly due to the presence of colloids and the simultaneous presence of ^{210}Po and ^{210}Pb, and consequently to the interference of ^{210}Pb decrease.

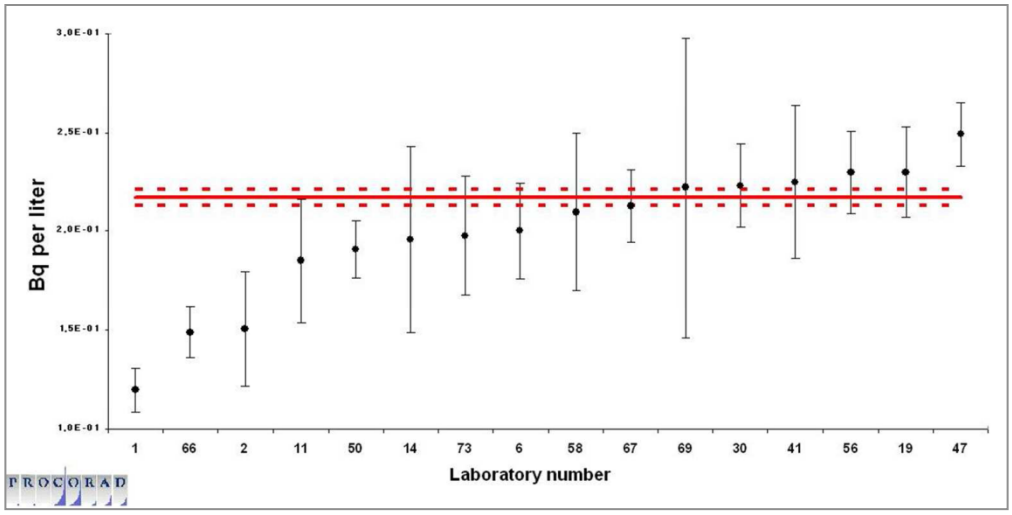


Figure 6: Proficiency testing results on biological samples for ^{210}Po organized by PROCORAD's association (2010). (Full line = certified value; dotted line = certified value ± uncertainty).

It is interesting to compare the results with the guidance level for members of the public of 0.1 Bq/L for ^{210}Po as a common natural and artificial radionuclide in drinking-water[47].

Another proficiency testing organized by PROCORAD's association, shows the performance of 16 laboratories on the determination of ^{210}Po inside urine samples. The method normally applies to a 24 hour urine sample. The urine is digested with nitric and

sulphuric acids, and then transferred into a round bottomed flask. After repeated nitric acid treatments, oxidation is completed. Polonium is precipitated on a manganese dioxide precipitate, which is centrifuged off, dissolved in hydrochloric acid, diluted with water and the polonium spontaneously deposited onto a silver disc, to produce a source suitable for alpha spectrometry. Figure 6 underlines the same discrepancies than for natural water with the effect of simultaneous plating of iron present in the urine sample. The target value was 0.217 ± 0.004 Bq/L and typical detection limits are around 0.005 Bq/L.

VIII. Background exposure risk in environment, water and food

This short paragraph is also not meant to be an exhaustive review of the literature covering all aspects of polonium exposure in environment but merely a short summarize on background exposure in environment, water and food. A recent review paper published in 2011 by Persson *et al.*[48], which includes more than hundred references, gives very useful and complete information about polonium in terrestrial environment and foodstuff.

^{210}Polonium and ^{210}Pb are naturally present in the terrestrial environment and are the final long-lived radionuclides in the decay of ^{238}U in the earth's crust. Some uranium ores can contain up to 100 μg ^{210}Po per ton.

The presence of ^{210}Po in the atmosphere is mainly due to the decay of ^{222}Rn diffusing from the ground. The range of activity concentrations of ^{210}Po in ground level air[48] is

0.03 - 0.3 Bq/m^3. Polonium-210 also can be emitted into the atmosphere during the calcining of phosphate rock as part of the production of elemental phosphorous or during volcanic eruption (*e.g.* Eyjafjallajökull eruption in 2010).

In drinking water from private wells the activity concentration of ^{210}Po is on the order of 7 - 50 mBq/L, whereas in rainwater and in marine waters it is on the order of 1 – 100 mBq/L and 1 – 5 mBq/L, respectively[48]. Recent measurement made in 2011 in some areas in Finland and USA have shown ^{210}Po activities in drinking water reaching until 7 Bq/L probably because of sulphur cycling involving microbial SO_4 reduction[49].

In aquatic environment (freshwater and marine), as polonium accumulates to plankton and subsequently to fish and mollusks the dose to human from marine food is substantial. It is often mentioned that the bioaccumulation of Po is higher in aquatic environment than in terrestrial environment so the Po in aquatic environment/food chain is important to mention. As the polonium concentration in fish can be about 1 Bq/kg and in mollusks even higher (~ 40 Bq/kg), the individuals that consume large amounts of marine products can get substantial dose[50-51].

Mosses and lichens efficiently capture ^{210}Po from atmospheric fallout and exhibit an inventory of ^{210}Po on the order of 0.5 - 5 kBq/m^2 in mosses and around 0.6 kBq/m^2 in lichens[48]. The activity concentration in lichens lays around 250 Bq/kg dry weight. Recent studies held in Nordic terrestrial ecosystems[52-53] have shown relatively high ^{210}Po concentration measured in Finland in wild mushrooms ranging from 10 – 1,200 Bq/kg dry weight[52], and activity concentrations of ^{210}Po in fauna (invertebrates, mammals, birds) ranging between 2 and 123 Bq/kg dry weight and in plants and lichens between 20 and

138 Bq/kg dry weight. The results showed that soil humus is an important reservoir for ^{210}Po and that fauna in close contact with this media may also exhibit elevated levels of ^{210}Po[53].

In some northern countries, reindeer and caribou graze lichen, resulting in an activity concentration of ^{210}Po of about 1 - 15 Bq/kg in meat from these animals. The food chain lichen-reindeer or lichen-caribou and man, constitutes a unique model for studying the uptake and retention of ^{210}Po (and ^{210}Pb) in humans. The effective annual dose due to ^{210}Po in people with high consumption of reindeer/caribou meat is estimated to be around 0.26 mSv/year[48].

In soils, ^{210}Po is adsorbed on minerals (and more particularly on clays). Po has also a high tendency to associate with organic matter. Organic matter increases Po retention when associated with minerals whereas it enhances Po mobility when present as colloids. The activity concentration varies with soil type and depth and also correlates with the amount of atmospheric precipitation. The average activity concentration levels of ^{210}Po in various soils typically are in the range 20 -240 Bq/kg but can reach 15,000 – 22,000 Bq/kg in specific countries due to the storage of uranium mining residues[54]. Several sources have reported that superphosphate fertilizers contain significant concentrations of ^{210}Po, which can provide a source of ^{210}Po to plants such as tobacco[55].

In general, plants become contaminated with radioactive nuclides both by deposition of radioactive fallout on the plants directly and by absorption from the soil. For ^{210}Po the main contamination pathway is foliar transfer[56]. In fresh leafy plants the level of ^{210}Po can be particularly high (few Bq/kg) as the result of the direct deposition of ^{222}Rn

daughters from atmospheric deposition. For example, tobacco is a terrestrial product with high activity concentrations of ^{210}Po and ^{210}Pb: the overall average activity concentration of ^{210}Po is 13 ± 2 Bq/ kg[48]. The average ^{210}Po activity content of one cigarette is about 20 mBq, which results in greater intakes in smokers compared to non-smokers. The activity of ^{210}Po in smoke inhaled from a cigarette varies greatly; an average value of 10 ± 6 mBq has been estimated.

Finally, the average median daily dietary intake of ^{210}Po for the adult world population has been estimated as 0.16 Bq/day, corresponding to an annual effective dose of 70 μSv/year. The average dietary intake of ^{210}Po from vegetarian food has been estimated as only 0.07 Bq/day, corresponding to an annual effective dose of 30.6 μSv/year[48].

Available values for the polonium transfer in terrestrial and freshwater environment

A series of IAEA reports including a recent Handbook of parameters[57] was published to provide radioecological concepts, models, parameters and data for assessing site-specific past, present and potential future radiation exposures of humans and other biota in terrestrial and freshwater environments in different climate conditions. A synthesis of the main transfer parameters available for polonium parameters is summarized in table 3.

Table 3: Transfer parameters of polonium in terrestrial and freshwater environment

Soil mobility	K_d* (L/kg) values in soils grouped according to the texture/organic matter criterion	All soils 210 (GSD=5.4)) Mineral 190 (GSD=5.1) Organic 6600
Soil to plant transfer	F_v # (dimensionless)	Cereals 2.4 x 10^{-4} Maize 2.4 x 10^{-4} Rice 1.3 x 10^{-2} Leafy vegetables 7.4 x 10^{-3} Non-Leafy vegetables 1.9 x 10^{-4}
Transfer to milk in animals	Transfer coefficient (d/L)	Cow's milk 2.1 x 10^{-4} (GSD=1.8) Goat's milk 2.3 x 10^{-3}
	Concentration ratio (Kg/L)	Cow 2.4 x 10^{-3}
Transfer to meat in animals	Concentration ratio (dimensionless)	Beef 1.4 x 10^{-1} (SD=1.3 x 10^{-1})
Transfer to freshwater fish	Concentration ratio (L/kg, fwt)	Fish 3.6 x 10^{-1} (GSD=4.3)

*K_d: distribution coefficient or ratio of the mass activity density (Bq/kg) of the specified solid phase (dry mass) to the volumetric activity density (Bq/L) of the specified liquid phase.# F_v: Concentration ratio or ratio of the activity concentration of radionuclide in the plant (Bq/kg dry mass) to that in the soil (Bq/kg dry mass).

IX. *In vivo* biokinetics following inhalation, ingestion and wounding

Polonium-210 is a health hazard only if it is taken into the body. External exposure is not a concern because ^{210}Po is an alpha emitter with an extremely low abundance of photon emissions. Polonium-210 is ubiquitous in the environment and is commonly taken into the body in small quantities in food, water, cigarettes and air, but intake of elevated

quantities clearly associated with adverse health effects (*e.g*. the Litvinenko poisoning) is rare.

Inhalation of ^{210}Po: Inhalation is of particular concern in the vicinity of a source of airborne dust such as a phosphate plant and in areas of high radon concentrations, or for cigarette smokers. Miners in uranium, niobium, silver, gold, nickel, phosphate and coal mines are also particularly affected by subject to elevated ^{210}Po exposure through mineral dust inhalation. Actual revision of ICRP 30[58] and 67[59] provides interesting information on inhalation of polonium compounds such as polonium oxide (PoO_2)[60], polonium chlorides ($PoCl_2$, $PoCl_4$)[61-63], polonium hydroxide ($Po(OH)_4$)[64-65], polonium under mineral dust form and polonium condensed with cigarette tar. For example, Cohen *et al.*[66-67] showed that the ^{210}Po that condenses with cigarette smoke tar is not readily soluble and is retained in the alveolar interstitial tissue of cigarette smokers. They showed a few years later in experimental studies on rats[68] that ^{210}Po lung clearance data fit a model with two phases of clearance: 90% was cleared at a rate of 0.036 day^{-1} and 10% was cleared at a rate of 5.5 x 10^{-3} day^{-1}. All these results are consistent with a moderate rate of absorption from the lungs to blood or "Type M" absorption as defined in ICRP Publication 66[69].

Ingestion of ^{210}Po: Polonium-210 is more absorbed by the gastro-intestinal tract to blood than some other alpha-emitting radionuclides (compared with 0.1% or less for plutonium-239 for example). Between 50% [58,70-71] and 90%[72] of the polonium taken in by ingestion will promptly leave the body in feces, meaning that only 10 to 50% is entering the

bloodstream. The absorption fraction f_A (0.1 to 0.5) strongly depends on the physiological state of the gastrointestinal tract (*e.g*. pH) and the composition of the diet, which affect polonium speciation and formation of either insoluble colloids or soluble complexes[73]: from 10% for inorganic compounds to 50% or more when polonium-210 is included in food chain[74]. In a six volunteers study concerning the absorption of polonium-210 from crabmeat, the absorbed fraction was estimated to be about 0.8[70].

Intake of ^{210}Po via the skin or wounds: Skin absorption of polonium (in the industrial form currently used (*e.g*. metal, oxide ...)) in humans does not exceed 2% of the dose per day[75]. In case of a puncture wound, the particles are directly implanted into the subcutaneous tissue or muscle. Polonium entering the body through a wound is likely to have relatively low transfer to blood due to the formation of colloids ($Po(OH)_4$ hydroxides). Limited results with intramuscularly injected soluble compounds of polonium (*e.g.* $Po(NO_3)_4$ (pH 2; 21 nM) in experimental animals (rats) have been published[76]. The results indicated strong retention at the wound site, with 74% of the injected dose remaining in the wound at 1 day, 50% at 4 days, and 26% at 14 days. These data are consistent with the strongly retained category of wound-site retention as represented in a wound model recently published by the U.S. National Council on Radiation Protection and Measurements (NCRP)[77].

However polonium, like other tetravalent elements, is subject to hydrolysis at weakly acidic and neutral pH. Depending on the mass, it may exist as soluble hydrolyzed products or colloids prior to contaminating a wound. In the latter case, the retention is expected to be described by the Colloid category of the NCRP[77], which represents slower

transfer to blood than the strongly retained category. When the specific activity of ^{210}Po is high, it is subject to auto-oxidation from the intense alpha radioactivity.

In vivo distribution and retention of ^{210}Po: The biological behavior of polonium has been extensively investigated by Leggett and Eckerman[78]. They reviewed records of about 1,500 former polonium workers and estimated urinary half-times for numerous cases of apparently elevated, acute exposure. Approximately 95% of the derived effective half-times were in the range 8-52 days corresponding to a range of biological half-times of 8.5-83 days. They also reviewed data on the biokinetics of polonium in laboratory animals. The systemic behavior of polonium is qualitatively similar among species in most respects, but some species differences have been identified[75,79].

The initial behavior of polonium in blood may depend on the route of exposure. After exposure by inhalation or wounds there is generally an early, rapid loss of polonium in urine[61] that appears to be absent or less pronounced after ingestion. Once in the blood, polonium in the Po(IV) valence state is mainly bound to citrates and bicarbonates and associated with red blood cells and plasma proteins[61,80-82].

Figure 7[78] shows the early distribution of polonium in target organs and tissues as determined in baboons[82-83], dogs[84] and a human subject[80].

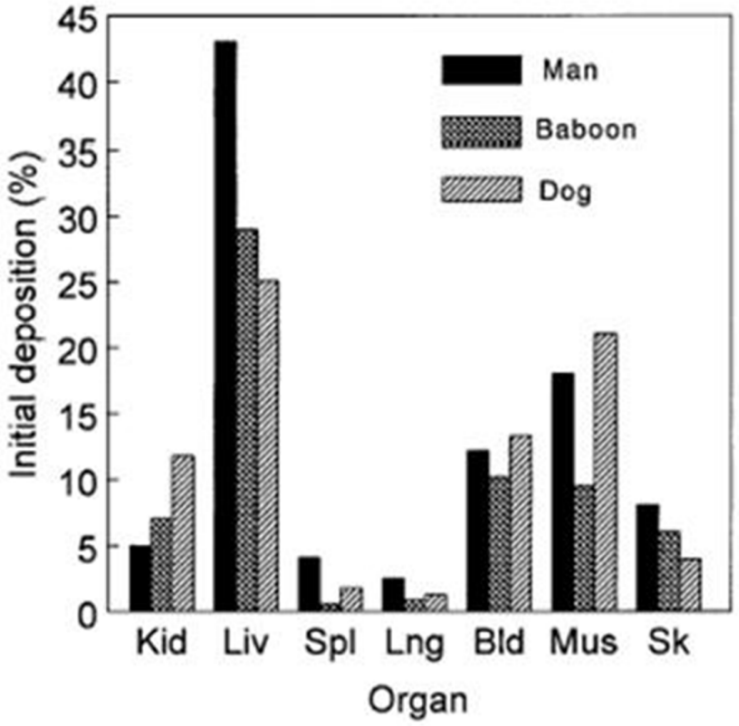


Figure 7: Comparison of initial distribution of polonium in different organs or tissues (kidneys, liver, spleen, lung, blood, muscles and skeleton) in baboons, dogs and a human subject[78].

In general, the liver, red bone marrow, kidneys and spleen concentrate polonium more than other tissues except for temporary deposition in the lung after inhalation of an insoluble form. It is estimated that approximately 40 to 45% of polonium absorbed from the gut will deposit in the liver (~35%), kidneys (~5%), 5% deposited in bone marrow and in spleen (~2%), and the remainder distributed throughout the body. These values have been revised after the ICRP 67[59] model, where the deposition had been described respectively as following: 30%, 10%, 10% and 5%. The retained polonium typically is removed from the body with a biological half-time of 30-50 days, but as indicated above there is considerable inter-subject variability in observed removal half-times.

The ratio of fecal to urinary excretion (F/U) of absorbed polonium has not been very well established for man. A selected value of 3 for use in radiation protection is a compromise, based on a fairly wide range of values determined for human subjects and non-human primates[78]. Natural urine ^{210}Po excretion rates fall in the range of about 5-15 mBq per day according to the living place and on individual habits of non exposed persons.

X. Radiotoxicity of polonium

As an alpha emitter (energy higher than 5 MeV) the polonium is a very radiotoxic agent only in case of internal contamination. The linear energy transfer (LET) of ^{210}Po is around 100 keV/μm in water, which is similar to biological tissues. Its average distance in such media is ~ 50 μm. This means it can penetrate cells of the human body, which are typically between 10 and 30 μm in diameter. The heavy alpha particles have devastating effects on cell structures and DNA of the tissues where polonium is deposited such as liver, kidneys, red bone marrow or spleen. Hence the incorporation of ^{210}Po can cause symptoms which are similar but not identical to the acute radiation syndrome (ARS)[85] caused by whole body gamma radiation, such as: i) bone marrow syndrome; ii) gastrointestinal syndrome and iii) central nervous system syndrome. Multi-organ failure and immunosuppression appear to be the main causes of death from ^{210}Po high contamination, and clinical severity is proportional to the exposure dosage.

Cell damages by alpha particles (*e.g.* ^{210}Po) appear to occur by direct damage as well as by 'bystander effect'. Cell damage by direct exposure is primarily due to single or

double-strand DNA breaks, resulting in both mutagenic and cytotoxic effects. In a series of studies[86-88] evaluating the effects of environmentally-relevant doses of alpha particles, Kadhim *et al.*[86] found that exposure on haematopoeitic stem cells resulted in chromosomal instability transmissible to subsequent culture generations. Alpha particles also appear to increase the frequency of sister chromatid exchanges[87]. Furthermore, DNA damage is not limited to cells directly hit by alpha particles. Experimental evidence suggests that DNA damage can also affect the unirradiated neighbors via cell signaling pathways, the so-called 'bystander effect'[88]. As expected with substances that cause DNA breaks, the most profound effect of alpha particles is on rapidly dividing cells, including those of the GI tract, bone marrow, skin, hair, and nails.

Remark: Concerns must be raised about the specific dangers to investigators and their labmates, associated with working with alpha emitters and particularly with polonium-210. Consequently the handling of polonium in research laboratories requires special safety precautions, such as wearing protective clothes, mask, gloves and glasses, but also specific monitoring, safe use in animals and disposal. A related case[2] occurred at the Curies' laboratory in 1941. S. Cotelle, a former technician of M. Curie's, inhaled a lethal amount of polonium dispersed after the explosion of a distillation vessel. She died two weeks later. I. Curie, who was standing behind S. Cotelle, was shielded by the body of her collaborator and felt no immediate harm.

For this reason it would be strongly recommended to work with an isotope of lower specific activity, such as ^{208}Po ($T_{1/2}$ = 102 y) or ^{209}Po ($T_{1/2}$ = 2.9 y).

Dosimetric aspect: In human dose calculations from alpha emitters such as ^{210}Po, the

absorbed dose in gray is multiplied by a radiation weighting factor w_R of 20 (ICRP 103[89]) to account the greater effectiveness of alpha particles in human carcinogenesis. A measurable quantity, directly related to the weighting factor w_R, is the relative biological effectiveness (RBE) for a given type of radiation. The RBE is defined[89] as the ratio of a dose of a low-LET reference radiation (*e.g.* X-rays or gamma rays) to a dose of the radiation considered that gives identical biological effect. Measurements of the alpha RBE in cell cultures are currently expected to yield different RBE values, depending on the doses, endpoint, cell type and species studies. Toxicological studies have shown that blood borne ^{210}Po can affect any tissue via endothelial cell injury. At high doses, hypoplasia, atrophy and hemorrhage occur in all body tissues but particularly in well-vascularized tissues, such as kidney[62]. Endothelial cell injury is considered the major cause of ^{210}Po-induced kidney damage in dogs[90]. Radiation damage in many tissues, such as fibrosis, necrosis, increased permeability, micro-hemorrhage, neovascularization and interruption of blood flow to normal tissues, may result from endothelial cell injury.

Polonium alpha particle RBE values were established and reported by Thomas *et al*.[91] for cultured porcine aortic endothelial cells. External alpha irradiation yielded more reliable results than internal alpha irradiation from ^{210}Po added to the medium under ^{210}Po-citrate form. Using the external alpha irradiation technique, RBE values were 21.2 ± 4.5 for cell viability, 12.9 ± 2.7 for decrease in live cell number and 5.3 ± 0.4 for lactate dehydrogenase (LDH) release to the medium but only 1.6 ± 0.1 for clonogenic survival. The low RBE for clonogenic survival response was mostly due to x-ray hypersensitivity of endothelial cells at low doses.

XI. Deterministic effects

Animal studies. ***Acute radiotoxicity:*** A number of median lethal systemic burden (LD_{50}) studies in animals (rats, cats, rabbits, and dogs) have been summarized by Cohen[82] and reviewed by Scott[73]. From these results, Scott[73] elaborated a risk model for estimating the risk of death from deterministic effects with polonium-210.

These studies focused on end-points such as $LD_{50/20}$ (*i.e.* lethal systemic burden for 50 % of the animals within 20 days), $LD_{50/30}$, and $LD_{50/40}$. These results obtained and expressed in terms of systemic body burdens (*e.g.* 1.1 - 2.6 MBq/kg body mass or 6 – 15 ng/kg body mass) indicate that ^{210}Po ingestion-related deaths occurring within a month of ingestion would suggest a very large intake of radioactivity, even though the mass of ^{210}Po associated with the intake may be microgram quantities[70]. Harrison *et al.*[92] reported similar results from a rat study showing that for systemic burdens > 4.4 MBq/kg body mass, 50% of the animals died within 7 days. This suggests a cause of death other than simply the hematopoietic syndrome, possibly a compounding of bone marrow failure with severe damage to tissues receiving higher doses including the gastric mucosa, kidneys, and liver.

Subacute and chronic radiotoxicity: At lower systemic burdens estimate (between 0.02 and 0.04 MBq/kg body mass), no death from deterministic effect is expected but clinical effects have been observed in animal studies such as life shortening and mild kidney lesions without hematological effects[92].

Human radiotoxicity. Except the poisoning of Alexander Litvinenko very few data exist concerning the human toxicity of polonium. One Russian accident has been described in which a worker inhaled a 530 MBq estimated dose of polonium. Lung doses were specifically high with 20 Gy at 1 day and 50 Gy at 3 days. The patient deceased 13 days after the contamination. First symptoms included vomiting, severe fever, decrease of red cells without diarrhea[76].

Otherwise Moroz and Parfenov[93] summarized changes in the livers of 10 children and 4 adolescents exposed accidentally by ingestion to polonium from polonium–beryllium sources. The amounts of polonium deposited in these two groups ranged from 20 kBq to 4,000 kBq. Just transitory changes were observed in liver function and decreased numbers of leukocytes and platelets were seen during the first few months after exposure.

Litvinenko's case: The death of Alexander Litvinenko on 23 November 2006 has brought into focus scientific judgments concerning the radiotoxicity of ^{210}Po and has heightened concerns about radiological terrorism[94]. Mr. Litvinenko was probably poisoned on 1st November 2006 and died 23 days later, on 23 November. The available information suggests that the polonium was administered orally and that multiple organ failure, probably connected with bone marrow syndrome, was responsible for his death[95]. Based on the calculated organ absorbed doses and different assumptions regarding blood absorption and lethal absorbed doses for red bone marrow, kidneys, liver, the minimum estimated amount of ^{210}Po which led to the death of A. Litvinenko is estimated to range from 27 to 1,408 MBq, about 0.2 to 8.5 µg, if only ^{210}Po was used as a poison[96].

These estimations are in agreement with animal data on the effects of ^{210}Po[78,92]. The data

obtained for a number of mammalian species are consistent with death occurring within 20 days of an uptake to blood of about 1–4 MBq/kg. This corresponds to absorption to blood of about 0.1–0.3 GBq or more in a 70 kg man, and intake by ingestion of 1–3 GBq or more, assuming 10% absorption. The animal studies show hematological changes characteristic of bone marrow failure (Figure 8). They also show gross damage to other organs, including the kidneys and liver, and to the gut mucosa after oral administration[75,92].

Figure 8: Autoradiograph of ^{210}Po in rat femur at 50 days after intravenous injection as the citrate, showing distribution throughout bone marrow (light area) and on extremal periosteal bone surfaces[92]

Stochastic effects There are only a few epidemiological cancer studies such as one including 22,000 nuclear workers having worked between 1951 and 1982. Among them, about 17% could have been exposed to polonium. An excess risk of 3 kidney cancers has been observed in this sub-group. Nevertheless methodological limits are reported such as the absence of individual dosimetry and coexposure factors consideration. The International Agency for Research on Cancer (IARC) concluded in the monography[97]

relative to internally deposited radionuclides that the epidemiological studies of nuclear industry workers exposed to polonium-210 are inadequate to allow a conclusion about cancer risk.

Treatment in case of internal intake. Severe damage to the bone marrow, gastrointestinal system, spleen, liver, kidney and potentially other organs would be expected in the case of a contamination with the alpha-emitter polonium-210. In case of an inhalation, the lung would also be severely damaged. The medical treatment (blood transfusion, cytokins, antibiotics…) used for counter the radiation effects to the bone marrow by reactivating stem cells should have to be completed for protecting the other organs such as spleen, liver and kidney… Development of new medical countermeasures is needed for clinical use to counter deterministic and eventually stochastic effects.

Currently treatments aim to decrease the radiological dose (*via* decorporation) by increasing the spontaneous excretion of polonium.

Limited decorporation research studies and therapies have been investigated for ^{210}Po. Chemically, polonium tends to bind (as a soft acid as defined by the HSAB Pearson classification[11]) most favorably with molecules having sulphur groups as opposed to carboxyl, catechol or pyridinone groups. Thus classical chelators such as EDTA (Ethylene Diamine Tetraacetic Acid) and DTPA (Diethylene triamine Pentaacetic Acid) are not useful for ^{210}Po decorporation.

Polonium's coordination chemistry is not well defined and consequently, little is known about its ability to form complexes with ligands. The published studies are more

interested in testing chelating agents already known to be efficient for other metal ions.

In U.S. Food and Drug Administration (FDA) Guidance for Industry[98], 2,3-dimercaptopropanol or British Anti-Lewisite (BAL) is recommended for internal contamination with polonium.

For polonium decorporation the Commission of the European Communities-Department of Energy guidebook for the treatment of internal radionuclide contamination[99] and the French National Guide on medical interventions in case of a radiological event[100] recommend the use of BAL. BAL is a lipid-soluble molecule developed during World War II for treating poisoning with the arsenic-based gas Lewisite. BAL is used for acute poisoning with arsenic, mercury, gold, and lead, as well as antimony, bismuth, chromium, copper, nickel, tungsten, or zinc. Although it has a long history of human use, it does have disadvantages, for example, a low therapeutic index or margin of safety, painful intramuscular injections, and a number of adverse effects.

Three other compounds that are chemical derivatives of BAL, DMPS (2,3-dimercaptopropane sulphonate), DMPA (N-(2,3-dimercaptopropyl) phthalamidic acid) and DMSA (meso-dimercapto succinic acid), have also been shown to decorporate ^{210}Po in experimental animals (rats)[101-103].

First studies in rats were initiated by Aposhian[101] with the 3 thiol-containing chelating agents DMPA, DMSA and DMPS. After 21 days, kidney levels of ^{210}Po in rats treated with DMPA were only 28% of those of the untreated controls and significantly lower than those receiving DMSA, DMPS, or N-acetyl-L-cysteine. DMPA and DMSA increased the urinary excretion of ^{210}Po 8-fold and 5-fold, respectively, as compared to

control animals. After DMPA treatment, the ^{210}Po levels of the spleen were 25% of the saline-treated control. The results indicate that DMPA has greater specificity in chelating and increasing the excretion of ^{210}Po than DMSA and appears to be a promising and consistent decorporating agent for ^{210}Po.

Both chelators (DMPS and DMSA) have been used in humans for heavy metal decorporation (primarily mercury and lead). Both are as effective in removing polonium as is BAL, and with fewer side effects, but none of these compounds has been used yet in humans for polonium decorporation.

Another compound, HOEtTTC (N'N-di[2-hydroxyethyl]ethylene-diamine-NN-biscarbodithioate), which is a derivative of DDTC (diethyldithiocarbamate), has been used to reduce the lethality of ^{210}Po in rats[104-105].

Volf *et al*.[103] reported some success in the decorporation of ^{210}Po using thiol chelating agents after contamination in rats by intramuscular injection of ^{210}Po simulating puncture wounds. Ten newly synthesized substances containing vicinal sulphydryl and carbodithioate groups were used, and their effect was compared with that of chelators clinically applicable in man (*e.g*. BAL, DMPS, DMSA, and DDTC). The results indicated: i) that complete removal of ^{210}Po from the injection site was achieved by only two local injections of DMPS; ii) many of the substances used merely induce translocation of ^{210}Po from the injection site into other tissues; iii) a combined local treatment at the injection site with DMPS plus repeated systemic, subcutaneous, treatments with HOEtTTC, a derivative of DDTC, results after 2 weeks in a reduction of the estimated total body retention of ^{210}Po to about one-third of that in untreated controls.

In the latter case the cumulative excretion of ^{210}Po increased from 8 to 54%, mainly via the feces.

The compound HOEtTTC has only been used in animals but does show promise for future use, provided adequate pharmacological studies are done.

Finally, recent studies performed by Levitskaia *et al*.[106] focused on two therapeutic drugs, Cuprimine™ (D-penicillamine) and Syprine™ (N,N'-bis-(2-aminoethyl)-1,2-ethanediamine dihydrochloride). These are oral therapeutics used for several decades to treat Wilson's disease (a genetic defect leading to copper overload) by chelation in accelerating excretion of internally deposited copper. Penicillamine is recommended for copper by ASN[100] in its French national guide.

In these studies (also conducted on cobalt), polonium was administered to animals by intravenous injection, followed by oral gavage doses of either Cuprimine or Syprine. Preliminary results showed that Cuprimine treatment was effective at reducing spleen levels of polonium compared to controls. Similarly, Syprine treatment produced statistically significant reductions of polonium in the spleen and skeletal tissues compared to control animals.

Nevertheless, one aspect of treatment for internally deposited ^{210}Po must be emphasized: all the experiments on ^{210}Po decorporation have been done under controlled experimental conditions (generally in rats) in which the chelating agent is given within minutes to hours after contamination. This is not realistic in the case of contamination of a member of the public (*e.g*., the Litvinenko case). It will take time to (i) identify that a radioactive material has been used, (ii) identify the substance, and (iii) be able to administer the right

drug. The longer the delay between the contaminating event and the initiation of therapy, the more radiation dose will have already been delivered, and the less effective will be the treatment for removing ^{210}Po from the body.

XII. Conclusion

Although polonium was discovered by P and M. Curie one hundred years ago using very efficient and basic separation chemistry, very little physical or radiochemical information has been developed on this element since this time.

Knowledge of the thermodynamic constant is very poor (*e.g.* there are no data on basic ligands such as phosphate, carbonate and sulfate) and information on interactions with natural or synthetic ligands encountered in biological and/or environmental media is very limited.

Transfer of polonium in the environment is better known and its biokinetics following internal contamination in rats is quite well described. Since the Litvinenko event in 2006, some new studies on this element have been published and new studies on decorporation have been triggered. There is a real need to apply speciation techniques to obtain useful thermodynamic data (stability constants) for this element.

Furthermore, a better comprehension of Po(IV) coordination properties should be a key issue for developing chelating agents specific for Po(IV).

Acknowledgment

Thanks to the French Toxicology programme.

Bibliographie de l'Article I

1. Curie, P., Sklodowska-Curie, M. (**1898**) Sur une substance nouvelle radioactive contenue dans la pechblende. *CR Acad Sci 127*, 175-178.
2. Adloff, J-P., Kauffman, B. (**2007**) The troubled story of polonium: an early controversy in radiochemistry. *Chem Educator 12*, 94-101.
3. Bagnall, K.W., Grudpan, K., Mabius, S., Manzel, H., Seidel,A., Tapper,W., Buschbeck, KC, Bius, S.M., Nzel, h.M., Pper, W.T. (**1990**) Gmelin: Handbook of Inorganic and Organometallic Chemistry: Po - Polonium. Supplement Vol 1: Element, Metal, Compounds, Chemistry in Solution.
4. IUPAC (**2011**) International Union of Pure and Applied Chemistry. Chemistry international. Marie Sklodowska Curie. 33, 1, 1-48. www.iupac.org/publications/ci/2011/3301
5. Atchison, F. (**1997**) Nuclide production in the SINQ Target. Report SINQ/816/AFN-702. Paul Scherrer Institut, Villigen, Switzerland.
6. Neuhausen, J., Köster, U., Eichler, B. (**2004**) Investigation of evaporation characteristics of polonium and its lighter homologues selenium and tellurium from Pb-Bi-eutectic. *Radiochimica Acta 92*, 917-923.
7. Heinitz, S., Neuhausen, J., Schumann, D. (**2011**) Alkaline extraction of polonium from liquid lead bismuth eutectic. *J Nuclear Materials 414*, 221-225.
8. Wheelwright, E.J., Swanson, J.L., Myers, T.R. (1980) Purification of polonium-210 using pyrochemical extraction. *Separation Science and Technology 15*, 987-997.
9. Näslund, J., Persson, I., Sandström, M. (**2000**) Solvation of the bismuth(III) ion by water, dimethyl sulfoxide, N,N'-dimethylpropyleneurea, and N,N-dimethylthioformamide. An EXAFS, large-angle X-ray scattering and crystallographic structural study. *Inorg Chem 39*, 4012-4021.
10. Figgins, P.E. (**1961**) The radiochemistry of polonium. US Atomic Energy Commission, NAS-NS 3037.
11. Pearson, R.G. (**1963**) Hard and soft acids and bases *J Am Chem Soc 85*, 3533-3539.
12. Ziv, D., Efros, I. (**1959**) Determination of the solubility of polonium hydroxide. *Radiokhim 1*, 290-294.
13. Bagnall, K., Robertson, D. (**1957**) Polonium monosulphide. *J Chem Soc* 204, 1044-1047.
14. IUPAC (**2004**) International Union for Pure and Applied Chemistry. Stability constant database, www.acadsoft.co.uk.

15. Starik, I., Ampelogova, N., Kuznetsov, B. (**1964**) Complex formation of polonium with the chloride ion in aqueous and aqueous-acetone solutions. *Radiokhim 6*, 507-509.
16. Ampelogova, N. (**1973**) Investigation of complex formation of polonium by an exhange method. *Radiokhim 15*, 823-829.
17. Ampelogova, N. (**1975**) Hydrolysis of polonium in perchlorate. *Radiokhim 17*, 69-75.
18. Starik, I., Ampelogova, N., Kuznetsov, B. (**1964**) Hydrolysis of polonium in perchloric acid solutions. *Radiokhim 6*, 501-506.
19. Bagnall, K. W., Freeman, J. H. (**1956**) Electrochemical studies on polonium. *J Chem Soc 544*, 2770-2774.
20. Pourbaix, M. (**1974**) Atlas of electrochemical equilibria in aqueous solutions, Houston, Texas, National Association of Corrosion Engineers, 2e ed., 644 p.
21. Ayala, R., Martinez; J.M., Pappalardo, R.R., Munoz-Paez, A., Sanchez Marcos, E. (**2008**) Po(IV) hydration: a quantum chemical study. *The Journal of Physical Chemistry B 112*, 5416-5422.
22. Ayala, R., Martinez, J.M., Pappalardo, R.R., Munoz-Paez, A., Sanchez Marcos, E. (**2009**) General quantum-mechanical study on the hydrolysis equilibria for a tetravalent aquo ion: the extreme case of the Po(IV) in water. *The Journal of Physical Chemistry B 113*, 487-496.
23. Ayala, R., Spezia, R., Vuilleumier, R., Munoz-Paez, A., Pappalardo, R.R., Sanchez Marcos E. (**2010**) An *ab initio* molecular dynamics study on the hydrolysis of the Po(IV) aquo ion in water. *The Journal of Physical Chemistry B 114*, 12866-12874.
24. Hataye, I., Suganuma, H., Sakata, M. Nagame, Y. (**1981**) Solvent extraction study on the hydrolysis of tracer concentration of polonium(IV) in perchlorate solutions. *J Inorg Nucl Chem 43*, 2101-2104.
25. Hataye, I., Suganuma, H., Sakata, M. Nagame, Y. (**1981**) Solvent extraction study on the hydrolysis of tracer concentration of polonium(IV) in nitrate solutions. *J Inorg Nucl Chem 43*, 2575-2577.
26. Suganuma H., Hataye,I. (**1981**) Solvent extraction study on the hydrolysis of tracer concentration of Po(IV) in chloride solutions. *J Inorg Nucl Chem 43*, 2511-2515.
27. Suganuma, H. (**1995**) Anion-exchange of the chemical species of tracer concentrations of polonium(IV) in chloride solutions. *J Radioanal Nucl Chem 191*, 265-272.
28. Ampelogova, N. (**1974**) Ion-exchange behavior of polonium in acid solutions. *Radiokhim 16*, *1*, 52-56.
29. Koch, H., Falkenberg, W.D. (**1967**) Solvent extraction Chemistry (Proc. Intern. Conf., Goteborg) pp 26-31, (Dryssen, D., Liljensen, J.O., Rydberg, J., Ed.) North-Holland Pub. Co. (Amsterdam).

30. Balistrieri, L.S., Murray, J.W., Paul B. (**1995**) The geochemical cycling of stable Pb, ^{210}Pb, and ^{210}Po in seasonally anoxic Lake Sammamish, Washington, USA. *Geochim Cosmochim Ac 59*, 4845-4861.
31. Benoit, G., Hemond, H.F. (**1988**) The biogeochemistry of ^{210}Pb and ^{210}Po in fresh waters and sediments. 14-08-0001-G1132, Department of Civil Engineering, Cambridge.
32. Swarzenski, P.W., McKee, B.A., Sorensen, K., Todd, J.F. (**1999**) Pb-210 and Po-210, manganese and iron cycling across the O_2/H_2S interface of a permanently anoxic fjord: Framvaren, Norway. *Mar Chem 67*, 199-217.
33. Hussain, N., Ferdelman, T.G., Churche, T.M., Luther, G.W. (**1995**) Bio-volatilization of polonium: results from laboratory analyses. *Aquatic Geochemistry 1*, 175-188.
34. Berry, J.A., Yui, M., Kitamura, A. (**2007**) Sorption studies of radioelements on geological materials. JAEA-Research 2007-074.
35. Suganuma, H., Samukawa, T., Hataye, I. (**1973**) Solution chemistry of polonium I: adsorption of polonium in acidic solution. Reports of the Faculty of Science, Shizuoka University, 8, 51.
36. Hataye, I., Suganuma, H., Ito, K., Kato, M. (**1974**) Solution chemistry of polonium II: deposition of polonium and radiocolloids in aged solution. Reports of the Faculty of Science,Shizuoka University, 9, 41.
37. Lanzola, E.E., Allegrini, M.E., Taylor, D.M. (**1973**) The binding of polonium-210 in rat tissues. *Radiat Res 56*, 370-384.
38. Aposhian, H.V., Bruce, D.C. (**1991**) Binding of polonium-210 to liver metallothionein. *Radiat Res 126*, 379-382.
39. Matthews, K.M, Kim, C.K, Martin P. (**2007**) Determination of ^{210}Po in environmental materials: A review of analytical methodology. *Applied Radiation and Isotopes 65*, 267-279.
40. Henricsson, F., Ranebo, Y., Holm, E., Roos, P. (**2011**) Aspects on the analysis of ^{210}Po. *J Environ Radio 102*, 415-419.
41. Lee, M.H., Lee, C.H., Song, K. Kim, C.K., Martin, P. (**2010**) Determination of polonium nuclides in a water sample with solvent extraction method. *Bull Korean Chem Soc 31*, 2488-2492.
42. Hataye, I., Suganuma, H., Shimizu, I. (**1991**) Mutual separation of ^{210}Po, ^{210}Bi, and ^{210}Pb with solvent extraction using copper dithizonate – CCl_4 and dithizone – CCl_4 solutions. *J Radioanal Nucl Chem 148*, 101-105.
43. Jokelainen, L., Vesterbacka, P., Letho, J. (**2010**) Method validation in solvent extraction for ^{210}Po determination from groundwaters. *Radiochimica Acta 98*, 91-97.

44. Lin, Z., Wu, Z. (**2009**) Analysis of polonium-210 in food products and bioassay samples by isotope-dilution alpha spectrometry. *Applied Radiation and Isotopes 67*, 907-912.
45. ISO (**2010**) International Organization for standardization. NF ISO 13161. Water quality - measurement of polonium-210 concentration activity in water by alpha spectrometry.
46. Fairman, W.D., Sedlet, J. (**1968**) Direct determination of lead-210 by liquid scintillation. *Anal Chem 40*, 13, 2004-2008.
47. WHO (**2011**) World Health Organization. Guidelines for Drinking-Water Quality, Recommendations; Geneva, 2006; Vol. 1.
48. Persson, B.R., Holm, E. (**2011**) Polonium-210 and lead-210 in the terrestrial environment: a historical review. *J Environ Radioact 102*, 420-429.
49. Seiler, R.L., Stillings, L.L., Cutler, N., Salonen, L., Outola, L. (2011) Biogeochemical factors affecting the presence of ^{210}Po in groundwater. *Applied Geochemistry 26*, 526-539.
50. Fowler, S.W. (**2011**) ^{210}Po in the marine environment with emphasis on its behaviour within the biosphere. *Journal of Environmental Radioactivity 102*, 448-461.
51. Lee, C.W., Kang, M.J., Lee, W., Choi, G.S., Cho, Y.H., Kim, H.R., Chung, K.H. (**2009**) Assessment of ^{210}Po in foodstuffs consumed in Korea. *J Radioanal Nucl Chem 279*, 519-522.
52. Vaaramaa, K., Solatie, D., Aro, L. (**2009**) Distribution of ^{210}Pb and ^{210}Po concentrations in wild berries and mushrooms in boreal forest ecosystems. *Science of the Total Environment 408*, 84-91.
53. Brown, J.E., Gjelsvick. R., Roos, P., Kalas, J.A., Outola, I., Holm, E. (**2011**) Levels and transfer of ^{210}Po and ^{210}Pb in Nordic terrestrial ecosystems. *Journal of Environmental Radioactivity 102*, 430-437.
54. Thomas, P.A. (**2000**) Radionuclides in the terrestrial ecosystem near a Canadian uranium mill. *Health Phys 78*, 614-640.
55. Rego, B. (**2009**) A hidden history of cancer, radiation, and the tobacco industry. *Isis 100*, 453-484.
56. Francis, C.W., Chesters, G., Erhardt, W.H. (**1968**) ^{210}Po entry into plants. *Environ Sci Technol 2*, 691-695.
57. IAEA (**2010**) International Atomic Energy Agency Handbook of parameter values for the prediction of radionuclide transfer in terrestrial and freshwater environments. IAEA Technical reports Series N° 472, Vienne.
58. ICRP (**1979**) International Commission on Radiological Protection. Limits on Intakes of Radionuclides for Workers. ICRP Publication 30, Pt.1. Pergamon Press, Oxford. Ann. ICRP 2, (3/4).

59. ICRP (**1993**) International Commission on Radiological Protection. Age dependent Doses to Members of the Public from Intake of Radionuclides. Pt.2. ICRP Publication 67. Pergamon Press, Oxford. Ann. ICRP. 23, (3/4).
60. Scott, L.M., West, C.M. (**1975**) Excretion of ^{210}Po oxide following accidental inhalation. *Health Phys 28*, 563-565.
61. Smith, F.A., Morrow, P.E., Gibb, F.R. (**1961**) Distribution and excretion studies in dogs exposed to an aerosol containing polonium-210. *Am Ind Hyg Assoc J 22*, 201-208.
62. Casarett, L.J. (**1964**) Distribution and excretion of polonium-210. IX. Deposition, retention, and fate after inhalation by nose-only exposure. *Radiat Res 5*, 148-165.
63. Berke, H.L., Dipasqua, A.C. (**1964**) Distribution and excretion of polonium-210. VIII. After inhalation by the rat. *Radiat Res 5*, 133-147.
64. Thomas, R.G., Stannard, J.N. (**1964**) Distribution and excretion of polonium-210. VI. After intratracheal administration in the rat. *Radiat Res 5*, 106-123.
65. Morrow, P.E., Della Rosa, R.J. (**1964**) Distribution and excretion of polonium-210. VII. Fate of polonium colloid after intratracheal administration to rabbits. *Radiat Res 5*, 124-132.
66. Cohen, B.S., Eisenbud, M., Wrenn, M.E., Harley, N.H. (**1979**) Distribution of polonium-210 in the human lung. *Radiat Res 79*, 162-168.
67. Cohen, B.S., Eisenbud, M., Harley, N.H. (**1980**) Alpha radioactivity in cigarette smoke. *Radiat Res 83*, 190-196.
68. Cohen, B.S., Harley, N.H., Tso,T.C. (**1985**) Clearance of polonium-210-enriched cigarette smoke from the rat trachea and lung. *Toxicol Appl Pharmacol 79*, 314-322.
69. ICRP (**1994**) International Commission on Radiological Protection. Human respiratory tract model for radiological protection. ICRP Publication 66. Pergamon Press, Oxford. Ann. ICRP. 24. (1–3).
70. Hunt, G.J. and Allington, D.J. (**1993**) Absorption of environmental polonium-210 by the human gut. *J Radiol Prot 13*, 119-126.
71. Hunt, G.J., Rumney, H.S. (**2007**) The human alimentary tract transfer and body retention of environmental polonium-210. *J Radiol Prot 27,* 405-426.
72. ICRP (**2006**) International Commission on Radiological Protection. Human Alimentary Tract Model for Radiological Protection, ICRP Publication 100. Pergamon Press, Oxford. Ann. ICRP. 36, (1-2).
73. Scott, B.R. (**2007**) Health risk evaluation for ingestion exposure of humans to polonium-210. *Dose-response 5*, 94-122.
74. Ladinskaya, L.A., Parfenov, Y.D., Popov, D.K. Fedorova, A.V. (**1973**) ^{210}Pb and ^{210}Po content in air, water, foodstuffs, and the human body. *Arch Environ Health 27*, 254–258.

75. Stannard, J.N., Smith, F.A. (1964) Distribution and excretion of polonium-210. X. Species comparison. *Radiat Res 5*, 166-174.
76. Ilyin (**2001**) Radiation medicine. Guidance for medical researchers and health management vol 2 Radiation damage of humans. Ed A Yu Bushmanov *et al.* (Moscow) ISBN 5-86656-114-X.
77. NCRP (**2006**) National Council on Radiation Protection and Measurements. Development of a biokinetic model from radionuclide-contaminated wounds and procedures for their assessment, dosimetry and treatment. Bethesda, MD: NCRP; Report No. 156.
78. Leggett, R.W., Eckerman, K.F. (**2001**) A systemic biokinetic model for polonium. *Sci Tot Env 275*, 109-125.
79. Stannard, J.N. (**1964**) Distribution and Excretion of Polonium-210. I. Comparison of oral and intravenous routes in the rat. *Radiat Res 5*, 49-59.
80. Silberstein, H.E., Valentine, W.N., Minto, W.L., Lawrence, J.S., Fink, R.M. (**1950**) Studies of polonium metabolism in human subjects. Intravenous studies, comparison of intravenous studies, oral administration. In: Fink RM, editor. Biological studies with polonium, radium, and plutonium. New York: McGraw-Hill, 122-148.
81. Thomas, R.G. (**1964**) The binding of polonium by red cells and plasma proteins. *Radiat Res 5*, 29-39.
82. Cohen, N., Fellman, A. L., Hickman, D. P., Ralston, L. G., Ayres, L. S. (**1989**) Primate polonium metabolic models and their use in estimation of systemic radiation doses from bioassay data. Mound Laboratory.
83. Fellman, A., Ralston, L., Hickman, D., Ayres, L., Cohen, N. (**1994**) Polonium metabolism in adult female baboons. *Radiat Res 137*, 238-250.
84. Parfenov, Y.D., Poluboyarinova, Z.I. (**1969**) Dynamics of polonium-210 exchange in dogs after a single subcutaneous administration. In: Moskaleve YuI, editor. Radioactive isotopes and the body, AEC-tr-7195. Moscow: Izdatel'stvo Medisina, 128-135.
85. Mai, H.Le. (**2007**) Polonium 210, exposed. *J Med Toxicol 3*, 82-84
86. Kadhim, M.A., MacDonald, D.A., Goodhead, DT. (**1992**) Transmission of chromosomal instability after plutonium alpha-particle irradiation. *Nature 355*, 738-740.
87. Nagasawa, H., Little, J.B. (**1992**) Induction of sister chromatid exchanges by extremely low doses particles. *Cancer Res 52*, 6394-6396.
88. Zhou, H., Randers-Pehrson, G., Waldren, C.A., Vannais, D., Hall, E.J., Hei, T.K. (**2000**) Induction of a bystander mutagenic effect of alpha particles in mammalian cells. *Proc Natl Acad Sci 97*, 2099-2104.
89. ICRP (**2007**) International Commission on Radiological Protection. The 2007 recommendations of the international commission on radiological protection, ICRP Publication 103. Pergamon Press, Oxford. Ann. ICRP. 37, (2-4).

90. Bruenger, F.W., Lloyd, R.D., Taylor, G.N., Miller, S.C., Mays, C.W. (**1990**) Kidney deseases in beagles injected with polonium 210. *Radiat Res 122*, 241-251.
91. Thomas, P., Tracy, B., Ping, T., Baweja, A., Wickstrom, M., Sidhu, N., Hiebert, L. (**2007**) Relative biological effectiveness (RBE) of alpha radiation in cultured porcine aortic endothelial cells. *Int J Radiat Biol 83*, 171-179.
92. Harrison, J., Leggett, R., Lloyd, D., Phipps, A., Scott, B. (**2007**) Polonium-210 as a poison. *J Radiol Prot 27*, 17-40.
93. Moroz, B.B and Parfenov Y. D. (**1972**) Metabolism and biological effects of polonium-210. *Energy Rev 10*, 175-232.
94. Nemhauser, J.B. (**2010**) The polonium-210 public health assessment: the need for medical toxicology expertise in radiation terrorism events. *J Med Toxicol 6*, 355-359.
95. Jefferson, R.D., Goans, R.E., Blain, P.G., Thomas, S.H.L. (**2009**) Diagnosis and treatment of polonium poisoning. *Clinical Toxicology 47*, 379-392.
96. Li, W.B., Gerstmann, U., Giussani, A., Oeh, U., Paretzke, H. G. (**2008**) Internal dose assessment of ^{210}Po using biokinetic modeling and urinary excretion measurement. *Radiat Prot Biophys 47*, 101-110.
97. IARC (**2001**) Monographs on the evaluation of carcinogenic risks to human. World Health organization IARC Press, vol 78. Ionizing radiation. Part 2: some internally deposited radionuclides.
98. FDA (**2006**) U.S. Food and Drug Administration. Guidance for industry. Internal radioactive contamination—development of decorporation agents. Washington, DC: U.S. FDA, Center for Drug Evaluation and Research.
99. Gerber GB, Thomas RG. (**1992**) Guidebook for the treatment of accidental internal radionuclide contamination of workers. *Radiat Prot Dosim 41*, 1-50.
100. ASN (**2008**) Autorité de Sureté Nucléaire. Guide national d'intervention médicale en cas d'évènement nucléaire ou radiologique.
101. Aposhian, H.V. Dart, R.C., Aposhian, M.M., Dawson, B.V. (**1987**) Tissue decorporation of polonium-210 in rats by DMPA. *Res Connun Chem Pathol Pharmacol 58*, 157-171.
102. Bogdan, G.M., Aposhian, H.V. (**1990**) N-(2, 3-dimercaptopropyl)phtalamidic acid (DMPA) increases polonium-210 excretion. *Biol Met 3*, 232-236.
103. Volf, V., Rencova, J., Jones, M.M., Singh, P.K. (**1995**) Combined chelation treatment for polonium after simulated wound contamination in rats. *Int J Radiat Biol 68*, 395-404.
104. Rencova, J., Volf, V., Jones, M.M., Singh P.K. (**1994**) Decorporation of Po from rats by new chelating agents. *Radiat Prot Dosim 53*, 311-313.

105. Rencova, J., Svoboda, V., Holusa, R., Volf, V., Jones, M.M., Singh ,P.K. (**1997**) Reduction of subacute lethal radiotoxicity of polonium-210 in rats by chelating agents. *Int J Radiat Biol 72*, 341-348.
106. Levitskaia, T.G., Creim, J.A., Curry, T.L., Luders, T., Morris J.E., Woodstock, A.D., Levinson, B., Thrall, K.D. (**2010**) Evaluation of cuprimine and syprine for decorporation of ^{60}Co and ^{210} Po. *Health Phys 98*, 471-479.

Article 2 : A route for polonium-210 production from alpha irradiated bismuth-209 target

A route for polonium-210 production from alpha irradiated bismuth-209 target

I. Summary

A method is proposed for production and purification of polonium-210 *via* the ^{209}Bi (α, 3n) ^{210}At nuclear reaction. Bombardment of a bismuth-209 target was performed with a 37 MeV alpha beam that conducts to the production of astatine-210 (8.1 hrs), which decays to polonium-210. It is further purified from the bismuth target matrix by employing liquid–liquid extraction method using tributyl phosphate (TBP) in *para*-xylene from 7 M hydrochloric acid. Back extraction of polonium-210 was performed by 9 M nitric acid. This method allows to purify a tracer amount of Po-210 ($2.6\ 10^{-13}$ mol) from macroscopic amount of Bi ($2.8\ 10^{-2}$ mol).

Keywords:

Polonium-210; bismuth; α irradiation; liquid-liquid extraction.

II. Introduction

Although Pierre and Marie Curie made the discovery of the element polonium (Po) more than a century ago, physical and chemical properties of this element and its complexes are still barely known. This can be explained by two main reasons: first, polonium has forty-two known isotopes with a mass ranging from 188 to 220, all radioactive[1]. Second, one of its isotopes, polonium-210 (Po-210) that occurs naturally in the uranium-238

decay is very rare in nature which impedes its studies. The equilibrium ratio of U to Po is 1.19 10^{10}, so that the Po concentration in uranium ores is less than 0.1 mg/ton[2]. Po-210 is a high-energy alpha emitter (E= 5.305 MeV)[3] with a radioactive half life of 138.376 days. It is known for being one of the most toxic radionuclides due to its high specific activity (1.66 10^{14} Bq/g)[4]. It presents an internal radiation hazard due to its short range of alpha particles in biological tissues (40-50 μm) [5]. With the advent of cyclotrons and nuclear reactors and their intense fluxes, production of milligrams of Po-210 has become feasible. Bombardment of stable bismuth-209 (Bi-209) with intense neutron fluxes has been studied in nuclear reactors [6]. Bi-210 in its ground state Bi-210g (5.012 days) is produced by the Bi-209(n,γ)Bi-210 reactions and decays to ^{210}Po *via* β^- decay. The production cross-section of Bi-210 being about 15 mb, a Po-210 activity close to 4.10^3 GBq/kg of Bi-209 is obtained after 200 days of irradiation in a thermal neutron flux of 1.10^{14} n/cm^2.s[7]. Po-210 can also be produced in cyclotrons *via* the direct reaction Bi-209(α,t)Po-210 [8]. Another possibility is to produce polonium from the decay of At-210 obtained *via* the reaction Bi-209(α,3n)At-210 [9]. This reaction is used in the present work as an easy way to produce Po-210 for radiochemistry studies. For this application, the production of Po-210 with good radionuclide and chemical purities is required.

The separation of polonium includes several steps: (i) dissolution of the irradiated target, (ii) Bi-Po separation and (iii) reconditioning.

The bismuth target will not dissolve easily in hydrochloric acid solution alone. In other hand, bismuth could be dissolve in nitric acid and no special problems are presented. A mixture of hydrochloric acid and nitric acid in a proportion of 1:4 and 3:4 will dissolve

bismuth metal [10].

Many methods can be used for the separation of Po from Bi, such as electrodeposition,[11] solvent extraction and ion separation[12,13]. For simplicity of setting up, liquid-liquid extraction has been chosen. Several extracting agents [14-33] were used for the extraction of Po from different aqueous layers (HCl, HBr, HNO_3, H_2SO_4, $HClO_4$, H_3PO_4 or lactic acid) into various organic layer (carbon tetrachloride, chloroform, xylene, toluene or cyclohexane) (Table.1). For all of the previously mentioned methods, the extraction yield of polonium is significant. However, some of the papers mention a poor extraction of Po/ Bi separation [17] while others indicate a good separation but without quantitative data. In addition, most of the studies report the separation polonium-210 tracer from bismuth-210 tracer [27].

Table 1: Literature data of protocols for polonium-210 extraction using liquid-liquid extraction.

EXTRACTANT	Solvent	Aqueous phase	Po extraction (%)	Reference
Cupper-diethyldithiocarbamate	CHCl3	1.06 M HNO_3	55	17
	CHCl3	0.07-0.72 M HNO_3	65 -86	17
	CHCl3	0.03 M HNO_3	60	17
Triuylamine	Xylene	7M HCl	91	19
Tributylphosphate 10%	ortho-xylene	7M HCl	85	27
Tributylphosphate 10%	Decalin	7-9 M HCl	98	28
Tributylphosphate 20%	Dibutylether	6M HCl	99	29
Dibutyl carbitol		3M HNO_3	98	33

As it is mentioned before, a prerequisite in the approach is to dissolve Bi target before Po

extraction. Therefore, an extracting agent efficient in strong acidic conditions (HCl and HNO_3) is required. Among the different molecules proposed, TBP has been chosen in this work. The challenge of this work was to separate radiotracer quantity of polonium-210 from macroscopic quantities of bismuth using TBP. In order to optimize this purification method, several parameters were studied (solvent nature, acidity, aqueous medium, extraction time, TBP dilution, organic solvent) for defining the best conditions for polonium separation.

III. Materials and method

III.1. Chemicals

All solutions were prepared using Milli-Q water and all experiments were conducted at room temperature (22±3˚C). All the chemicals used were of analytical reagent grade.

Bismuth foils (chemical purity 99.999% content of Pb, Cu and Ag below 3, 2 and 2 ppm respectively, 25 x 25 mm^2, thickness of 1 mm), kapton Polyimide Film (75 µm , 25 x 25 mm^2), aluminium foil (chemical purity 99.999%, 25 x 25 mm^2, thickness of 1 mm), silver foil (chemical purity 99.999%, 25 x 25 mm^2, thickness of 0.25 mm) were purchased from Goodfellow and used as received. Po-210 (2 M HCl) and Bi-207 (1 M HCl) tracers were purchased from Eckert Ziegler and CERCA LEA, AREVA, France, respectively. Both are used after reconditioning and diluting into the appropriate medium.

III.2. Irradiation

The targets are bismuth foil (Bi-209) placed behind an aluminium foil in a capsule. The ARRONAX cyclotron is designed to deliver a fixed 67.4 MeV alpha beam. In order to decrease the energy of the alpha beam, a degrader system was used. This system is built up and placed along the alpha beam line (Figure 1).

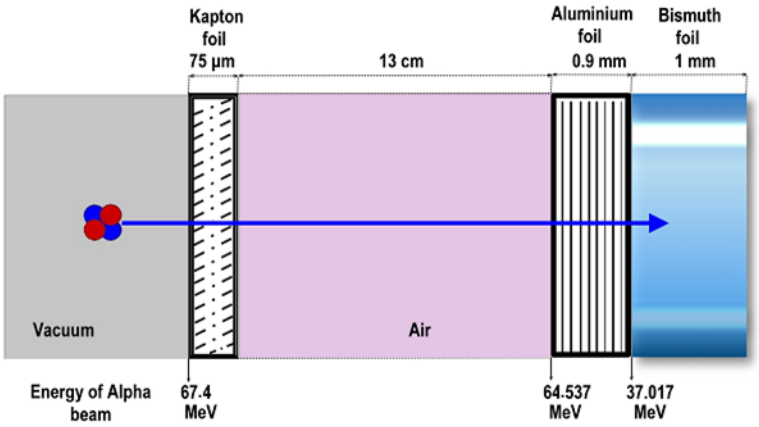


Figure 1: Schematic diagram for the irradiation setup of bismuth-209 foil by a 37 MeV alpha beam.

The target was placed in air 13 cm away from the end of the beam line which is closed by a kapton foil. The alpha beam used for irradiation is extracted at 67.4 MeV. Typical irradiation stands one hour with an average beam current of 0.2 µA. According to literature cross section data of α-particle induced reactions on Bi-209, an energy of 37 MeV was selected for optimal production of At-210 (Figure 2)[8,34-35]. An aluminum foil of 0.9 mm thick is used to degrade the incident energy .The target and its degrader foil are presented in Figure 1. The bismuth foil was ensured by an air cooling system during irradiation. After the end of irradiation, the target cell containing the aluminium and bismuth foils was left in a lead container for a week, allowing At-210 to decay into Po-210.

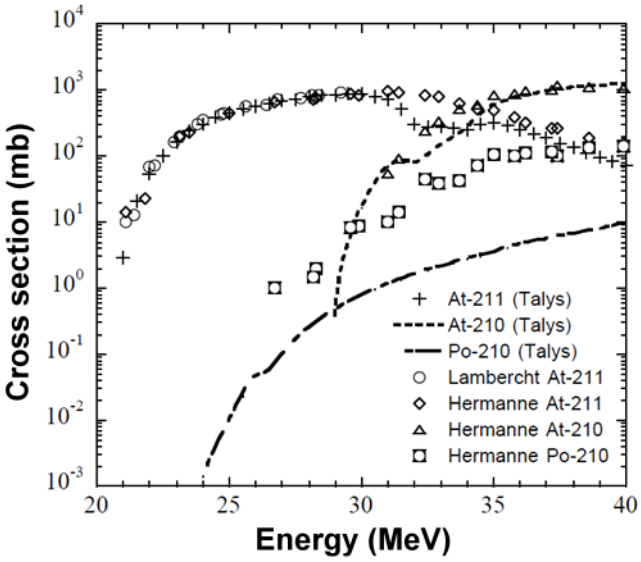


Figure 2: Direct production cross-section for At-211, At-210, and Po-210 by nuclear reaction of Bi-209(α,2n)At-211, Bi-209(α,3n)At-210 and Bi-209(α, t)Po-210, respectively.[8,32]

III.3. Batch experiments

Tubes of polypropylene were used for liquid/liquid batch experiment, due to the adsorption of polonium on glass [25] which causes difficulty in the accurate determination of polonium activity. Before polonium addition, the organic layer was pre-equilibrated with the aqueous solution, i.e. the composition does not change in presence of the organic layer.

2 mL of organic and aqueous solution were brought in contact. After equilibrium of the biphasic system, an aliquot of polonium (300 Bq) and/or bismuth (100 Bq) was added and the tubes were shaken for 90 min. This time proved to be sufficient to achieve distribution equilibrium of polonium/ bismuth between the phases.

After phase separation, an aliquot of the aqueous and organic phases were withdrawn to derive the percentage of extraction, %E, corresponding to:

$$\%E = \left(\frac{A_{org}}{A_{org} + A_{aq}} \right) \left(\frac{V_{aq}}{V_{org}} \right) \times 100 \qquad \text{eq.(1)}$$

where, A_{org} and A_{aq} define the polonium activities measured in the organic and aqueous phases, respectively. V_{org} and V_{aq} define the volume of organic and aqueous phases, respectively.

For the back-extraction, %BE is defined as:

$$\%BE = \left(\frac{A_{aq}}{A_{org} + A_{aq}} \right) \left(\frac{V_{org}}{V_{aq}} \right) \times 100 \qquad \text{eq.(2)}$$

III.4. **Analytical Tools**

The radionuclide composition was checked by gamma spectrometry. A gamma spectrometer from ORTEC, using a high purity germanium detector with 1.82 keV resolution at 1.33MeV, was used to quantify the production of gamma rays-emitting radionuclides, and more particularly At-210 and At-211, after Bi-209 irradiation [8,36].

After the dissolution of the irradiated bismuth foil in 7 M HCl solution, an alpha spectrometer was used for the determination of polonium-210 activity. Sample for alpha spectrometry was prepared by spontaneous deposition of Po-210 on silver discs [37-39] in a water bath (80˚C) for two hours. The detector consists of a 24 mm ruggedized surface

barrier detector connected to a 7401 VR Canberra spectrometer. Spectra are obtained by a TRUMP 8 K multichannel buffer card on PC. Efficiency of the detector is determined by a multi-alpha surface source with geometry identical to the Po-silver sample disks (Figure 3).

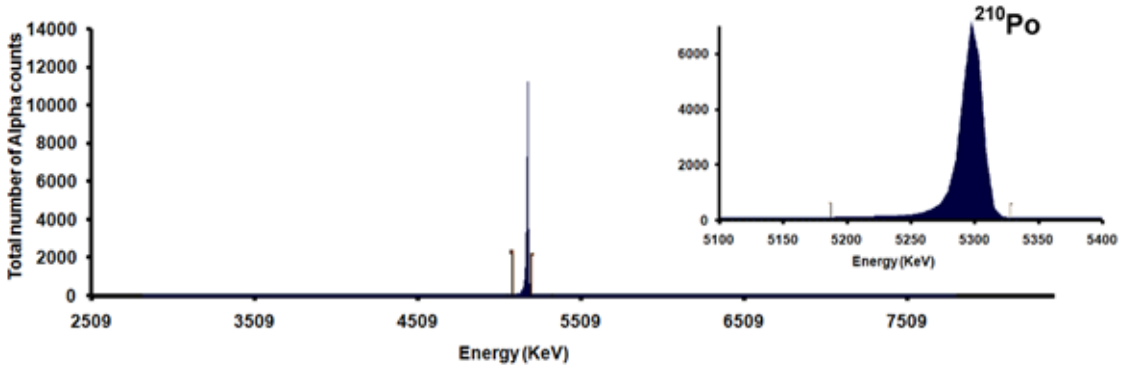


Figure 3: Alpha spectrum of the irradiated bismuth target after dissolution in 2 M HCl and spontaneous deposition on a silver disk.

For batch experiments, activity of the radionuclides (Po-210 or Bi-207) was determined by liquid scintillation counting using a Packard 2550 TR/AB Liquid Scintillation analyzer. The samples were prepared by mixing an aliquot of 0.2 mL of the solution to measure with 2 mL of 1 M HCl and 2.2 mL of ultimate gold AB scintillation cocktail. The measuring time was fixed at 1 hour. The quenching arising from the organic solvent was taken into account according to the following relation:

$$A = A_m\,(-9.10^{-5}\times Tsie^3 + 673\ 10^{-4}\times Tsie^2 - 17.26\times Tsie + 1565.8) \qquad \text{eq.(3)}$$

A_m being the activity measured by liquid scintillation and *Tsie*, an independent parameter from polonium analyses defined by the apparatus to determine the quenching parameter.

Determination of stable bismuth-209 concentration was performed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES, Thermo Scientific iCAP 6500) with a plasma power of 1150 W. These analyses were done with a rate of 0.50 L/min auxiliary gas, 12.00 L/min gas cooling and 0.45 L/min for gas nebulizer. The wavelength (λ) used for bismuth analysis was 223.061 – 306.770 nm. Mass spectrometer - Electrospray (MS-ESI) was used to analyze the hydrolysis of tributyl phosphate in 8 M nitric acid solution. The analysis of phosphate ions in the final purified polonium solution was done by Ion-exchange chromatography ICS2500 with a conductivity detector. These analyses were done using AS18 (4 x 250 mm) column type with a 50 µL injection loop, 1 mL/ min flow and 120 mA current suppression.

IV. Results and discussion

IV.1. Production of Po-210

Using SRIM-2008 software [40], calculation shows that the bombardment of Bi-209 foil (1 mm) by an alpha particle beam with energy of 37.02 MeV is achieved by using a 0.9 mm aluminium foil. The energy of the delivered alpha beam arrived on the aluminium foil with a value of 64.5 MeV and an average current being of 0.2 µA for one hour. The stopping point inside the bismuth target was measured to be 0.314 mm at 37.02 MeV.

The produced activity can be determined numerically using the following formula:

$$A = \Phi_0 \frac{\rho}{A} N_a (1 - \exp(-\lambda t)) \int_{E_{\min}}^{E_{\max}} \frac{\sigma(E)}{\frac{dE}{dx}} dE \qquad \text{eq.(4)}$$

Where, ϕ_o, ρ, A, N_a, λ and σ(E) are the incident particle number (particle/s/cm^2), target density, target number of mass, Avogadro number, radioactive constant (s^{-1}) and production cross section, respectively.

$\frac{dE}{dx}$ is the energy loss calculated using the Bethe formulae:

$$-\frac{dE}{dx} = \frac{K.Z_p^2.Z.N_A}{\beta^2.A}\left[\ln(\frac{2.m_e.\gamma^2.\beta^2}{I}) - \beta^2\right] \qquad \text{eq.(5)}$$

K, m_e, Z_p, Z, I, γ and β correspond to a constant (equal to 0.307 MeV.cm^2), electron mass, projectile charge, target charge, ionization potential and relativistic coefficients associated to the projectile, respectively.

The theoretical activity calculated based on the irradiation parameters (0.2 μA, 1 hr) amounts to 6.34 MBq of At-210. The experimental results shows the production of 4.11 MBq of At-210 which corresponds to the production of almost 10 kBq of Po-210.

The analysis of the irradiated Bi-209 target by using gamma spectrometry (Figure 4), shows the production of At-210 and At-211 which decay to Bi-207 ($t_{1/2}$=32.9 y) and Bi-206 ($t_{1/2}$=6.423 d), respectively. No other γ ray emitting radionuclides were found. After bismuth target irradiation, the foil is kept 7 days in a lead container for the total decay of At-210 into Po-210.

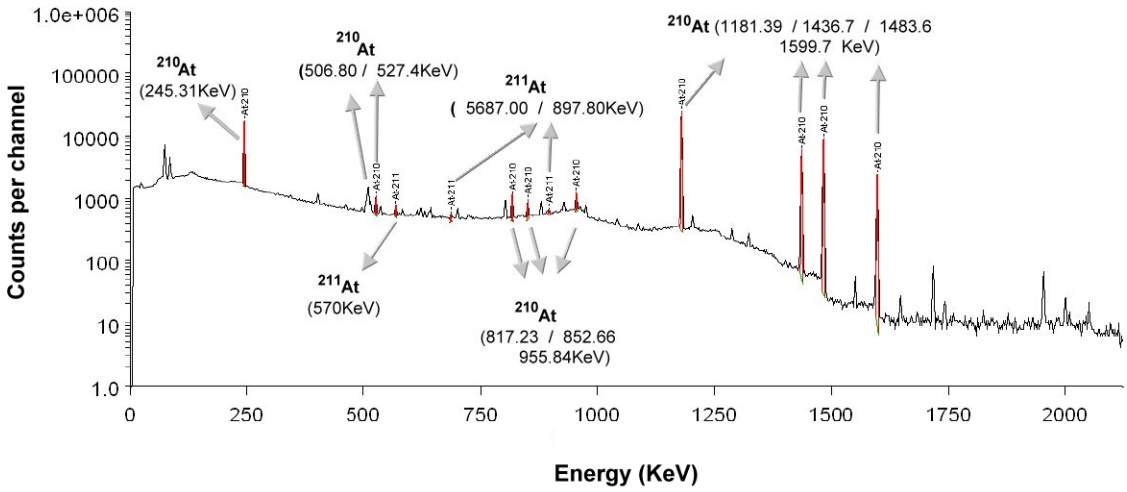


Figure 4: Gamma spectrum obtained for a Bi-209 foil after irradiation with a 37.0 MeV alpha beam with a current of 0.2 µA for 1 hour.

IV.2. **Polonium / Bismuth batch experiments**

The method for separation of polonium is essentially based on the work of Chen *et al.* [27], *i.e.* the extraction is performed in HCl whereas the back extraction is realized in nitric acid. However several optimization steps were studied in order to find the best conditions for achieving a high extraction yield of polonium-210 and a high selectivity for polonium extraction over bismuth.

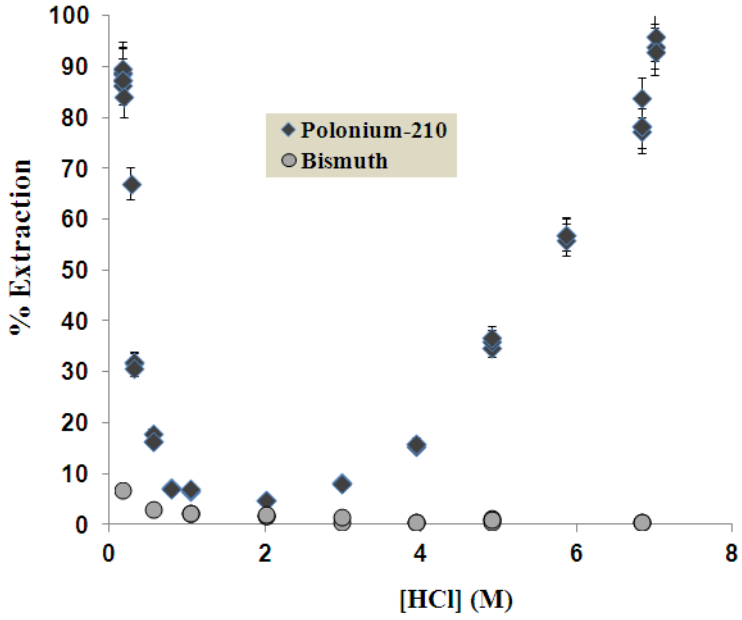


Figure 5: Effect of the nature and acidity of aqueous layer on the extraction of polonium-210 and bismuth-207 in 10% TBP- para-xylene. The batch activity used were 300 Bq and 100 Bq , for Po-210 and Bi, respectively.

These experiments were done with synthetic solutions containing either Po-210 or Bi-207. The influence of the solvent nature containing TBP (10%) was first studied from 6.9 M aqueous hydrochloric acid. Hexane, *para*-xylene, toluene, *ortho*-xylene, and chloroform were studied. Results show no effect of the investigated solvent on the extraction of Po with an approximate extraction percentage of 83.0 ± 6.0 %, except for chloroform for which a negligible extraction value is found. *Para*-xylene was chosen for the further studies.

The extraction of polonium from hydrochloric acid solution is strongly dependent on the acid concentration of the aqueous phase. Investigation of the extraction of Po and Bi as a function of hydrochloric concentration in 10 % TBP / para-xylene is presented in Figure

5. A good polonium extraction value of 89.0 ± 5.0 % was obtained at 0.17 M HCl. However, when the acidity increases, the extraction values decrease until it reaches a minimum value of 5.0 ± 0.3 % at 2 M HCl. After 2 M HCl, the extraction value of Po increases again and reaches its maximum value of 96.0 ± 4.7 % at 7 M HCl. For Bi, the extraction is weak in the acidic range studied with a maximum extraction value of 6.7 ± 0.5% for 0.17 M HCl. In conclusion, a good compromise (high extraction yield, good selectivity) would be to work at 0.1 M or 7 M HCl, with a preference for the latter condition.

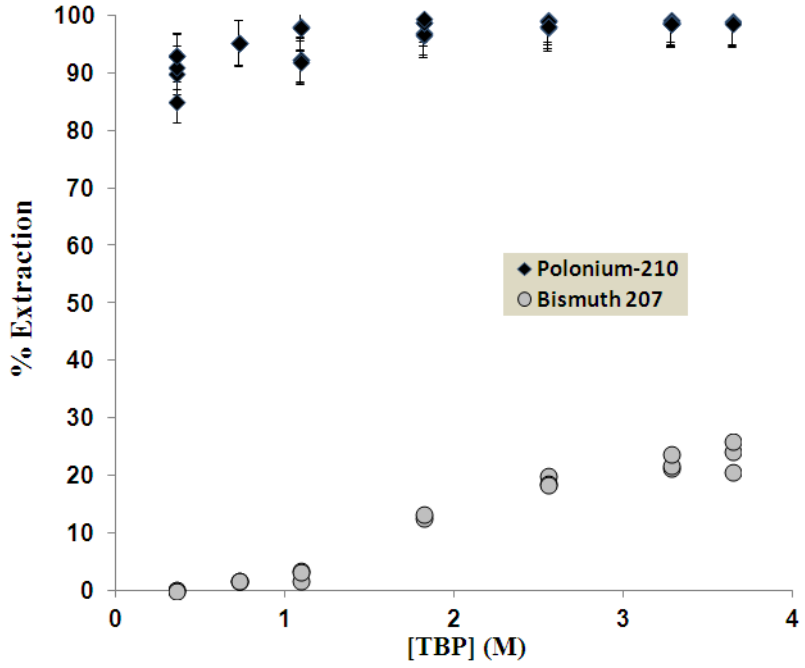


Figure 6: Effect of the concentration of tributyl phosphate on the extraction of polonium-210 and bismuth from aqueous layer of 7M HCl. The batch activity used were 300 Bq and 96.1 Bq , for Po-210 and Bi-207, respectively.

Then, the influence of the concentration of tributyl phosphate in para-xylene from 7 M aqueous hydrochloric acid was performed (Figure 6). The extraction of Po is greater than

90 % whatever the concentration of TBP studied with a slight increase, as the TBP concentration increases. For Bi, the extraction is almost equal to zero for a TBP concentration below 1 M. Above 1 M, Bi extraction becomes significant with 25 % of Bi extracted at 3.5 M of TBP. To have a good compromise between extraction yields on selectivity, a concentration of 0.36 M in TBP (or 10 % in weight) was selected for the extraction step.

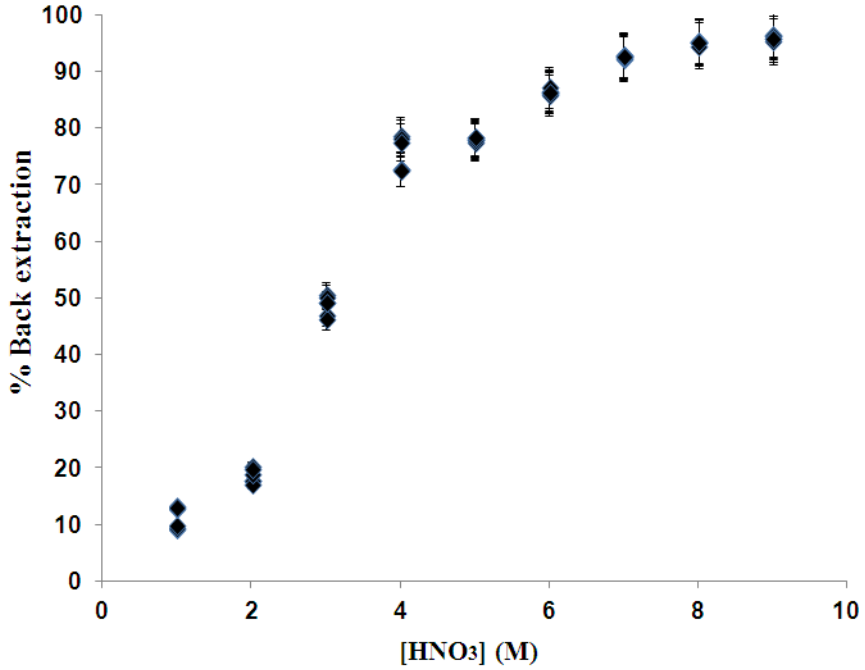


Figure 7: Effect of the concentration of nitric acid on the back-extraction of polonium-210 from organic layer (10%TBP-paraxylene). The batch polonium activity used was 288 Bq.

The following step is to back extract polonium to a new aqueous layer to be used for further studies. Investigation shows nitric acid to be the convenient aqueous layer for polonium back extraction [41]. Back extraction of Po in *para*-xylene is studied as

function of HNO_3 concentration (Figure 7). Back extraction of polonium increases as the acidity increases and reaches a maximum value of 96.0 ± 4.0 % for a solution of 9 M HNO_3. This can be explained by the increasing competition between Po and HNO_3 for TBP [42]. The potential degradation of TBP can contribute to explain the important back-extraction. Indeed according to the literature, at high concentrations of nitric acid, TBP can be hydrolyzed like it occurs in reprocessing spent reactor fuels [43, 44]. To confirm the hydrolysis of TBP at high acidic nitric acid solution, the aqueous phase (8 M HNO_3) obtained after extraction was analyzed by mass spectrometer. Results show the presence of phosphoric acid, monobutyl phosphate and dibutyl phosphate which are the hydrolyzed products of tributyl phosphate. Thus, the concentration of 9 M nitric acid will be chosen for the purification process.

Finally, the idea was to test the protocol optimized for the polonium extraction and back-extraction to see the behavior of bismuth using the whole procedure. The final step was to confirm the absence of cold bismuth in the back extracted solution. As the analysis using ICP-OES gives signals below the detection limit of ICP-OES (<1 ppb), absence of bismuth-209 can be guaranteed.

IV.3. Target dissolution and Po/ Bi separation

In this part, we want to use the results previously presented in order to obtain the best conditions for the Po/ Bi separation from the irradiated target. The first step is the transfer all the polonium from the target into solution. The depth of penetration determined by SRIM-2008 software corresponded to 0.314 μm. To recover all produced Po-210, a total dissolution of the irradiated foil (thickness of 1 mm) is required. The easiest way was to dissolve directly the target in 0.1 M or 7 M HCl (see previous part). However the solubility of metallic Bi is not important in HCl. It was then decided to follow the work of Bhatki *et al.* [10] and to use 10 M HNO_3. The next step is then the reconditioning of the extraction medium after evaporation. In order to remove the trace quantities of nitric and nitrous acids, two evaporation cycles in 7 M HCl using IR-lamp are done. IR-lamp was used for acidic solution evaporation to minimize the loss of polonium due to its high volatility [45-47]. 8.0 ± 0.5 moles of nitric acid was used per mole of dissolved bismuth; the off gases appear to be nitric oxide and nitrogen dioxide [33].

Solubility tests showed that an HCl concentration lower than 3.5 M was sufficient for a complete dissolution of the residue. An attempt was done to decrease the HCl concentration to 0.1 M, after complete dissolution of the target, with the idea to precipitate bismuth while keeping polonium in solution. However, a strong decrease in polonium concentration was observed indicating a co-precipitation process in agreement. This was already evidenced by Holgye [48]. Therefore, it was decided to use 7 M HCl for the extraction step.

Alpha spectrometry analysis of the dissolved Bi foil shows only one peak at 5.3 MeV,

which corresponds to the presence of Po-210 (Figure 3). Gamma spectrometry analysis indicates the presence of Bi-207 and Bi-206, produced from the decay of At-211 and At-210, respectively.

After the extraction of polonium in the presence of 10% TBP in *para*-xylene, the trace quantities of bismuth extracted in the organic phase were removed by washing two times with a new equilibrated layer of 7 M HCl. The affinity of Bi at this acid concentration is largely favorable for the aqueous phase. The extraction yield for Po was 96.0 ± 5.0 %.

The following step was to back extract polonium from the previously extracted organic phase to 9 M HNO_3.The percentage of polonium back extracted was 96.0± 4.0 % and the percentage of the total recovery yield of polonium in this methodology was 85.2 ± 4.5%. Bismuth was confirmed to be under the detection limit.

The final step was to evaporate the nitric acid and to recondition in the appropriate medium such as $HClO_4$ or HCl for the radiochemical studies. The phosphoric acid coming from the degradation of TBP (see previous part) was not observed in the reconditioned solution (Ionic chromatography, DL = 50 ppb). The complete methodology is depicted in Figure 8.

V. Conclusion

The bombardment of bismuth-209 target with a 37 MeV alpha particle beam conducts to production of astatine-210 (8.1 hrs) which decays to polonium-210. The produced radiotracer of polonium is purified from macroscopic quantities of bismuth by a wet

methodology using liquid–liquid extraction. The procedure is presented in Figure 8. Results confirm that polonium can be extracted (96.0 ± 4.7%) to an organic phase containing 10% TBP in *para*-xylene from 7 M HCl while the bismuth nuclides remain in the aqueous phase. Moreover, the back-extraction of polonium from the organic phase can be performed by using a 9 M HNO_3 phase (96.0 ± 4.0%). The optimized process leads to a solution of Po-210 with a global recovery yield of 85.2 ± 4.5% with good radionuclide and chemical purities.

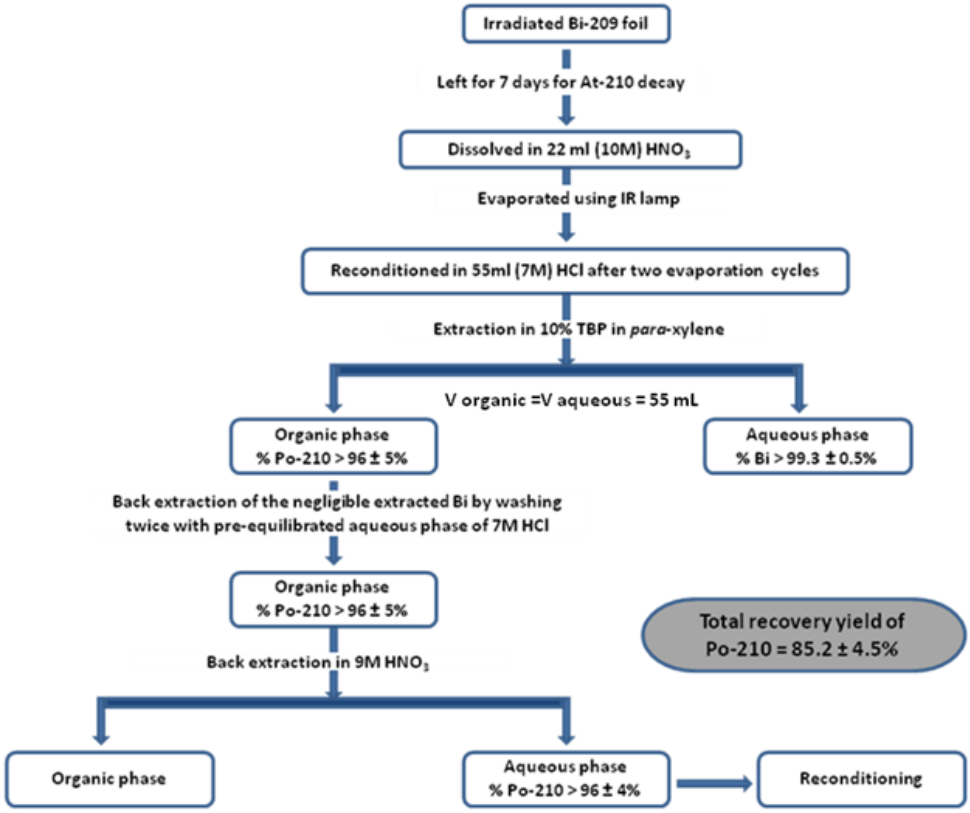


Figure 8: Methodology followed to recover Po-210 ($2.6\ 10^{-13}$ mol) from the irradiated bismuth target ($2.8\ 10^{-2}$ mol).

Acknowledgement

The authors thank the National Center of scientific research (CNRS) and the Region Pays de Loire for the grant allocated to Ali Younes to perform his thesis. We thanks Veronique Baty for the ionic chromatography analysis.

Bibliographie de l'Article II

1. Fry, C., Thoennessen, M. (**2013**) Discovery of the thallium, lead, bismuth, and polonium isotopes. *Atomic Data and Nuclear Data Tables*. 99,365–389.
2. Figgins, P. E.: The radiochemistry of Polonium. National Academy of Sciences Nuclear Science Series U.S. Atomic Energy Commission. NAS-NS 3037.
3. Lederer, C. M, Hollander, J. M., Perlman, I. (**1967**) TABLEOFISOTOPES. John Wiley & Sons, Inc.
4. Scott, B. R. (**2007**) Health risk evaluations for ingestion exposure of humans to polonium-210. *Dose Response.* 5, 94–122.
5. Harrison, J., Leggett, R., Lloyd, D., Phipps, Scott, B. (**2007**)Polonium-210 as a poison. *J. Radiol. Prot.* 27, 17-40.
6. Vogelsang, W. F., White, A. M., Wittenberg, L., Sze, DK. (**1984**) Polonium production in the Mars reactor . *Fusion technology institute, University of Wisconsin,Madison.* UWFDM-573.
7. Mikheev, N. B. (**1978**) Polonium.ChemikerZeitung. (UCRL-Trans-12034).
8. Hermanne, A., Tarkanyi, F., Takacs, S., Szucs, Z., Shubin, YN., Dityuk, AI. (**2005**) Experimental study of the cross-sections of alpha-particle induced reactions on 209Bi. *Appl. Radiat. Isot*. 63, 1-9.
9. Kelly. E.L., Segrè. E. (**1949**) Some excitation functions of bismuth. *Physical Review*. 75,999.
10. Bhatki, KS. (**1977**) Radiochemistry of bismuth, NAS-NS-3061. National Academy of Sciences - National Research Council.
11. Meyer, S., Schweidle, E. (**1906**) *Sitzungber. Akad. Wiss. Wien*, Abt.IIa. 115, 697.
12. Reischmann, F. J., Trautmann, N., Herrmann, G. (**1984**) Chemistry at low concentrations: polonium at a level of 108 to 105 atoms. *J. Radiochim. Acta*. 36, 139–143.
13. Vajda, N., LaRosa, J., Zeisler, R., Danesi, P., Kis-Benedek, GY. (**1997**) A novel technique for the simultaneous determination of ^{210}Pb and ^{210}Po using a crown ether. *J. Environ. Radioactiv.* 37, 355–372.
14. Martin, P., Hancock, GJ. (**2004**) Routine analysis of naturally occurring radionuclides in environmental samples by alpha-particle spectrometry. Supervising Scientist Report 180, AGPS, Canberra.
15. Clayton, RF., Bradley, EJ. (**1995**) A cost-effective method for the determination of ^{210}Po and ^{210}Pb in environmental samples. *Sci. Total Environ*. 173/174, 23–28.
16. Smithson, G., Muzaffer, F., Petrow, M. (**1979**) Radiochemical determination of lead-210 in environmental samples resulting from uranium mining-milling operations-Radiochemical procedures for determination of selected members of the uranium and thorium series. Report 78-22, Appendix B, CANMET, Ottawa.
17. Wai, CM., Lo, JM. (**1982**) Extraction and separation of ^{210}Pb, ^{210}Bi and ^{210}Po by diethyldithiocarbamate. *Radiochem. Radioanal. Lett.* 50, 293–298 .

18. Roseberry, LM., Scott, TG. (**1985**) Radiochemical analysis of ^{210}Po in coal gasification samples. J. *Radioanal. Nucl. Chem. Lett*. 93, 271–278.
19. Sheppard, Warnock (**1964**) The distribution of bismuth (III) and polonium(IV) between trilaurylamine solution of xylene and hydrochloric and hydrobromic acid solution. *J. Inorg. Nucl. Chem*. 26, 1421-1427.
20. Ibrahim, SA., Whicker, FW. (**1987**) Plant accumulation and plant/soil concentration ratios of ^{210}Pb and ^{210}Po at various sites within a uranium mining and milling operation. *Env. Exp. Bot*. 27, 203–213.
21. Hataye, I., Suganuma, H., Sakata, M., Nagame, Y. (**1981**) Solvent extraction study on the hydrolysis of tracer concentration of Po(IV) in perchlorate solutions. *J. inorg. nucl. Chem*.43(10), 2101-2104.
22. Suganuma, H., Hataye, I. (**1981**). Solvent extraction study on the hydrolysis of tracer concentration of Po(IV) in chloride solutions. *J. inorg. nucl. Chem*. 43(10), 2511-2515
23. Hataye, I., Suganuma, H., Sakata, M. (**1981**) Solvent extraction study on the hydrolysis of tracer concentration of Po(IV) in nitrate solutions. *J. Inorg. Nucl. Chem*. 43(10), 2575-2577.
24. Johansson, M., Skarnemark, G. (**2001**) Extraction of polonium from aqueous lactic acid solutions using dioctyl sulphide, Cyanex 272, Cyanex 301 or Cyanex 302 in toluene. *J. Radioanal. Nucl. Chem*. 250, 473–476.
25. Matsuura, N., Ouchi, A., Kojima, M. (**1961**) Studies on extraction of polonium(IV) by hexone from acid Solution .*Bulletin of the Chemical Society of Japan*. 34(3), 411-416.
26. Sheppard, J. C. (**1967**) The distribution of polonium-210 and bismuth-210 between linear aliphatic ethers and nitric acid solutions . *J.Inorg, nucl. Chem*. 29, 848-853.
27. Chen. YM., Shu, RY. (**1966**) *J. Chin. Chem. Soc*. 13, 82-89.
28. Bagnell, K. W., Robertson, D. S. (**1957**) Solvent extraction studies with polonium. *J. Chem. Soc.* 509-512.
29. Karracker, D. G., Templeton, D. H. (**1951**).*J. Phys. Rev*. 81, 510
30. Jia, G., Torri, G., Petruzzi, M. (**2004**) Distribution coefficients of polonium between 5% TOPO in toluene and aqueous hydrochloric and nitric acids. *Appl. Radiat. Isotopes*. 61, 279–282
31. Case, GN., McDowell, WJ. (**1982**) An improved sensitive assay for polonium-210 by use of a background-rejecting extractive liquid scintillation method. Talanta. 29, 845–848.
32. Jia, G., Torri, G. (**2008**) Distribution coefficients of polonium between 0.75 M HDEHP in cyclohexane and aqueous hydrochloric and nitric acids. *The Open Inorganic Chemistry Journal*. 2, 18-21.
33. Schulz, W., Richardson, G. L. (**1968**) Dibutyl Carbitol Solvent Extraction of Polonium-210 from Nitric Acid Solutions of Irradiated Bismuth. *Industrial & Engineering Chemistry Process Design and Development*. 7(1), 149-156.
34. Maiti, M., Lahiri, S. (**2009**) Theoretical approach to explore the production routes of astatine radionuclides. *Phys. Rev. C* 79, 024611.

35. Lahiri, S., Maiti, M. (**2012**) Recent developments in nuclear data measurements and chemical separation methods in accelerator production of astatine and technetium radionuclides. *Radiochim. Acta*. 100, 85-94.
36. Lambrecht, RM., Mirzadeh, S. (**1984**) Cyclotron isotopes and radiopharmaceuticals-XXXV astatine-211. *Int. J. Appl. Radiat. Isot*. 36, 443-50.
37. Flynn, WW. (**1968**) The determination of low levels of polonium-210 in environmental materials. *Anal. Chim. Acta*. 43, 221.
38. Hasanen, E. (**1977**) Radiochem. Radioana. Lett. 31, 214.
39. Feldman, I., Frisch, M. (**1956**) Precision plating of polonium. *J. Anal. Chem*. 28, 2024.
40. SRIM **2008**, http://www.srim.org/srim/srim2008.htm
41. Meinke, W. (**1949**). American Report AECD-2738. Sec. 84-1.
42. Naganawa, H., Tachimori, S. (**1997**) Complex formation between tributyl phosphate and nitric acid and the hydration of the complexes in dodecane. *Bull. Chem. Soc. Jpn*., 70, 809-819.
43. Naylor, A. (**1968**), TBP extraction systems-TBP and diluent degradation, KR-126. Aere Harwell, Oxon, Great Britain.
44. Richardson, G. L. (**1973**) The effect of high solvent radiation exposure on TBP processing of spent LMFBR fuels, HEDL-TME-73-51, Hanford Engineering Development Laboratory.
45. Martin, A., Blanchard, RL. (**1969**) The thermal volatilisation of caesium-137, polonium-210 and lead-210 from in vivo labelled samples. *Analyst*. 94, 441–446.
46. Cleary, JJ., Hamilton, EI. (**1968**) Loss of polonium-210 on dry ashing rat tissues in a muffle furnace. *Analyst*. 93, 235–236.
47. Mabuchi, H. (**1963**) On the volatility of some polonium compounds. *J. Inorg. Nucl. Chem*. 25, 657–660.
48. Holgye, Z. (**2007**) Coprecipitation of polonium with bismuth phosphate. *Journal of Radioanalytical and Nuclear chemistry*. 274(3), 647-649.

Article 3 : Po(IV) speciation in HCl solution using solvent extraction by tributylphosphate and trioctylamine

Po(IV) speciation in HCl solution using solvent extraction by tributylphosphate and trioctylamine

I. Abstract

The extraction mechanism of Po(IV) from hydrochloric acid solution by tributylphosphate and trioctylamine has been investigated as a function of acid (0.1-10M) and extracting agent (0.36-3.64 M) concentration. Experimental data were modeled to determine the formed species as well as the associated formation constants. Preliminary extraction experiments were done by trioctylamine (TOA) in order to determine the species existing in the aqueous phase. The results show the existence of a species holding a charge -2 and confirm the presence of $PoCl_6^{2-}$ as a major polonium species in concentrated hydrochloric acid (6-12M). Above 6M, another species is formed corresponding to the neutral aqueous species $PoCl_4$. This species dominated Po speciation up to 0.2M HCl after which a mixed hydroxo-chloro complex $Po(OH)_mCl_z$ (m+z=4) appears in the aqueous phase.

The obtained information was used to determine the extraction mechanism occurring in the presence of TBP. The theoretical model fits the experimental results considering the existence of the following extracted polonium species: $Po(OH)_2Cl_2.2TBP$, $PoCl_4.4TBP$ and $HPoCl_5.2TBP$ in an acidic range of 0.1-1, 1-2.9 and 2.9-8 M, respectively. The information taken all together support a complete extraction scheme composed of the five

following reactions:

$PoCl_4 + 2Cl^- \rightleftharpoons PoCl_6^{2-}$ Logβ=4.6± 0.2

$Po(OH)_2Cl_2 + 4Cl^- + 2H^+ \rightleftharpoons PoCl_6^{2-} + 2H_2O$ Logβ=2.7± 0.8

$PoCl_6^{2-} + 2\overline{TBP} + 2H_2O \rightleftharpoons \overline{Po(OH)_2Cl_2(TBP)_2} + 2H^+ + 4Cl^-$ LogK=2.18± 0.06

$PoCl_6^{2-} + 4\overline{TBP} \rightleftharpoons \overline{PoCl_4(TBP)_4} + 2Cl^-$ LogK=3.35± 0.08

$PoCl_6^{2-} + 2\overline{TBP} + H^+ \rightleftharpoons \overline{HPoCl_5(TBP)_2}.+H_2O + Cl^-$ LogK=2.14± 0.07

Keywords:

Polonium, tributylphosphate, trioctylamine, extraction mechanism, liquid-liquid extraction.

II. Introduction

The early fundamental investigation of polonium chemistry was complicated by the absence of a stable isotope and the availability of only extremely small quantities of this element. For example, M. Curie and A. Debierne needed several tons of pechblende to extract 2 mg of Po which was used for its discovery.[1] This was the case until 1944, when the method of polonium production and purification was improved allowing for some long-life polonium isotopes, such as polonium-209 (half-life of 109 years) and polonium-210 (half-life of 138.47 day) to be produced artificially by nuclear reactions. However,

the study of Po remains a challenge as all the investigations of the chemical properties of polonium are conducted at trace levels. Thus, specific tools are required to evaluate the chemistry of polonium and the use of spectroscopic methods is excluded because of the manipulation of trace amounts.

Most of the basic chemical properties of polonium were identified and published in the 1960s[2]. The oxidation state of polonium has been reported to be ±II, III, IV and VI [3], but the most stable oxidation state in aqueous solution is the quadrivalent one [4-6]. Po(VI) is expected to only exist in the solid state and oxidation states +II and +III in aqueous reducing conditions. The study of the behavior of Po(IV) in aqueous solution is complicated, due to several phenomena. First, Po(IV) is a highly reactive species which has the notable the tendency to hydrolyze easily in solution[7], preventing the polonium ion from remaining in a truly dissolved state. Secondly, when relative high activities are used, the radiocolloids / precipitate form in an aged, weakly acidic solution[7, 8], Thirdly, polonium is adsorbed onto glassware[9] which causes difficulty in the accurate determination of polonium activity, and thus a poor reproducibility of the analytical data.

The early assumption about the formation of polonium-ion complexes was based on its high solubility in acidic solutions (HCl, HF, oxalic and citric acids)[2]. A special interest was devoted to study Po chemistry in HCl medium. The speciation of polonium in aqueous solution was studied by electromobility [2], coprecipitation [10], electrochemistry [3, 11], ion-exchange [5, 12-15] and solvent extraction[7, 9, 11, 16]. Figure.1 gives a state of the art about what is known in the HCl range between 0.1 and 12M.

The high Po solubility in HCl was mainly explained by the formation of the species $PoCl_6^{2-}$ in strong acidic conditions[2]. There is an indirect evidence of the existence of this species by Staritzky [17]who has prepared compounds of the type M_2PoCl_6 [M = Cs, Rb, K, NH_3, and $(CH_3)_4N$] on the milligram scale, which were identified by optical crystallographic studies. X-ray diffraction measurements of the cesium salt were also made. Attempts to assess the HCl concentration range where $PoCl_6^{2-}$ predominates remain scarce. Marcus [18] made a compilation of available data to propose the speciation diagram given in Figure 1. According to the review, $PoCl_6^{2-}$ would exist in a wide range of HCl concentration from 2 to 12M. A formation constant of around 10^{14} was proposed by Bagnall and Freeman[19]. Below 2M, mixed hydro-chloro and hydroxo-complexes begin to form.

The most interesting and direct measurements were done in the fifties by spectrophotometry with macroscopic quantities of Po (~3.10^{-5}M) [20-23]. Several parameters were studied ($[H^+]$, $[Cl^-]$) in order to assess the stoichiometry of the species. They observed the existence of one species in the range 0.5-5M which was characterized by an absorption band at ~418 nm. The absorption band was used for the calorimetric determination of Po. The species was qualified as a chloro-complex of Po of the form $PoCl_{n+b}$ (n=2) as no H^+ ion effect was observed. Below 0.5M, this species was shown to transformed in another one characterized by an absorption band at 344nm. The study evidenced the existence of a mixed chloro-hydroxo-complex, $Po(OH)_aCl_b$ (a=1).

A comparison between the review of Marcus and the work of Hunt[20] highlights the lack

of reliable information. More recent work [9, 14, 16, 24] were made without attempting to clarify the discrepancy. Suganuma et al.[16, 25] further complicated the picture by proposing another mixed hydro-chloro complex at 1M, $Po(OH)_2Cl_4^{2-}$ and by studying Po liquid-liquid extraction with dithizone as a function of H^+/Cl^- concentration.

The aim of this work is to propose a coherent picture of Po speciation in the HCl concentration range between 0.1 and 10M using available data and new data determined by liquid-liquid extraction methods. Experiments were first realized with triocylamine (TOA) in order to assess the charge/nature of the species existing in the aqueous phase. The data are completed with experiments realized with tri-butyl-phosphate (TBP). The characterization of the extraction and complexation reactions occurring in both aqueous and organic phases is supported by a modeling approach.

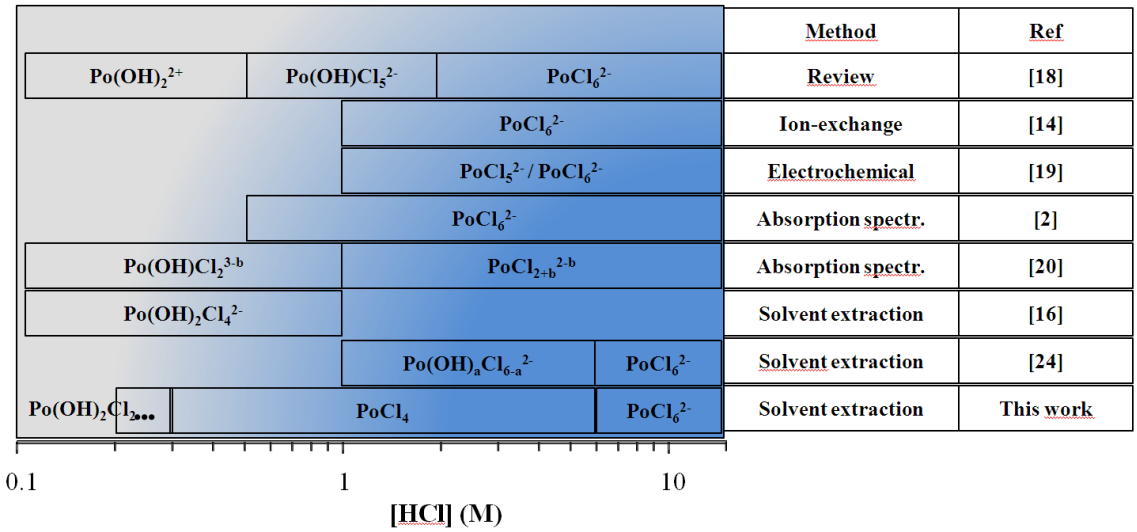


Figure 1: Predominance of Po species in HCl; state of the art.

III. EXPERIMENTAL SECTION

III.1. Materials

The organic solvent p-xylene (>99%, Fluka), the extractants tributylphosphate (>99%, Sigma Aldrich) and trioctylamine (98%, Sigma Aldrich) were used to prepare the organic solutions. Hydrochloric acid (>99%, Fluka), and MilliQ water (18.2 MΩ.Cm) from Millipore were used to prepare the aqueous solutions. All these commercial products were used without further purification.

Polonium-210 radionuclide was produced and purified according to the protocol developed by A. Younes et al. [26]. To prevent potential unsuitable complexation, the polonium solution was evaporated to dryness and recovered in the appropriate medium; the procedure was repeated two times.

III.2. Apparatus and procedure

Polypropylene tubes were used due to limit Po adsorption on the walls. Before polonium addition, the extracting agents were dissolved in p-xylene and pre-equilibrated for 2 h by shaking with the appropriate acidic medium.

The solvent extraction experiments were performed at equal volume (5mL) and at room temperature (22 ± 3˚C). Po-210 activities in aqueous and organic phases were determined to calculate the Po distribution ratio (D_{Po}), *i.e.* the measurement of the efficiency by which TBP-complexed polonium is transferred to the organic phase. It is defined as:

$$D = \frac{\sum \overline{[Po]}}{\sum [Po]} = \left(\frac{A_{org}}{A_{aq}}\right)\left(\frac{V_{aq}}{V_{org}}\right) \qquad \textbf{Eq. 1}$$

where $\overline{[Po]}$ and $[Po]$ are the total species of polonium in the organic and aqueous phases, respectively.

A_{org} and A_{aq} define the polonium activities measured in the organic and aqueous phases, respectively.

V_{org} and V_{aq} define the volume of the organic and aqueous phases, respectively.

The uncertainty of the partition coefficient, σ_D, was calculated according to:

$$\sigma_D = D \times \sqrt{\left(\frac{\sigma_{A_{org}}}{A_{org}}\right)^2 + \left(\frac{\sigma_{A_{aq}}}{A_{aq}}\right)^2 + \left(\frac{\sigma_{V_{org}}}{V_{org}}\right)^2 + \left(\frac{\sigma_{V_{aq}}}{V_{aq}}\right)^2} \qquad \textbf{Eq. 2}$$

σ_{Aorg}, σ_{Aaq}, σ_{Vorg} and σ_{Vaq} define the uncertainties of the polonium activity measured in the organic and aqueous phases and of the volume of the organic and aqueous phases, respectively.

The activity of polonium was determined by liquid scintillation counting using a 2550 TR Liquid Scintillation analyzer from Packard or a 1440 Quantulus from Perkin Elmer. The samples were prepared by mixing an aliquot of 0.4 mL of the aqueous/organic solution to be measure with 1.8 mL of 1 M HCl and 2.2 mL of ultimate gold AB scintillation cocktail. The measurement time was fixed at 1 hour. The quenching arising from the organic solvent was taken into account according to the following relation:

$$A = Am(-9.10-5 \times Tsie3 + 67310-4 \times Tsie2 - 17.26 \times Tsie + 1565.8) \qquad \textbf{Eq. 3.}$$

A_m being the activity measured by liquid scintillation and *Tsie*, an independent parameter from polonium analyses defined by the apparatus to determine the quenching parameter. The associated uncertainties were determined by the Poisson Law.

III.3. Modeling

a) Extraction by TOA

Trioctylamine is protonated in contact with hydrochloric acid solution [27]and the extraction of HCl could not be neglected under these conditions. The mechanism of HCl extraction by TOA can be represented as:

$$\overline{[CH_3(CH_2)_7]_3N} + HCl \Leftrightarrow \overline{[CH_3(CH_2)_7]_3NH^+Cl^-} \qquad \textbf{Eq. 4}$$

According to Kojima et al.[28], TOA is totally associated with HCl in a hydrochloric acid solution with a concentration ranging between 0.01 and 10 M. In this HCl concentration range, this equilibrium could therefore be neglected by considering TOA in hydrochloric acid solution as TOA.HCl or $\overline{[CH_3(CH_2)_7]_3NH^+Cl^-}$

The TOA.HCl formed will serve as an ion exchanger for anionic metal complexes. Only the polonium aqueous complexes which are present in the anionic form will exchange chloride ions and thus be extracted into the organic phase. The resulting equilibrium can be written as:

$$Po_a(OH)_mCl_z^{na-m-z} + (z+m-na)\overline{[CH_3(CH_2)_7]_3NH^+Cl^-} \Leftrightarrow \overline{([CH_3(CH_2)_7]_3NH)_{z+m-na}Po_a(OH)_mCl_z} + (z+m-na)Cl^- \qquad \textbf{Eq. 5}$$

where $Po_a(OH)_mCl_z^{na-m-z}$ represent the major aqueous species of polonium in the defined conditions with (na-m-z) lower than zero.

All experiments were performed at ultra-trace concentration (Po concentration ~10^{-13}M). We can safely eliminate the possible formation of polynuclear polonium species, *i.e.* a=1. According to the literature[5, 29, 30], the most stable oxidation state of polonium in aqueous solutions is +4 (n=4) and the coordination number is accepted as 6 [31-33]. Moreover, as each phase is neutral, the extracted polonium species must also be neutral. Considering these different information, equilibrium 5 can be re-written:

$$Po(OH)_mCl_z^{4-m-z} + (z+m-4)\overline{[CH_3(CH_2)_7]_3NH^+Cl^-} \Leftrightarrow \overline{([CH_3(CH_2)_7]_3NH)_{z+m-4}Po(OH)_mCl_z} + (z+m-4)Cl^- \quad \textbf{Eq. 6}$$

The associated thermodynamic constant can be expressed as:

$$K_{m,z} = \frac{\overline{[([CH_3(CH_2)_7]_3NH)_{z+m-4}Po(OH)_mCl_z]}[Cl^-]^{z+m-4}}{[Po(OH)_mCl_z^{4-z-m}][\overline{([CH_3(CH_2)_7]_3NH^+Cl^-]}^{z+m-4}} \frac{\overline{\gamma_{Po}} * \gamma_{Cl^-}^{z+m-4}}{\gamma_{Po(OH)_mCl_z} * \overline{\gamma}^{z+m-4}_{[[CH_3(CH_2)_7]_3NH^+Cl^-]}} \quad \textbf{Eq. 7}$$

where the extracted species of polonium are denoted as Po for simplicity.

$[C]$ and $[\bar{C}]$ are the molar concentrations of C species in the aqueous and organic phases, respectively. $\bar{\gamma}$ and γ are the activity coefficients in the aqueous and organic phases, respectively.

By rearranging Eqs. (2) and (7), the following expression is obtained:

$$LogD = \sum_{m,z}(\log K_{m,z} - \log\frac{\overline{\gamma_{Po}} * \gamma_{Cl^-}^{z+m-4}}{\gamma_{Po(OH)_mCl_z} * \overline{\gamma}^{z+m-4}_{[[CH_3(CH_2)_7]_3NH^+Cl^-]}} - (m+z-4)\log[Cl^-] - (m+z-4) * \log\overline{[[CH_3(CH_2)_7]_3NH^+Cl^-]} - \log\alpha_{Po(OH)_mCl_z}) \quad \textbf{Eq. 8}$$

where $\alpha_{Po(OH)_m Cl_z}$ is a Ringböm coefficient (also named complexation coefficient), linked to the total aqueous speciation of polonium $\alpha_{Po(OH)_m Cl_z} = [Po]_T / [Po(OH)_m Cl_z^{4-m-z}]$. The global partition coefficient is the summation of the different extracted species.

As the liquid/liquid extractions were done at ultra-trace polonium concentration, the organic activity coefficients were assumed constant. Eq. (8) can be written as:

$$LogD = \sum_{m,z} (\log C_1 + \log \gamma_{Po(OH)_m Cl_z} - (m+z-4)\log a_{Cl^-} + (m+z-4) * \log[\overline{[CH_3(CH_2)_7]_3 NH^+Cl^-}] - \log \alpha_{Po(OH)_m Cl_z}) \quad \textbf{Eq. 9}$$

where C1 is constant.

According to Eq. (9), the stoichiometric coefficient (m+z-4) could be found by analyzing the slope of log D *vs.* $\log[\overline{[CH_3(CH_2)_7]_3 NH^+Cl^-}]$. Consequently, the charge of the major aqueous species of polonium could also be determined.

In addition, the complexation coefficient could be established by studying the partition coefficient *vs.* chloride activity for constant TOA concentration.

b) Extraction by TBP

The extraction of polonium by TBP may be represented by the general expression presented below, considering the different species which can be characterized by the extraction with TOA:

$$\boldsymbol{Po(OH)_m Cl_z^{4-m-z} + d\overline{TBP} \rightleftharpoons \overline{Po(OH)_b Cl_c (TBP)_d} + (b-m)H^+ + (m-b)H_2O + (z-c)Cl^-} \quad \textbf{Eq. 10}$$

with b+c=4 and m+z=4 or 6

The associated thermodynamic constant can be expressed as:

$$K_2 = \frac{\overline{a_{Po}} a_H{}^{b-m} a_{H_2O}^{m-b} a_{Cl}^{z-c}}{a_{Po(OH)_m Cl_z} \overline{a_{TBP}}^d} = \frac{[\overline{Po}]\overline{\gamma_{Po}}[Cl^-]^{z-4+b}\gamma_{Cl}{}^{z-4+b}[H^+]^{b-m}\gamma_H{}^{b-m} a_{H_2O}^{m-b}}{[Po(OH)_m Cl_z{}^{4-m-z}]\gamma_{Po(OH)_m Cl_z}[\overline{TBP}]^d \overline{\gamma_{TBP}}^d}) \qquad \textbf{Eq. 11}$$

By rearranging Eq. (2) and Eq. (11), as for the extraction by TOA, the general expression of the partition coefficient of polonium between both phases at trace concentration can be written as:

$$\boldsymbol{D = \frac{K_2 * \gamma_{Po(OH)_m Cl_z} * [\overline{TBP}]^d \overline{\gamma_{TBP}}^d}{\overline{\gamma_{Po}}[Cl^-]^{z-4+b}\gamma_{Cl}{}^{z-4+b}[H^+]^{b-m}\gamma_H{}^{b-m} a_{H_2O}^{m-b} \alpha_{Po(OH)_m Cl_z}}} \textbf{Eq. 12}$$

$$\boldsymbol{\log D = \log K_2 - (m-b) * \log a_{H2O} + \log \frac{\gamma_{Po(OH)_m Cl_z} * \overline{\gamma_{TBP}}^d}{\overline{\gamma_{Po}}\gamma_{Cl}{}^{z-4+b}\gamma_H{}^{b-m}} - (z-4+b)\log[Cl^-] + d * \log[\overline{TBP}] - (b-m)\log[H^+] - \log \alpha_{Po(OH)_m Cl_z}} \qquad \textbf{Eq. 13}$$

As previously, the activity coefficients of organic species could be considered constant during experiments.

$$\boldsymbol{\log D = \log C_2 - (m-b) * \log a_{H2O} + \log \frac{\gamma_{Po(OH)_m Cl_z}}{\gamma_{Cl}{}^{z-4+b}\gamma_H{}^{b-m}} - (z-4+b)\log[Cl^-] + d * \log[\overline{TBP}] - (b-m)\log[H^+] - \log \alpha_{Po(OH)_m Cl_z}} \qquad \textbf{Eq. 14}$$

Consequently, under fixed aqueous conditions (ionic strength and concentration of HCl), the number of TBP involved can be proven by varying the concentration of TBP. Then, by knowing the speciation of polonium in aqueous medium, the stoichiometric coefficients of Eq. (13) involving chloride ions and protons could be determined. The deviation between integer slopes and experimental slopes could be explained by the non-ideal behavior in concentrated medium. When necessary, activity coefficients in aqueous solution were calculated using the SIT theory [34].

All the fittings in this work were done by using Excel software and solver. The associated uncertainties were determined by using the Excel Macro from Robert de Levie[35].

IV. Results and discussion

IV.1. Kinetic aspects

The extraction mechanisms and the associated thermodynamic data could be determined if the equilibrium is reached. In the first step, the time necessary to reach equilibrium was studied for both extraction systems. As shown in Figure 2, the partition of Po(IV) between the organic phase containing 0.04 M TOA in para-xylene and an aqueous phase of 10 M HCl reached equilibrium within less than 100 min.

Concerning the extraction of polonium by TBP, several extractions were carried out using equal volumes of pre-equilibrated 10% TBP in a para-xylene organic phase and an aqueous phase containing polonium in different HCl concentrations. As shown in Figure 2, the distribution of Po(IV) between both phases reached equilibrium within 20 min in a variable range of hydrochloric acid concentration. Based on these results, 40 min shaking was adopted in all cases to ensure the existence of partition equilibrium.

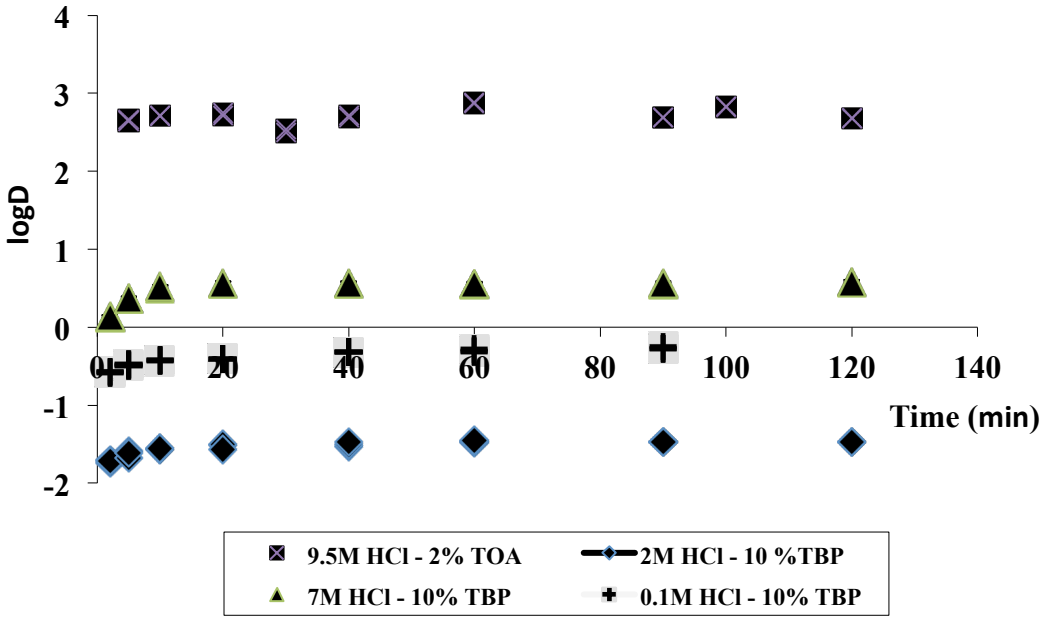


Figure 2: **Effect of shaking time on the polonium distribution coefficient using different extractant (TBP or TOA) in para-xylene from HCl.**

IV.2. Extraction study with TOA

Our first objective was to confirm the existence of $PoCl_6^{2-}$ expected in strong HCl concentration. According to Eq. (9), for a constant hydrochloric acid concentration, the charge of the aqueous species is found by studying the partition coefficient as a function of TOA concentration. From Figure 3, a slope equal to 1.78 ± 0.16 can be obtained by plotting logD(Po) *vs.* log[TOA] which indicates that (m+z) is equal to 6. Moreover, in 10 M HCl, the existence of hydroxide complexes can be neglected (m=0). This confirms the presence of $PoCl_6^{2-}$ as a major polonium species in acidic media.

Eq.(8) can thus be written as :

$$LogD = Log\,K - \log\frac{\overline{\gamma_{Po}} * \gamma_{Cl^-}^2}{\gamma_{PoCl_6^{2-}} * \overline{\gamma}_{[[CH_3(CH_2)_7]_3NH^+Cl^-]}^2} - 2\log[Cl^-] + 2 * \log\overline{[[CH_3(CH_2)_7]_3NH^+Cl^-]} - \log\alpha_{PoCl_6^{2-}} \quad \textbf{Eq. 15}$$

where, $\log\alpha_{PoCl_6^{2-}}$ is equal to 1.

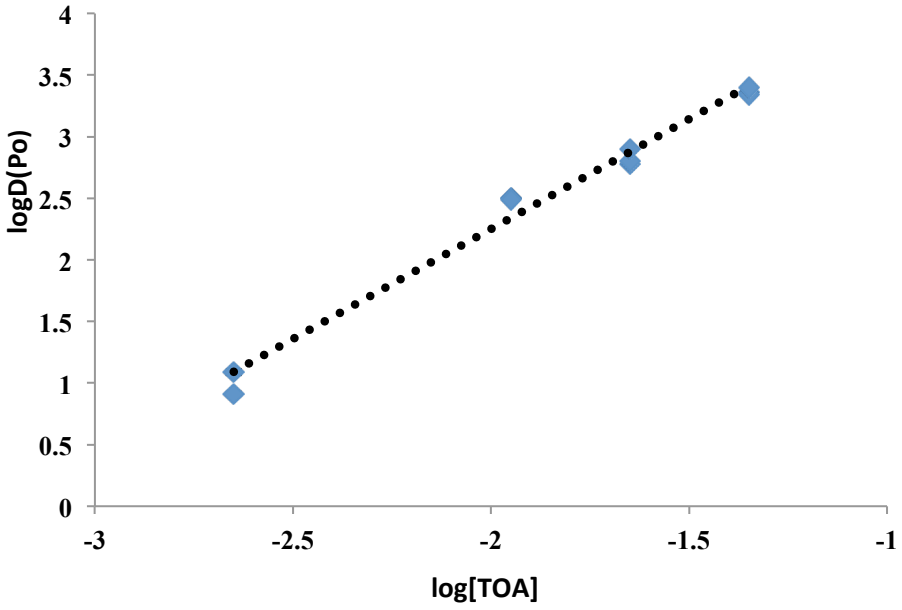


Figure 3: Influence of TOA concentration in *para*-xylene on polonium extraction using 10 M HCl solution; Po activity = 280 Bq; The line represents a linear tendency curve.

When the acid concentration decreases, other species are proposed in the literature (Figure 1). This change of speciation was investigated by studying polonium extraction as a function of hydrochloric acid activity with TOA (Figure 4). Note that the activity coefficients were determined using Specific Interaction Theory (SIT) [34]. The value of the activity coefficient between H^+ and Cl^- interaction is available in the literature[34] and amounts to 0.12. Our results show the existence of $PoCl_6^{2-}$ as a major species in the

concentrated medium. However, no specific interaction parameter between this species and H^+ is reported in the literature. Because thorium and polonium possess the same ionic charge and ionic radius, the available data for thorium can be considered for polonium. However, data are unavailable for $ThCl_6^{2-}$ species. Therefore, the specific interaction parameter for $PoCl_6^{2-}$ has been considered as a fitting parameter in our modeling.

If $PoCl_6^{2-}$ is the major aqueous species over the total range of Cl^- activity (10^{-1}- $10^{2.5}$ M), the logarithm of the partition coefficient must decrease with a slope equal to **-2** as a function of chloride activity (Eq. (9)). Except in the a_{Cl^-} range above ~$10^{1.8}$ M (or for HCl concentration >6M), the predicted tendency is not observed. A constant distribution coefficient value of $10^{3.4}$ is measured in the HCl concentration range between 0.2 and 6 M (or aCl^- range between $10^{-0.5}$ and $10^{1.2}$ M). Below $a_{Cl}=10^{-0.5}$ ([HCl]=0.2 M), the D value decreases.

This behavior can be explained by the formation of other non-extractable cationic or neutral Po species in the aqueous solution which compete with the extraction of $PoCl_6^{2-}$ by TOA. This is thermodynamically related by an increase in the value of Ringböm coefficient.

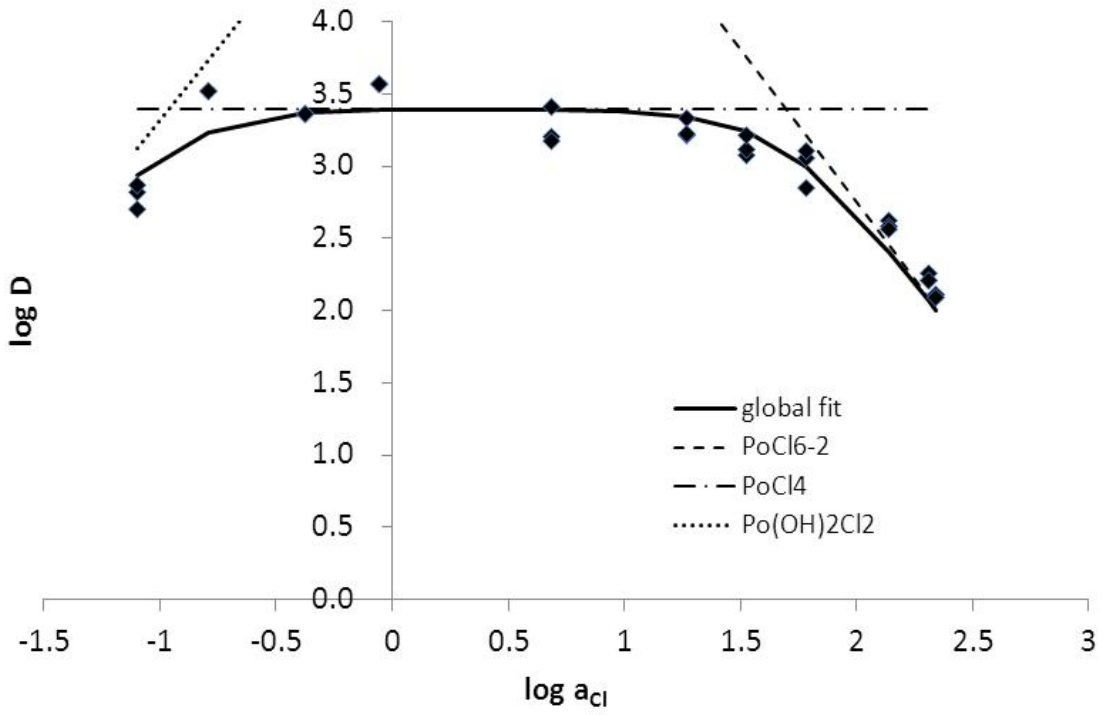


Figure 4: Extraction of $PoCl_6^{2-}$ by 0.04 M TOA in para-xylene (Po activity = 280 Bq) as a function of chloride activity. The linear fit represents the extraction of $PoCl_6^{2-}$ as a function of the major species in aqueous solution according to Eq.9.

To be coherent with the spectrophotometric results of Hunt et al.[20], two species were considered, one in the concentration range between 0.5 and 6M (i.e. the one characterized by an absorption band of 418 nm and corresponding to $PoCl_{n+b}$ (n=2)) and the other dominating Po speciation below 0.5M (i.e. the one characterized by an absorption band of 318 nm and corresponding to $Po(OH)_aCl_b$ (a=1)). A good agreement between the experiment and the calculation was obtained when the neutral complex of the general form $Po(OH)_mCl_z^{4-m-z}$ is considered with m+z equal to 4. As it is impossible to define m and z by the fitting approach considering the available experimental data, literature data were used in order to identify them.

According to Hunt et al[20], in the concentration range between 0.5 and 6 M, no effect of H+ was observed, suggesting that m=0. The weak influence of H^+ in the speciation was also observed by Suganuma [16] by liquid-liquid extraction in the pH range 0-0.5. This allows the conclusion that the species corresponds to PoCl4.

Danon & Smith [14] showed that the adsorption of polonium is negligible at concentrations of hydrochloric acid greater than 0.2 M on a cation exchange resin suggesting the presence of an anionic or neutral species. The anionic character of the species was further evidenced based on data realized with the anion-exchange resin where a strong sorption was observed in the HCl concentration range between 0.1 and 12M. These data are coherent with previous studies performed by cataphoresis experiments[2] that showed that polonium ions migrated toward the anode for $[H+] \geq 0.5M$ As a conclusion, Danon & Smith stated "Since under these conditions the adsorption by the cation-exchange resin is negligible and essentially all polonium migrates in the electric field to the anode, $PoCl_{2+b}$ is probably a negatively charged complex, possibly $PoC1_6^{-2}$". This result does not agree with our results that propose $PoCl_4$. It is worth saying, as it is also indicated in the paper of Danon, that the identification of the charge of a species by adsorption studies on exchangers should be taken with great care when working at ultra-trace concentrations. We notably showed that cationic forms of astatine, the radioelement next to Po in the periodic table, can be sorbed both on anionic and cationic exchangers [36]. However, the divergence between the charge of the proposed species and the results of the

electromobility measurements remains however difficult to understand. This will be further discussed in the next part of the paper.

For the lowest value of HCl activity (<0.2 M), the decreasing partition coefficient evidenced can be explained by the formation of another aqueous species $Po(OH)_mCl_z^{4-m-z}$ with **m+z=4.** In this acidic pH range, we cannot neglect the possible formation of mixed a hydroxo-chloro complex and the stoichiometry of the species cannot be completely determined. According to the literature, there are two possibilities; Suganuma[16, 25] studied the H^+ dependence by liquid liquid extraction and came to the conclusion that two H^+ ions were exchanged in the 0.5-0.1 pH range. This would indicate the existence of the species $Po(OH)_2Cl_2$. A pH dependence was also studied by Hunt regarding the evolution of the absorbance of the two complexes as a function of H^+ concentration (0.5-0.1). In this case, they came to the conclusion that there is one proton exchanged which would indicate the existence of the species $Po(OH)Cl_3$. The picture is further complicated by the number of average chloride ions determined to be bound to Po at pH=1, i.e. 3.5, by studying the dependence of the distribution ratio on the concentration of the chloride ion.

In conclusion, the experiments performed in the presence of TOA enabled the confirmation of (i) the existence of $PoCl_6^{2-}$ in strong acidic conditions. To our knowledge, this is the first experiment to unequivocally identify the charge -2 (ii) the potential presence of two other neutral species, $Po(OH)Cl_3$ or $Po(OH)_2Cl_2$ and $PoCl_4$, predominant in the HCl concentration range of 0.1-0.5 and 0.5-6 M, respectively.

IV.3. Extraction study with TBP

a) Influence of TBP concentration

For each domain of HCl concentration in which there was a single dominant species (i.e. the Ringböm coefficient was constant), the influence of TBP concentration on the Po distribution coefficient was studied. The results are presented in Figure 5. According to Eq. (14), the slope of log D(Po) *vs.* log[TBP] at a constant acidity indicates the difference in the average number of tributylphosphate molecules of the species of polonium extracted into the organic phase.

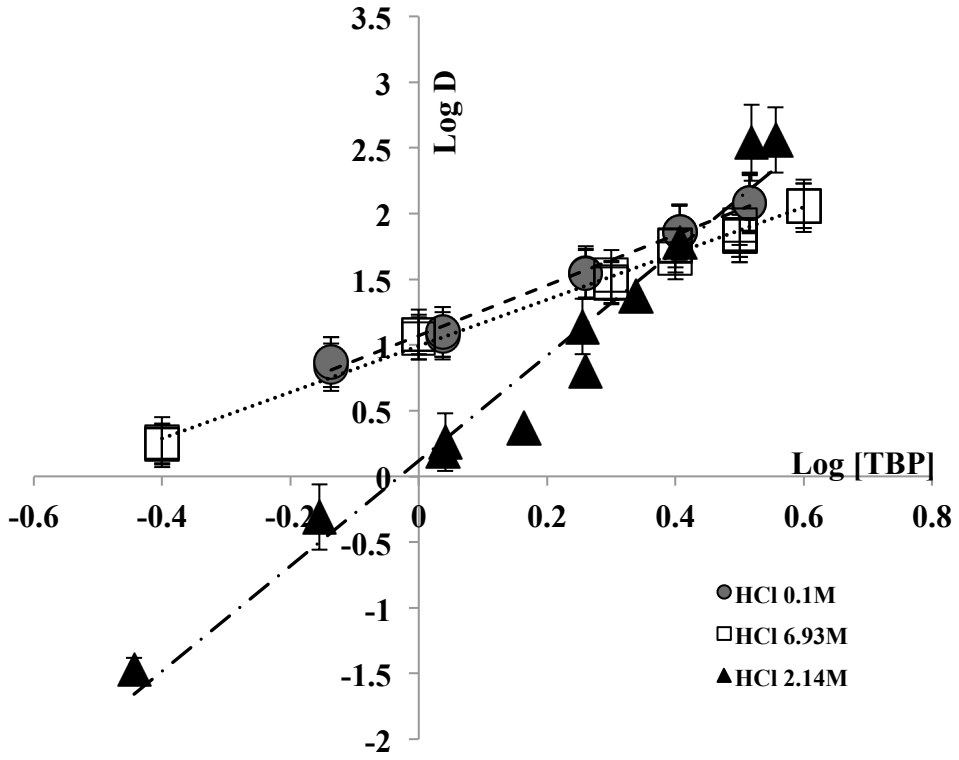


Figure 5: Influence of TBP concentration and HCl concentration on the partition coefficient of polonium. Po activity = 280 Bq . The lines correspond to linear tendency curves.

For the highest concentration of hydrochloric acid, the slope is equal to 1.76 ± 0.06. This can be explained by the involvement of two TBP molecules in the extraction mechanism of the polonium-chloride complex. This is in agreement with the work of Bagnall et al.[37], who reported the extraction of a Po species bound to two TBP molecules from 8 M HCl in decalin. This number of TBP molecules has often been observed for similar extraction conditions with other metal ions like Pt. In contrast, as the concentration of hydrochloric acid decreases to 2.14M, a slope of 4.0 ± 0.5 exhibited. This could be explained by the extraction of another polonium species with 4 TBP molecules in interaction with Po. This result confirms that at least two different aqueous species exist in the HCl concentration range 2-10M. Considering that $PoCl_6^{2-}$ has been shown to be present at 10M, we can exclude its existence at 2M. This simply excludes the conclusion of the work of Danon[14] who proposed that $PoCl_{2+b}$ species identified by spectrophotometry corresponds to $PoCl_6^{2-}$.A careful analysis of the literature data show that Bagnell et al. indicate the extraction of polonium with three molecules of TBP at a 6M HCl concentration. This result agrees with our work, since 6M corresponds to transient range where both extraction mechanisms with 2 and 4 TBP molecules occur.

In the diluted HCl medium, a slope equal to 1.9 ± 0.1 was observed which corresponds to the involvement of two TBP molecules in the extraction mechanism. The results confirm the initial conclusion that at least three different aqueous polonium species were extracted by TBP in the HCl concentration range studied.

b) Extraction mechanism as a function of HCl

concentration

To elucidate the extraction mechanisms of polonium by TBP, the following step was to determine the number of H^+ and Cl^- involved by studying D_{Po} variation as a function of HCl concentration. In this study, polonium was extracted by TBP in HCl concentration range from 0.1 M and 8M HCl. The influence of HCl concentration on the distribution ratio of polonium using 10 % TBP in para-xylene, shows an important variation (figure.6). The partition coefficient decrease from HCl 0.1M to 0.9M to reach a plateau equal to 0.06. Then the partition coefficient increases with HCl concentration from 2M to 8M. A similar curve determined in the similar experimental conditions (same TBP concentration but different solvent) was obtained by Bagnall [37] and Haissinsky [38].

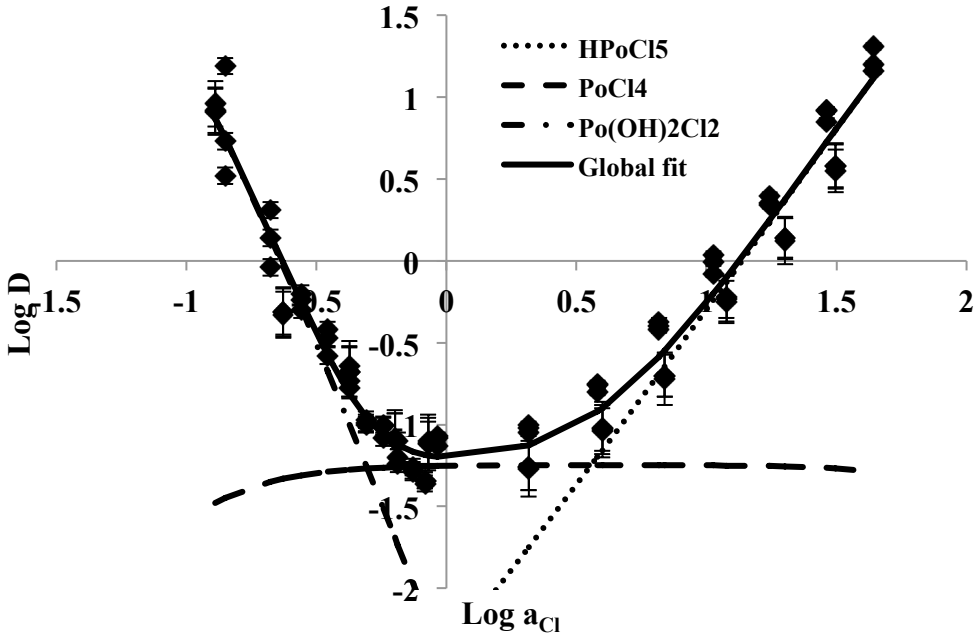


Figure 6: **Partition coefficient of polonium as a function of hydrochloric acid activity.10% TBP in *para*-xylene; Po activity= 280 Bq. The line represents the fitting made according to eq.16 with the parameters presented in table 2.**

As $PoCl_6^{2-}$ was evidenced in this study in concentrated hydrochloric acid, $PoCl_6^{2-}$ was substituted in Eq. (10) and Eq. (14) to define the general extraction mechanism which can be written as:

$$\boldsymbol{PoCl_6^{2-} + d\overline{TBP} \rightleftharpoons \overline{Po(OH)_bCl_{4-b}(TBP)_d} + bH^+ + (-b)H_2O + (2+b)Cl^-} \qquad \textbf{Eq. 16}$$

$$\boldsymbol{\log D = \log C_2 + b * \log a_{H2O} + \log \frac{\gamma_{PoCl_6}}{\gamma_{Cl}{}^{6-c}\gamma_H{}^b} - (2+b)\log[Cl^-] + d * \log[\overline{TBP}] - b\log[H^+] - \log \alpha_{PoCl_6}} \qquad \textbf{Eq. 17}$$

As the study of polonium extraction as a function of HCl concentration was reached at a fixed TBP concentration (10%) and polonium was used at trace concentration, $[H^+]$ and $[Cl^-]$ are assumed to be equal. Moreover, HCl is responsible for the ionic strength so the activity coefficients of both species are equal. Eq. (17) could be simplified as:

$$\boldsymbol{\log D - \log \gamma_{PoCl_6} + \log \alpha_{PoCl_6} = \log C_3 + b * \log a_{H2O} - (2+2b)\log a_{HCl}} \qquad \textbf{Eq. 18}$$

The complexation coefficient used to fit the extraction of polonium was determined previously from the study with TOA.

To fit the experimental results, at least three different extracted species needed to be considered as a function of hydrochloric acid activity. In concentrated medium, a slope equal to 0 was determined (figure 6) and thus $HPoCl_5$ species had to be considered to explain the increase in extraction with HCl activity. In these conditions, the major aqueous species evidenced previously was $PoCl_4$. Consequently, an HCl molecule seemed to be extracted simultaneously with $PoCl_4$ and 2 TBP molecules (previously determined).

When the acidity decreased, $PoCl_4$ seemed to be extracted alone because a slope equal to

-2 had to be used to fit the experimental data for hydrochloric acid concentrations between 0.9 and 2 M (figure 7).

In more diluted media, where $PoCl_4$ is no longer the major species, the extraction data can be explained considering the extraction of $Po(OH)_2Cl_2$ (slope equal to -6), which is stabilized in organic solution. The extracted species may be related to the one evidenced in aqueous solution from the study with TOA, i.e. $Po(OH)_mCl_z$ (m+z=4) with m=2 and z=2.

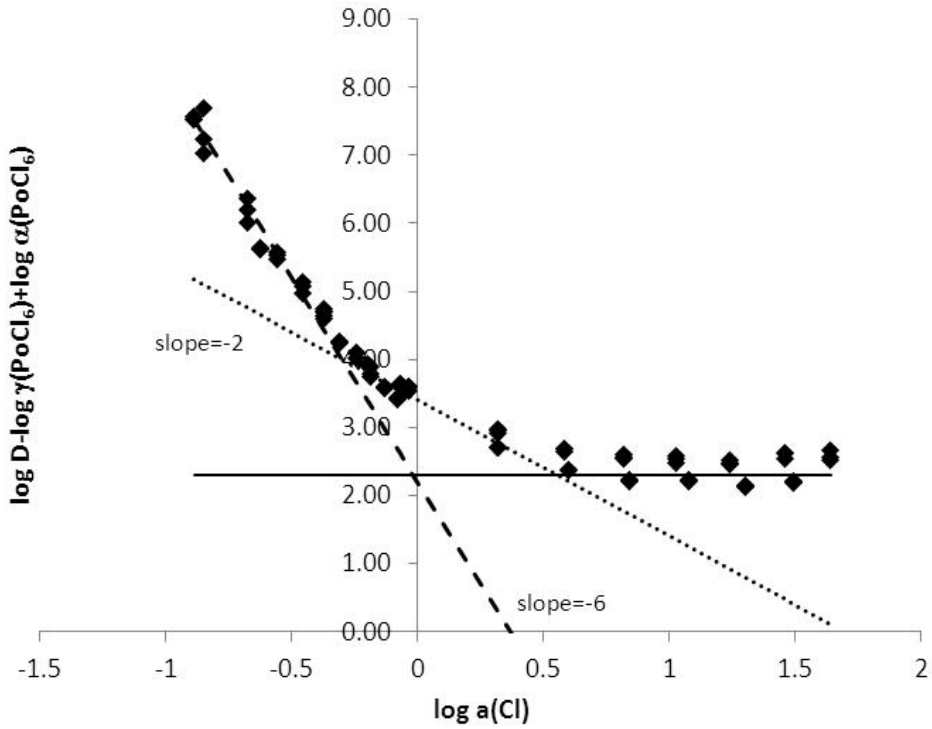


Figure 7: **$(\log D - \log \gamma_{PoCl_6} + \log \alpha_{PoCl_6})$ as a function of logarithm of chloride ions activity (TBP 10% in para-xylene).**

c) Modeling results

The experimental result fits the theoretical modeling by considering five equilibriums which represent the aqueous polonium speciation and the extracted polonium species.

Table 1: Fitted thermodynamic data of polonium speciation (SIT parameter for $PoCl_6^{2-}$: ε=-0.01± 0.008)

Reaction	**logarithm of thermodynamic constants**
Aqueous equilibrium:	
$PoCl_4 + 2Cl^- \rightleftharpoons PoCl_6^{2-}$	4.6±0.2
$Po(OH)_2Cl_2 + 4Cl^- + 2H^+ \rightleftharpoons PoCl_6^{2-} + 2H_2O$	2.7±0.8
Extraction equilibrium :	
${PoCl_6}^{2-} + 2\overline{[CH_3(CH_2)_7]_3NH^+Cl^-} \rightleftharpoons \overline{([CH_3(CH_2)_7]_3NH)_2PoCl_6} + 2Cl^-$	
${PoCl_6}^{2-} + 2\overline{TBP} + H^+ \rightleftharpoons \overline{HPoCl_5(TBP)_2} + Cl^-$	2.14± 0.07
${PoCl_6}^{2-} + 4\overline{TBP} \rightleftharpoons \overline{PoCl_4(TBP)_4} + 2Cl^-$	3.35± 0.08
${PoCl_6}^{2-} + 2\overline{TBP} + 2H_2O \rightleftharpoons \overline{Po(OH)_2Cl_2(TBP)_2} + 2H^+ + 4Cl^-$	2.18± 0.06

The stability constant for the equilibrium between $PoCl_4$ and $PoCl_6^{2-}$ amounts to $10^{-4.6}$. From the constants given in table 1, we can determine a value equaling $10^{-3.07}$ which is higher than our value. This discrepancy could be explained by the inaccuracy of the value determined by Starik et al[39]. Indeed, they proposed 6 successive thermodynamic constants as a function of HCl concentration. However, the calculation of the polonium speciation diagram presented in Starik studies[39], showed $PoCl_6^{2-}$ to be the major species (>99%) over the total range of HCl concentration studied!

Table 2: Stability constant data of polonium-chloride complex.

Species	Temperature		Medium	Equilibrium constant	Method of determination	Reference
$[PoCl]^{3+}$	22-25 °C	I=1M	4-6 M HCl	$LogK_1 = 2.56$	Liquid-liquid extraction	[40]
		I=1M	1 $H(ClO_4)$	$LogK_1 = 2.34$	Cation exchange	[40]
$[PoCl_2]^{2+}$	22-25 °C	I=1M	4-6 M HCl	$Log\beta_2 = 4.8$	Liquid-liquid extraction	[40]
		I=1M	1 $H(ClO_4)$	$Log\beta_2 = 4.42$	Cation exchange	[40]
$[PoCl_3]^{+}$	22-25 °C	I=1M	4-6 M HCl	$Log\beta_3 = 6.88$	Liquid-liquid extraction	[40]
		I=1M	1 $H(ClO_4)$	$Log\beta_3 = 6.34$	Cation exchange	[40]
$[PoCl_4]$	22-25 °C	I=1M	4-6 M HCl	$Log\beta_4 = 8.85$	Liquid-liquid extraction	[40]
		I=1M	1 $H(ClO_4)$	$Log\beta_4 = 8.53$	Cation exchange	[40]
$[PoCl_5]^{-}$	22-25 °C	I=1M	4-6 M HCl	$Log\beta_5 = 10.6$	Liquid-liquid extraction	[40]
		I=1M	1 $H(ClO_4)$	$Log\beta_5 = 10.08$	Cation exchange	[40]
$[PoCl_6]^{2-}$	22-25 °C	I=1M	4-6 M HCl	$Log\beta_6 = 11.92$	Liquid-liquid extraction	[40]
	22-25 °C	I=1M	1 $H(ClO_4)$	$Log\beta_6 = 11.57$	Cation exchange	[40]
	22-25 °C	I=0M		$LogK_6 = 2.3$	Liquid-liquid extraction	[40]
	22°C	I=1M		$Log\beta_6 = 14$	Electrochemical	[19, 41, 42]

V. Conclusion

The extraction of polonium by TOA indicates the presence of three major polonium species in hydrochloric acid solution. $PoCl_6^{2-}$ species dominates in concentrated hydrochloric acid medium ([HCl]>6M). Below 6M, $PoCl_4$ appears the major species that governs Po speciation. Finally, in diluted acidic medium, our results show the presence of a third species $Po(OH)Cl_3$ or $Po(OH)_2Cl_2$.

The extraction of polonium by TBP shows the presence of three extracted species which are attached to a different number of TBP molecules. They are not directly related to

species proposed to exist in aqueous phase. $PoCl_6^{2-}$ doesn't appear to be extracted; this is comprehensible as it is a strong complex where the coordination sphere is complete. $PoCl_4$ appears to be extracted (sphere not completed) with a priori two possibilities, either with one HCl and two TBP or without HCl but with 4 TBP. Finally, the results in dilute medium indicate the extraction of $Po(OH)_2Cl_2$ by two TBP molecules which might correspond to the one evidenced by the TOA study. Po behavior was quantitatively described with the constants given in table 2.

Acknowledgement

The authors thank the French National Center of Scientific Research (CNRS) and the Pays de Loire Region for the doctoral grant allocated to Ali Younes.

Bibliographie de l'Article III

1. Ansoborlo, E.; Berard, P.; Den Auwer, C.; Leggett, R.; Menetrier, F.; Younes, A.; Montavon, G.; Moisy, P., Review of Chemical and Radiotoxicological Properties of Polonium for Internal Contamination Purposes. *Chemical Research in Toxicology***2012***,* 25, (8), 1551.
2. Moyer, H. V.; Gnagey, L. B.; Rogers, A. J., *POLONIUM*. United States Atomic Energy Commission: Oak Ridge, Tennesse, July **1956**.
3. Bagnall, K. W., *Chemistry of the rare elements, Polonium- Actinium*. Butterworths: London, **1957**; p 25 and 51.
4. Ampelogova, N., *Radiokhim* **1974,** 16, (1), 52.
5. Koch, H.; Schmidt, H., *Z. Naturforsch* **1963,** 18b, 936.
6. Starik, I.; Ampelogova, N.; Kuznetsov, B., *Radiokhim* **1964,** 6, 507.
7. Suganuma, H.; Samukawa, T.; Hataye, I., *Solution Chemistry of Polonium I: Adsorption of Polonium in Acidic Solution*. **1973**; p 51.
8. Hataye, I.; Suganuma, H.; Ito, K.; Kato, M., *Solution Chemistry of Polonium II: Deposition of Polonium and Radiocolloids in Aged Solution*. **1974**; p 41.
9. Matsuura, N.; Ouchi, A.; Kojima, M., Studies on extraction of polonium(IV) by hexone from acid Solution. *Bulletin of the Chemical Society of Japan* **1961,** 34, (3), 411-416.
10. Guillot, M., *J. chim. phys.* **1931,** 88, 92.
11. Starik I.E.; Ampelogova, N., *Radiokhimiya* **1961,** 3, 261.
12. Starik, I. E.; Ampelogova, N.; S., K. B., *Radiokhimiya* **1964,** 6, 524.
13. Ampelogova, N., *Radiokhim* **1973,** 15, 823.
14. Danon, J.; Zamith, A. A. L., Ion-Exchange and Solvent-Extraction Studies with Polonium. *The Journal of Physical Chemistry* **1957,** 61, (4), 431-434.
15. Ampelogova, N., *Radiokhimiya* **1973,** 16, 56.
16. Suganuma, H.; Hataye, I., Solvent extraction study on the hydrolysis of tracer concentration of Po(IV) in chloride solutions. *Journal of Inorganic and Nuclear Chemistry* **1981,** 43, (10), 2511-2515.
17. Staritzky US Atomic Energy Commission Rep.LA 1286: 1951.
18. Marcus, Y., Metal-chloride complexes studied by ion exchange and solvent extraction methods: II Transition-metal elements and the hexavalent actinides. *Coordination Chemistry Reviews* **1967,** 2, (3), 257-297.
19. Bagnall, K. W.; Freeman, J. H., Electrochemical studies on polonium. *Journal of the Chemical Society (Resumed)* **1956**, 2770-2774.
20. Hunt, D. J. *Absorbancy Studies of Polonium Complexes in Chloride Solutions*; MLM-979; Mound Laboratory: **1954**.
21. McCluggagd. W.C. *Quarterly Progress Report*; MLM-405-2; **1949**; p 71.
22. Progress Report, report WASH-82, December **1952**; p 11.
23. Progress Report, report WASH-86, April **1953**; p 6.
24. Sheppard, J. C.; Warnock, R., The distribution of bismuth (III) and polonium (IV) between trilaurylamine solutions of xylene and hydrochloric and hydrobromic acid solutions. *Journal of Inorganic and Nuclear Chemistry* **1964,** 26, (8), 1421.

25. Suganuma, H., Anion-exchange of the chemical species of tracer concentrations of polonium(IV) in chloride solutions. *Journal of Radioanalytical and Nuclear Chemistry* **1995,** 191, (2), 265-272.
26. Younes, A.; Montavon, G.; Alliot, C.; Mokili, M.; Haddad, F.; Deniaud, D.; Champion, J., A route for polonium 210 production from alpha irradiated bismuth-209 target. *Journal of Radiochemical Acta* (to be published).
27. Fu, J.; Nakamura, S.; Akiba, K., Separation and recoverey of Gold,Platinum and Palladium by trioctylamine membrane. *Analytical Sciences* **1995,** 11, 149-153.
28. Kojima, T.; Fukutomi, H.; Kakihana, H., Extraction of hydrochloric acid by Tri-n-octylamine in benzene. *Bulletin of the Chemical Society of Japan* **1969,** 42, 875-880.
29. Starik, I. E.; Ampelogova, N. I.; Kuznetsov B. S., *Radiokhimiya* **1964,** 6, 519.
30. Ampelogova, N. I., *Radiokhimiya* **1974,** 17, 68.
31. Ayala, R.; Martinez, J. M.; Pappalardo, R. R.; Munoz-Paez, A.; Marcos, E. S., Po(IV) Hydration: A Quantum Chemical Study. *The Journal of Physical Chemistry B* **2008,** 112, (17), 5416-5422.
32. Ayala, R.; Martinez, J. M.; Pappalardo, R. R.; Munoz-Paez, A.; Sanchez Marcos, E., *J. Phys. Chem. B* **2009,** 113, 487.
33. Ayala, R.; Spezia, R.; Vuilleumier, R.; MartiÌ□nez, J. M.; Pappalardo, R. R.; Sainchez Marcos, E., An Ab Initio Molecular Dynamics Study on the Hydrolysis of the Po(IV) Aquaion in Water. *The Journal of Physical Chemistry B* **2010,** 114, (40), 12866-12874.
34. Grenthe, I.; Wanner, H., guidelines for the extrapolation to zero ionic strength. France, **2000**.
35. Levie, R. d., *Advanced Excel for scientific data analysis*. New York, **2004**.
36. Champion, J.; Alliot, C.; Huclier, S.; Deniaud, D.; Asfari, Z.; Montavon, G., Determination of stability constants between complexing agents and At(I) and At(III) species present at ultra-trace concentrations. *Inorganica Chimica Acta* **2009**, (362), 2654–2661.
37. Bagnall, K. W.; Robertson, D. S., Solvent extraction studies with polonium. *Journal of the Chemical Society (Resumed)* **1957**, 509-512.
38. Haïssinsky, M.; Pluchet, E., Extraction du polonium par solvants et potentiels normaux du polonium réduit. *Journal of Inorganic and Nuclear Chemistry* **1966,** 28, (12), 2861-2871.
39. Starik, I. E.; Ampelogova, N. I., *Radiochimiya* **1965,** 7, 658.
40. Sillén, L. G., *Stability Constants of Metal Ion Complexes*. Special publication n°25 ed.; Burlington House, London, **1971**; p 187.
41. Figgins, P. E., *The Radiochemistry of Polonium*. NAS-NS: **1961**; p 3037.
42. L.G. Sillén, *Stability Constants of Metal Ion Complexes*. Special publication n°17 ed.; Burlington House, London, **1964**; p 300.

Article 4 : Synthesis of a Novel Hexadentate Chelating Agent "$N_2S_2O_2/N_4O_2$" for Po(IV) Complexation

Synthesis of a Novel Hexadentate Chelating Agent "$N_2S_2O_2/N_4O_2$" for Po(IV) Complexation

I. Abstract

Polonium is a high-energy alpha emitter, which presents an internal radiation hazard due to its low range of alpha particles in biological tissues (40-50 μm). It is considered an important component of natural radiation affecting humans. Poisoning by polonium is well-known from the case of Alexander Litvinenko who, in the absence of a special chelating agent, died in a very short period. In this context, there is a need to design a specific decorporation agent for polonium. A novel water-soluble multidentate "$N_2S_2O_2/N_4O_2$" ligand complexing agent was designed and synthesized. The synthesis of 2,2'-(2,2'-((5-(2-aminoethoxy)-1,3-phenylene)bis(methanylylidene))bis(1-(carboxymethyl)hydrazin-1-yl-2-ylidene))bis(thiazole-4-carboxylic acid) "$N_2S_2O_2/N_4O_2$" is based on the condensation of the bis(thiosemicarbazone) with the synthesized dialdehyde followed by a reaction with α-bromoester to afford the ligand core, bis(thiaziolohydrazine). This ligand presents *a priori* good characteristics for polonium complexation, *i.e.* a platform with four soft heteroatoms (N/S) and two additional pendant carboxylic groups to complete the octahedral coordination shell suitable for polonium (IV) complexation. Its strong affinity for polonium was studied and verified at pH=7.4 and compared with other known chelating agents such as BAL, DTPA and EDTA. The

results show the formation of stable polonium complexes in order of stability: $N_2S_2(N_4)$ < DTPA << BAL < $N_2S_2O_2$ (N_4O_2).

Keywords:

"$N_2S_2O_2/N_4O_2$" Synthesis, Po(IV) complexation, BAL, DTPA, Dithizone

II. Introduction

Early in November 1913, Sir William Ramsay believed that polonium (Po) had therapeutic qualities for the treatment of diseases which had hitherto not been treated[1]. There was enormous hope but this rapidly diminished after the discovery of polonium toxicity. Polonium-210 is a radioactive decay product in the natural uranium-238 decay series and one of the granddaughters of radon-222. It is a high alpha emitter with a half-life of 138.47 days, which presents a radiation hazard when ingested or inhaled. The emitted alpha particles have a range of 40-50 mm in biological tissue and are easily stopped by the stratum corneum [2]. Thus, external contamination by polonium does not cause radiation sickness. Although polonium-210 is present in the environment at extremely low concentration[3, 4], it is commonly taken into the body by consuming food, water, cigarettes and air, and is considered one of the most toxic elements ($LD_{50/30}$ =1.1-2.6 MBq/kg body mass or 6-15 ng/kg body mass)[5, 6]. Unlike the other alpha emitters, its radioactivity and high specific activity (166 TBq/g)[7] make it a very dangerous substance.

Interest in polonium in clinical toxicology was stimulated by the poisoning of Alexander

Litvinenko on November 1, 2006. He died 23 days later, after multiple organ failure[8]. The toxicokinetics of polonium have mostly been investigated in animals. Studies in humans following occupational inhalation incidents have met with analytical problems[8]. After internal contamination, 50 to 90% of the polonium ingested swiftly leaves the body in feces.[5, 9-11] The polonium absorbed from the gut enters the blood stream where it is concentrated initially in red blood cells [12] and then in the liver, kidney, spleen, bone marrow, gastrointestinal tract and gonads[3]. Unlike other alpha emitters that usually deposit in bone, polonium has a strong tendency to deposit in soft tissues[3]. The approximate fatal oral amount is probably of the order of 10-30 μg[8]. Owing to its high irradiation for several months after contamination, polonium deposition in the body tissues may present a serious risk of subacute and chronic damage. Consequently, an effective decorporation treatment is crucial.

Knowledge of the complexation chemistry of polonium in biological media is not very well defined. This is due to its adsorption onto surfaces and formation of colloids even at very low concentration. In non-complexing medium, polonium tends to form a Po(IV) hydroxide species [13]. The most stable oxidation state of polonium in solution is the quadrivalent one[14-16]. In blood medium, Po(IV) is mainly bound to citrates and bicarbonates; it is also associated with blood cells, especially erythrocytes and plasma proteins[6, 17-19].

Decorporation research studies and therapies are limited by this lack of information, which can be explained by the extremely small amounts of polonium available and the

inadequacy of the tested chelating agents. There is little information about the interaction of polonium with ligands and its affinity for certain amino acids and proteins in biological media[20, 21]. Most of the previously tested chelating agents were designed as antidotes for other toxic metals and not specifically for polonium decorporation. The classic chelating agents, such as diethylene triamine pentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA), are not suitable for polonium decorporation due to its binding properties and its high affinity for molecules having a sulfur group [22]. Clinical studies have been carried out to determine the effectiveness of chelation treatment of polonium as a function of time, dosage, and route of chelate administration[23]. Thiol-containing compounds (for example, dimercaprol (BAL)) and substances containing vicinal sulfhydryl and carbodithioate chelating agents have been used for polonium decorporation studies [23-27]. The results indicate that some of these chelating agents can completely remove polonium from the injected site merely by translocation into other tissues. This is often at the expense of increased retention in the kidneys and sometimes in the liver and brain. Consequently, repeating the systemic treatment is critical to bind polonium translocated into the blood.

In the present work, the interest of a hexadentatebis(thiosemicarbazide) "$N_2S_2O_2/N_4O_2$" to serve as a ligand for polonium complexation is studied. Thiosemicarbazide compounds are known to be numerous and possess important biological activities, such as antitumor, antiprotozoal, antibacterial and antiviral. All these activities have been shown to involve interaction with metal ions, which gives them their essential interest[28, 29]. Some thiosemicarbazide compounds, like ATSM that have a common diacetylbis(4-

methylthiosemicarbazone) scaffold, serve as carriers for radiotracers such as copper isotopes[30-36]. Others are proposed for the complexation of copper for treating Alzheimer's disease. Moreover, ^{64}Cu-ATSM is one of the two lead compounds for PET (Position emitting tomography). The interest in these ligands relies on their rapid complexation kinetics, the efficiency of their synthesis and their ability to form neutral complexes.

The work is divided in two parts. The first part aims at developing an easy way to synthesize the chelating agent. Its ability to complex Po(IV) is studied in the second part and is compared with two reference ligands: BAL and DTPA. The important of the two carboxylic acid arms to complete Po(IV) coordination sphere is assessed by comparing Po(IV)- $N_2S_2(N_4)O_2$ and Po(IV)- $N_2S_2(N_4)$ system.

III. Materials and methods

III.1. Chemicals

All experiments were conducted at room temperature (22±3 ˚C) and all commercially available reagents were purchased from either Sigma-Aldrich, Merck or Fischer and used without further purification. All solvents were distilled and stored under an atmosphere of argon. THF was distilled over sodium benzophenone. DMF was distilled from potassium hydroxide. Air-sensitive reagents were transferred by syringe or with a double-ended needle. Yields refer to chromatographically and spectroscopically (^{1}H, ^{13}C) homogeneous material.

Polonium-210 was produced and purified according to the protocol developed by A.

Younes et al.[37]. To prevent potential unsuitable complexation, the polonium solution was evaporated to dryness and recovered in the appropriate medium; the procedure was repeated two times.

III.2. Chelating agent synthesis

Ligand synthesis: Purification by column chromatography was performed with 70–230 mesh silica gel. TLC analyses were carried out on alumina sheets precoated with silica gel 60 F254 and visualized with UV light; R_f values are given for guidance. The ^{1}H NMR spectra were recorded on a Bruker Avance-300 Ultra Shield spectrometer operating at 300 MHz and the ^{13}C NMR spectra were recorded with a 100 MHz or a 75 MHz spectrometer. Chemical shifts are expressed in ppm downfield from TMS. Data are reported as follows: chemical shift [multiplicity (s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad), coupling constants (*J*) in Hertz, integration]. Number of attached proton(s) in the ^{13}C NMR spectra was elucidated using DEPT.

Diethyl 5-hydroxyisophthalate. 2. 5-Hydroxyisophthalic acid (16.47 mmol) was dissolved in ethanol. A catalytic amount of sulfuric acid was added and the reaction was stirred for 15 h under reflux. The solution was reduced under pressure. The residue was dissolved in 100 mL of ethyl acetate, washed twice with aqueous $NaHCO_3$ and once with brine. Then the organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. Colorless oil, yield: 95%. **^{1}H NMR($CDCl_3$, 300 MHz):** 1.41 (t, 6H, C*H*$_3$, J_3=

7.2 Hz), 4.40 (q, 4H, CH_2, J_3= 7.2 Hz),6.74 (bs, 1H, O*H*), 7.81 (d, 2H, *H*ar, J_4= 1.2 Hz), 7.81 (t, 1H, *H*ar, J_4= 1.2 Hz).**^{13}C NMR (75 MHz, CDCl$_3$):** 14.4 (2 *C*H_3), 61.7 (2 *C*H_2), 121.0 (2 *C*ar), 123.0 (*C*ar), 132.6 (2 *C*ar), 156.5 (*C*ar), 166.1 (2 *C*O_2Et). **MS (EI) m/z (%):** 238 (43, $M^{+\bullet}$), 210 (21), 193 (69), 165 (56), 137 (40), 92 (100). **MS (MALDI) m/z (%):** 261 ($M+Na^+$), 499 ($2M+Na^+$), 737($3M+Na^+$).

Diethyl 5-(2-((*tert*-butoxycarbonyl)amino)ethoxy)isophthalate. 3. K_2CO_3 (32.94 mmol, 2 eq) and **2** (16.47 mmol, 1 eq) were dissolved in 50 mL of DMF. The solution was stirred for 30 min followed by the addition of *tert*-butyl 2-bromoethylcarbamate (32.94 mmol, 2 eq) and the total mixture was stirred for 4 days at 40 °C. When the reaction was completed (checked by TLC), 50 mL of water and 50 mL of ethyl acetate were added. The mixture was extracted four times with 50 mL of ethyl acetate. The combined organic layers were collected, dried over Na_2SO_4, filtered and solvent was removed under reduced pressure. The crude product was then purified by column chromatography on silica using DCM and DCM:ethyl acetate (94:4) as eluent. White powder, yield: 90%. Melting point 59°C. **^{1}H NMR (CDCl$_3$, 300 MHz):** 1.40 (t, 6H, CH_3CH_2O, J_3 = 7.2 Hz), 1.45 (s, 9H, C(CH_3)$_3$), 3.56 (m, 2H, CH_2), 4.10 (t, 2H, CH_2, J_3 = 5.1 Hz), 4.39 (q, 4H, CH$_3$CH_2O, J_3 = 7.2 Hz), 4.95 (bs, 1H, N*H*Boc), 7.73 (d, 2H, *H*ar, J_4 = 1.2 Hz), 8.28 (t, 1H, *H*ar, J_4 = 1.2 Hz). **^{13}C NMR (CDCl$_3$, 75 MHz):** 14.4 (2 *C*H_3CH_2O), 28.6 (3 C(*C*H_3)$_3$), 40.5 (*C*), 61.5 (2 CH$_3$*C*H_2O), 68.2 (*C*e), 79.9 (*C*(CH$_3$)$_3$), 120.0 (2 *C*ar), 123.5 (*C*ar), 132.6 (2 *C*ar), 156.0 (*C*ar), 158.9 (N*C*O_2*t*Bu), 165.7 (2 *C*O_2Et). **MS (MALDI) m/z (%):** 404 ($M+Na^+$), 785 ($2M+Na^+$).

***Tert*-butyl (2-(3,5-bis(hydroxymethyl)phenoxy)ethyl)carbamate. 4.** $LiAlH_4$ (12.0 mmol, 3 eq) was suspended in 20 mL of dry THF at 0 °C and a solution of **3** (4.0 mmol, 1 eq) in 10 mL of THF was added dropwise. The solution was stirred for 2 h under reflux and 10 h at room temperature. The reaction was stopped by the dropwise addition of 3 mL of ethyl acetate, followed by 1.5 mL of ethanol and 15 mL of brine. The suspension was filtered and washed twice with 5 mL of ethanol. The filtrate was concentrated under reduced pressure and the residue was directly engaged in the further reaction. The residue was added to 20 mL of a solution of methanol and triethylamine (9/1, v/v) and the solution was stirred at room temperature for 24 h. Solvents were evaporated under reduced pressure. 2 mL of ethyl acetate, 10 mL of ethanol and 30 mL of brine solution were added then the solution was filtered on a micropore plate. The filtrate was concentrated under reduced volume to give a pale yellow oil. Pale yellow oil, yield: 72%. **^{1}H NMR (300 MHz, [D6]DMSO):** 1.37 (s, 9H, C(C*H*$_3$)$_3$), 3.26 (m, 2H, C*H*$_2$), 3.91 (t, 2H, C*H*$_2$, J_3 = 5.7 Hz), 4.42 (s, 4H, C*H*$_2$OH), 5.29 (bs, 2H, O*H*), 6.72 (s, 2H, *H*ar), 6.82 (s, 1H, *H*ar), 7.02 (bs, 1H, N*H*Boc). **^{13}C NMR (75 MHz, [D6]DMSO):** 28.0 (3 C(*C*H$_3$)$_3$), 39.6 (*C*), 62.7 (2 *C*H$_2$OH), 66.3 (*C*), 77.6 (*C*(CH$_3$)$_3$), 110.7 (2 *C*), 116.7 (*C*), 143.7 (2 *C*), 155.4 (*C*), 158.2 (N*C*O$_2$*t*Bu).

***Tert*-butyl 2-(3,5-diformylphenoxy)ethylcarbamate. 5.** (1.03 mmol, 1 eq) was added to a vigorously stirring mixture of PCC (3.09 mmol, 3 eq) and celite (1.3 g) in 10 mL of dichloromethane. The mixture was stirred for 3 h at room temperature, and monitored for

completion by TLC. The reaction mixture was filtered over cotton and then over a short pad of silica gel (5 cm) before being concentrated under reduced pressure to give a crude product, which was column chromatographed on silica gel, eluting with petroleum ether:diethyl ether (30:70) as eluent. Colorless solid, yield: 64%. Melting point 69°C. **^{1}H-NMR (300 MHz, $CDCl_3$):** 1.45 (s, 9H, C(CH_3)$_3$), 3.58 (m, 2H, CH_2), 4.15 (t, 2H, CH_2, J_3 =5.1 Hz), 4.95 (bs, 1H, N*H*Boc), 7.65 (d, 2H, *H*ar, J_5=1.34 Hz), 7.97 (s, 1H, *H*ar), 10.05 (s, 2H, C*H*O). **^{13}C-NMR (75 MHz, $CDCl_3$):** 28.5 (3 C(CH_3)$_3$), 40.3 (*C*), 68.4 (*C*), 80.0 (*C*(CH_3)$_3$), 120.0 (2*C*), 124.3 (*C*), 138.8 (2 *C*), 155.9 (*C*), 160.2 (N*C*O_2*t*Bu), 190.5 (2 *C*HO). **MS (MALDI) m/z (%):** 316 ($M+Na^+$), 348 $(M+Na+MeOH)^+$.

***Tert*-butyl 2-(3,5-bis(-(2-carbamothioylhydrazono)methyl)phenoxy)ethylcarbamate. 6.** Thiosemicarbazide (2.19 mmol, 2 eq) and **5** (1.10 mmol, 1 eq) were suspended in 4 mL of ethanol in a 10 mL-microwave reactor. The reactor was placed in the microwave oven and heated from room temperature to 120 °C (optic fiber) in 5 min by monomode microwave irradiation (power: 150 W, stirring: 50%, ventilation: 1/3), maintained at 120 °C for 20 min (power: 50 W, stirring: 50%, ventilation: 1/3) and left to cool down to room temperature for 5 min (power: 0 W, stirring: 50%, ventilation: 3/3). The mixture was filtered and washed with cold ethanol. Off white powder, yield: 92%. Melting point 237°C. **^{1}H NMR (300 MHz, [D6]DMSO):** 1.37 (s, 9H, C(CH_3)$_3$), 3.28 (m, 2H, CH_2), 4.05 (m, 2H, CH_2), 7.01 (bs, 1H, N*H*Boc), 7.42 (s, 2H, *H*ar), 7.68 (s, 1H, *H*ar), 8.01 (s, 2H, C*H*N), 8.15 (bs, 2H, NH_2), 8.25 (bs, 2H, NH_2), 11.51 (bs, 2H, N*H*). **^{13}C NMR (75 MHz, [D6]DMSO):** 28.2 (3 C(CH_3)$_3$), 39.8 (*C*), 66.8 (*C*), 77.8 (*C*(CH_3)$_3$), 113.9 (2*C*), 119.8 (*C*), 136.0 (2*C*), 141.6 (2*C*HN), 155.7 (*C*), 159.02 (N*C*O_2*t*Bu), 178.1 (2*C*S). **MS**

(MALDI) m/z (%): 462 ($M+Na^+$).

Diethyl 2,2'-(2,2'-((5-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-1,3-phenylene)-bis(methanylylidene))-bis(hydrazin-1-yl-2-ylidene))-bisthiazole-4-carboxylate. 7. *tert*-butyl 2-(3,5-bis((2-carbamothioylhydrazono)methyl)phenoxy)ethylcarbamate (0.543 mmol, 1 eq) was dissolved in 38 mL of DMF. After total dissolution, 1.055 mL of Et_3N was added and the solution was shaken for 5 min. Ethyl bromopyruvate (1.085 mmol, 2 eq) was added and the solution immediately turned dark orange. The reaction was stirred for 3 h at room temperature under an inert system and was concentrated under reduced pressure. The residue was suspended in 150 mL of dichloromethane and 150 ml of saturated NH_4Cl solution. The organic layer and the solid formed between the two phases were washed with 5 mL of methanol and then the solid compound was filtered on a micropore plate. The solid was a pale orange color, yield 40%. **^{1}H NMR (300 MHz, [D6]DMSO):** 1.28 (t, 6H, *CH*$_3$, *J*$_3$ = 7.1 Hz), 1.38 (s, 9H, C(C*H*$_3$)$_3$), 3.3 (m, 2H, *CH2*), 4.03 (m, 2H, *CH2*), 4.24 (q, 4H, *CH2*, *J*$_3$ = 7.1 Hz), 7.06 (bs, 1H, N*H*Boc), 7.22 (s, 2H, *H*ar), 7.54 (s, 1H, *H*ar), 7.79 (s, 2H, *CHN*), 7.98 (s, 2H, *Har*), 12.4 (bs, 2H, N*H*). **^{13}C NMR (75MHz, [D6]DMSO):** 14.1 (2C), 28.2 (3 C(*C*H$_3$)$_3$), 45.7 (*C*), 60.3 (*C*), 66.6 (2*C*), 77.8 (*C*(CH$_3$)$_3$), 112.8 (2*C*), 117 (2*C*), 119.1 (*C*), 136.1 (2*C*), 141.1(2*C*HN), 142,8(2*C*), 155.6 (N*C*O$_2$*t*Bu), 159 (*C*), 160.9 (2*C*O$_2$*Et*), 168 (2*C*). **MS (MALDI) m/z (%):** 654 ($M+Na^+$).

Diethyl 2,2'-(2,2'-((5-(2-((tert-butoxycarbonyl)amino)ethoxy)-1,3 phenylene)bis(methanylylidene))bis(1-(2-(tert-butoxy)-2-oxoethyl)hydrazin-1-yl-2

ylidene))bis(thiazole-4-carboxylate). N_2S_2/N_4. A mixture of compound **7** (112 mg, 0.17 mmol), ethanol (12 mL) and 2 M aqueous solution of NaOH (12 mL) was stirred for one night at 60 °C. The solution was evaporated to dryness, and the residue was dissolved in HCl solution (2 M, 12 mL). The mixture was shaken for 3 h at room temperature. The solvent was evaporated under reduced pressure and the residue was dissolved in cooled acidic water. The dark orange precipitated compound was filtered on a micropore plate. Brown solid, yield: 80%. **^{1}H NMR (300 MHz, [D6]DMSO):** 3.28 (m, 2H, *CH_2*), 4.29 (m, 2H, *CH_2*), 7.27 (s, 1H, Har) , 7.72 (s, 2H, *H*ar), 8.06 (s, 1H, *CHN*), 8.34 (bs, 4H, *CHN* and *NH_2*). **MS (MALDI) m/z (%):** 498 ($M+Na^+$).

2,2'-(2,2'-((5-(2-aminoethoxy)-1,3phenylene)bis(methanylylidene))bis(hydrazin-1-yl-2-ylidene))bis(thiazole-4-carboxylic acid). 8. Compound **7** (0.717 mmol, 1 eq) was dissolved in 38 mL of DMF. After total dissolution, it was added dropwise to a reaction beaker of potassium butoxide in 20 mL of DMF over an ice bath. The mixture was shaken for 30 min before addition of *tert*-butyl 2-bromoacetate (2.866 mmol, 3 eq). The solution turned brown and the reaction mixture was stirred for 4 h 30 min at room temperature. The solution was evaporated under reduced pressure. The residue was suspended in 100 mL of dichloromethane and 100 mL of NH_4Cl. The mixture was extracted four times with 50 mL of dichloromethane. The combined organic layers were pooled, dried over Na_2SO_4, filtered and solvent was removed under reduced pressure. The crude product was then purified by column chromatography on silica using DCM and DCM:ethyl acetate (20:80) as eluent to obtain a white solid. Yield: 12%. **^{1}H NMR (300 MHz, [D6]DMSO)**: 1.29 (t, 6H, *CH3*, J_3 = 7.1 Hz), 1.38 (s, 9H, C(*CH_3*)$_3$), 1.43 (s,

18H, C(C*H*$_3$)$_3$), 3.28 (m, 2H, *CH*$_2$), 4.05 (m, 2H, *CH*$_2$), 4.26 (q, 4H, *CH*$_2$, *J*$_3$ = 7.1 Hz), 5.02 (bs, 4H, *CH*$_2$), 7.07 (bs, 1H, N*H*Boc), 7.25 (s, 2H, *H*ar), 7.69 (s, 1H, *H*ar), 7.89 (s, 2H, C*HN*), 7.98 (s, 2H, *H*ar). **^{13}C NMR (75MHz, [D6]DMSO):** 14.1 (2C), 27.6 (6 C(*C*H$_3$)$_3$), 28.2 (3 C(*C*H$_3$)$_3$), 46.9 (*C*), 60.4 (3 C(*C*H$_3$)$_3$), 66.6 (2*C*), 77.76 (*C*), 82.1 (*C*), 113.5(2*C*), 116.9 (2*C*), 121.9 (*C*), 136.1 (2*C*), 137.9 (2*C*), 142,5(2*C*), 155.68(*C*O$_2$*t*Bu), 159.1(*C*), 160.72(2*C*O$_2$*Et*), 165.7(2*C*O$_2$*t*Bu), 169.2(2*C*). **MS (MALDI) m/z (%):** 882 (M+Na$^+$).

2,2'-(2,2'-((5-(2-aminoethoxy)-1,3-phenylene)bis(methanylylidene))bis(1-(carboxymethyl)hydrazin-1-yl-2-ylidene))bisthiazole-4-carboxylic acid. "$N_2S_2O_2/N_4S_2$". A mixture of 2,2'-(2,2'-((5-(2-aminoethoxy)-1,3phenylene)bis(methanylylidene))bis(hydrazin-1-yl-2-ylidene))bis(thiazole-4-carboxylic acid) **8** (62 mg, 0.14 mmol), ethanol (12 mL) and 2 M aqueous solution of NaOH (12 mL) was stirred for 12 h at 60 °C. The solution was evaporated to dryness, and the residue was dissolved in HCl solution (2 M, 12 mL). The mixture was shaken for 3 hrs at room temperature. Solvent was evaporated under reduced pressure and the residue was dissolved in cooled acidic water. The yellowish-white precipitated compound was filtered on a micropore plate. Yield: 80%. **^{1}H NMR (300 MHz, [D6]DMSO):** 3.2 (m, 2H, *CH*$_2$), 4.1 (m, 2H, *CH*$_2$), 4.84 (bs, 4H, *CH*$_2$), 7.1 (s, 2H, *H*ar), 7.62 (s, 1H, *H*ar), 7.74 (s, 2H, *CHN*), 7.82 (s, 2H, *H*ar). **MS (MALDI) m/z (%):** 614 (M+Na$^+$)

III.3. Polonium complexation methodology

A displacement method based on liquid/liquid separations (DMLL) was used in this study. A given species is characterized by a given distribution coefficient (D) and a change in D arising from a perturbation of the system indicates a change in speciation. A quantitative analysis of the data based on the law of mass action gives the number of protons (pH perturbation) or ligands ([ligand] perturbation) exchanged in the chemical process.

Tubes of super-polyethylene, from PerkinElmer and resistant to dichloromethane, were used for this DMLL methodology in order to limit polonium adsorption onto the container walls. In all cases, 5 mL of organic and aqueous phases were contacted. Before polonium addition, the system was pre-equilibrated, *i.e.* the aqueous solution (with or without the ligand) was put into contact with the organic phase in order to reach conditions where the aqueous composition did not change in the presence of the organic phase. After equilibrium of the biphasic system, an aliquot of polonium stock solution was added and the tubes were shaken for 2 h. This time proved to be sufficient to achieve distribution equilibrium between the phases for all the studied systems. After phase separation, aliquots of the aqueous and organic phases were withdrawn to derive the distribution coefficient D:

$$D = \frac{A_{org}}{A_{aq}} \qquad \textbf{Eq. 1}$$

A_{org} and A_{aq} define the polonium activities measured in the organic and aqueous phases, respectively. The pH of the solution was systematically controlled/measured at equilibrium. For all experiments, a concentration of 10^{-5} M dithizone was fixed in the organic phase (chloroform). Preliminary extraction tests showed a decomposition of the extracting agent when a concentration higher than 10^{-5} M was used[38]. 0.1 M $NaClO_4$ was used to fix the ionic strength in the aqueous solution.

Two series of experiments were performed. In the first, the pH effect on Po extraction by dithizone (HDz) was studied in the pH range 2-8. The objective of this experiment was to characterize the formation of the hydrolysis species of Po in order to consider them in the modeling of the complexation data. The second series aimed to characterize the complexation strength between Po and the complexing agents; all experiments were carried out at pH=7.4 using 10^{-3} M HEPES as a buffer.

Po-210 activity was determined by liquid scintillation using a Packard 2550 TR Liquid Scintillation analyzer. The samples were prepared by mixing an aliquot of 2 mL of the solution to be measured and 3 mL of ultimate gold AB scintillation cocktail. The measuring time was fixed at 1 h. Quenching arising from the organic solvent was taken into account according to the following relation:

$$A = A_m (5.10^{-12} \times TSIE^5 - 10^{-8} \times TSIE^4 + 10^{-5} \times TSIE^3 - 4.310^{-3} \times TSIE^2 + 0.9587 \times TSIE + 10.988)$$

Eq. 2

where A_m is the activity measured by liquid scintillation and TSIE is an independent parameter from polonium analysis defined by the apparatus to determine the quenching parameter.

III.4. Modeling of Po/ligand interaction

The simplest model was used to describe what happens in the biphasic system:

$$Po + \overline{HDz} \Leftrightarrow \overline{Po(HDz)} \qquad \textbf{Eq. 3}$$

$$Po + nL \Leftrightarrow PoL_n \qquad \textbf{Eq. 4}$$

$$PoL_n + \overline{HDz} \Leftrightarrow \overline{PoL_n(HDz)} \qquad \textbf{Eq. 5}$$

where Po represents the polonium species in the aqueous phase at pH=7.4 in the absence of the complexing agent, and L is the complexation agent ($N_2S_2O_2$, N_2S_2, DTPA or BAL). The extraction by HDz of the two species (Po and PoL) present in solution is described by a simple distribution coefficient between the aqueous and organic phases. The complexation constant associated with equilibrium (Equation 4), describing the complexation strength of the ligand, is a conditional constant which can be applied only in the experimental conditions of the study. n represents the number of ligands interacting with Po.

In conditions where ligand concentration is in excess with respect to Po concentration,

$$\frac{D}{D_0} = \frac{D_0 + KD_1 [L]_{tot}^n}{D_0 (1 + K[L]_{tot}^n)} \qquad \textbf{Eq. 6}$$

where **D** represents the experimental distribution coefficient in the presence of ligand in the aqueous phase, $\mathbf{D_0}$ the distribution coefficient of the Po species in the absence of ligands in the aqueous phase, $\mathbf{D_1}$ the distribution coefficient of the PoL complex, **K** the conditional complexation constant between ligand and dithizone, $[L]_{tot}$ the total concentration of the ligand and **n** the number of ligand molecules contributing to polonium complexation.

In the fitting procedure, D_0 is a fixed parameter. Several extraction tests were carried out over a long period with different batches of Po in order to determine the D_0 value in the experimental conditions of this work (*i.e.* BAL concentration of 10^{-5}, pH=7.4). It varied from 7 to 28 with a mean D value of 16.9 and an error of 14.6 (95% confidence interval).

This marked deviation is a problem generally found when working at ultra-trace concentrations ($[Po]_{tot}=4.10^{-13}$ M). The uncertainty becomes lower when the test of repeatability (at least 3 experiments run in parallel) is carried out for one given Po batch at a given moment, *i.e.* the error (2σ) decreases to around 2-10%. It is systematically higher than that which can be calculated from the uncertainties associated with measurements by liquid scintillation (typically below 2%). Finally, an experiment without ligand was systematically carried out in parallel with the complexation test to determine the D_0 value and the reproducibility error was systematically taken for the calculations presented in this paper.

IV. Results and Discussion

IV.1. Design of polonium decorporation agent

The design of the novel ligand was based on the properties and behavior of polonium in aqueous medium. It has been reported that polonium exists in aqueous solution in a stable quadrivalent state with a coordination number of six [39-41]. Its high ionic potential and polarizing action indicate the strength of hydroxyl complexes of polonium. Moreover, polonium has a high affinity for thiol groups, which was investigated by the strong interaction of polonium with metallothionein protein [21]. $N_2S_2O_2/N_4O_2$ is an ambidentate chelating agent with two thiazole rings that are bound by a single bond to the ligand core, thus enabling their rotation and providing the flexible coordination ability of the

compound. This ambidentate ligand can be either an $N_2S_2O_2$, N_3SO_2 or N_4O_2 ligand (Figure. 1). It presents *a priori* good characteristics for polonium complexation, *i.e.* a platform with four soft heteroatoms (N/S) and two additional pendant carboxylic groups to complete the octahedral coordination shell suitable for Po(IV) complexation. Furthermore, this ligand is water-soluble and could be bound to a biological vector *via* the NH_2 group, if necessary.

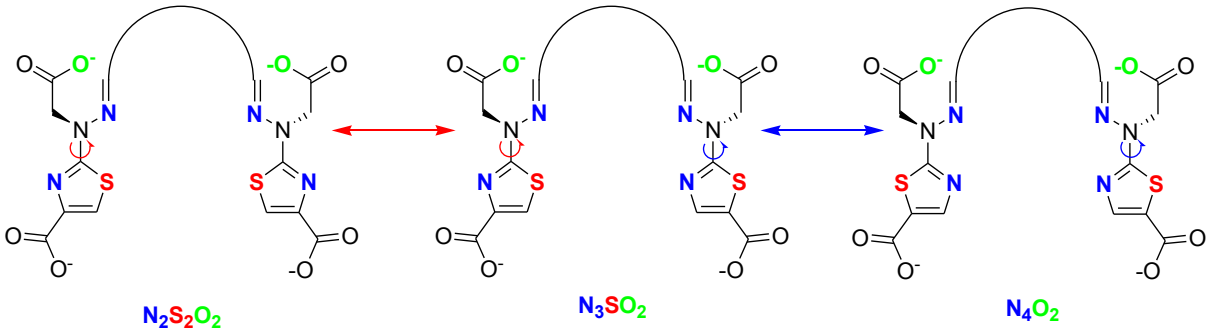


Figure. 1. **$N_2S_2O_2$ multidentate ligand structures.**

IV.2. **Polonium decorporation agent synthesis:**

The strategy used to synthesize *2,2'-(2,2'-((5-(2-aminoethoxy)-1,3-phenylene)bis(methanylylidene))bis(1-(carboxymethyl)hydrazin-1-yl-2-ylidene))bis(thiazole-4-carboxylic acid)* **$N_2S_2O_2$** was based on the biscoupling reaction between the di-carbonyl compound and two thiosemicarbazines which gives the bis(thiosemicarbazone) compound. This latter product was treated with commercially available ethyl bromopyruvate to give the corresponding *S*-alkyl salt which undergoes a spontaneous cyclization, followed by deamination of the cycloadduct to produce the

tetradentatebis(thiazole) "N_2S_2/N_4". The insertion of the two carboxyl arms for the "N_2S_2/N_4" was performed by a reaction with α-bromoketone to generate "$N_2S_2O_2/N_4O_2$".

The synthesis begins by esterification of commercially available 5-hydroxyisophthalic acid **1** to generate diethyl-5-hydroxyisophthalate **2** in 95% yield (Figure 2). Reaction of diethyl-5-hydroxyisophthalate with *N*-Boc-2-bromoethylamine offers the grafting of a protected ethyl amine chain and thus the production of diethyl-5-(2-((*tert*-butoxycarbonyl)amino)ethoxy)isophthalate **3** (90%). The selected protected alkylamine compound is *N*-Boc-2-bromoethylamine due to its commercial availability. However, the ethyl chain can act as a spacer with the additional amine that could be grafted onto a biological binding vector. Consequently, a longer chain was not chosen to avoid aliasing the terminal amine which participates in polonium complexation. The principle of this reaction is based on the deprotonation of the phenol to generate the active phenolate which reacts in a nucleophilic attack on the halogenated derivative under elevated temperature. The following step is the synthesis of *tert*-butyl (2-(3,5-diformylphenoxy)ethyl)carbamate **5**. The first experiment showed that the reduction of diester **3** into dialdehyde **5** could be achieved by a two-step reaction. According to Star et al.[42], the ester can be converted into alcohol **4** (*tert*-butyl 2-(3,5-bis(hydroxymethyl)phenoxy)ethylcarbamate) by reaction with lithium aluminum hydride. Then, the generated dialcohol **4** undergoes an oxidation step using pyridiniumchlorochromate[43] to offer dialdehyde **5** with a good yield. However, our results show that the use of lithium aluminum hydride would cleave the Boc-protected

amines with a methylation of the terminal amine. The complementary reaction to solve this would be the insertion of the "Boc" functional group after the reaction with lithium aluminum hydride. However, the reaction of diester with dibutyl lithium aluminum hydride at a very low temperature (<-80 °C) provided the dialdehyde with a 40% yield. The synthesis of dialdehyde using the one-step reaction with dibutyl lithium aluminum hydride gives a lower yield (40%) than the three-step reaction using lithium aluminum hydride (46%).

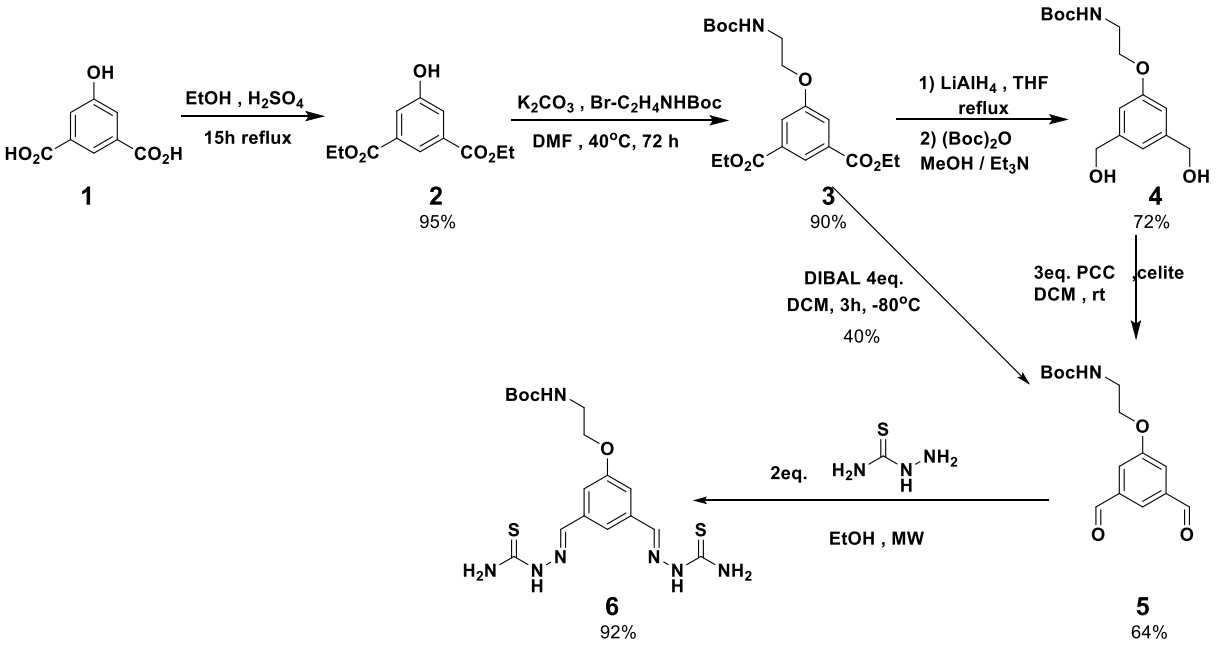


Figure. 2 The synthesis of bis(thiosemicarbazone) 6.

The protected *tert*-butyl (2-(3,5-diformylphenoxy)ethyl)carbamate **5** was engaged in a double condensation reaction with the commercially available thiosemicarbazide (92%). Previous studies showed that this reaction works faster (15 min) with a clean product by using microwave irradiation rather than a classic heating system. The bis(thiosemicarbazone) **6** produced was converted into bis(thiazadiene) **7** by a double

condensation reaction with commercially available ethyl bromopyruvate in DMF (Figure 3). The use of triethylamine in this reaction is necessary to trap the released hydrogen bromide which could react with the protected amine. The product can undergo deprotection of the implanted ethyl acetate group in the thiazole ring and the *tert*-butyl carbamates thus forming the tetradentate "N_2S_2/N_4" chelating agent, or it can be treated in a further step to implant the two additional carboxylic acid arms to offer a hexadentate "$N_2S_2O_2/N_4O_2$" chelating agent.

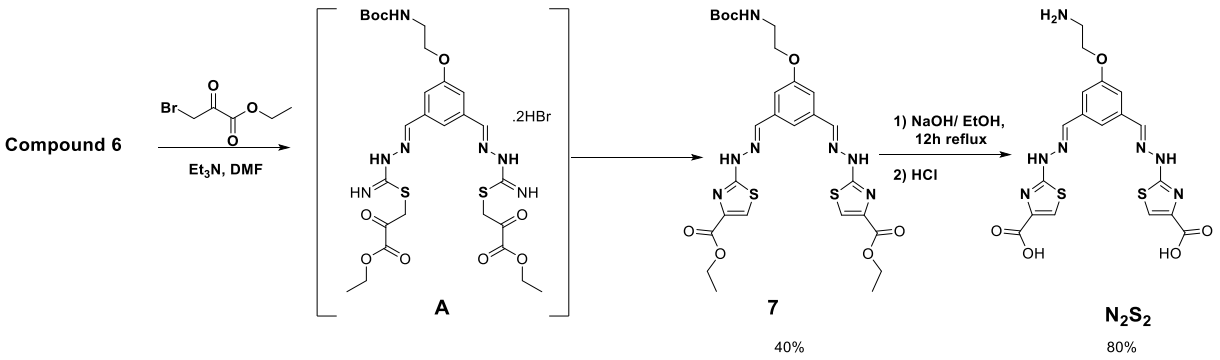


Figure. 3 Synthesis of "N_2S_2 /N_4" 7

The reaction of the compound with *tert*-butyl-2-bromoacetate in DMF provided the corresponding tetraester **8** in moderate yield (Figure 4). The final step was the deprotection of esters and amino functions, which includes reflux with a sodium hydroxide-ethanol mixture to cleave the implanted ethyl acetate groups in the thiazole ring and the *tert*-butyl groups on the implanted carboxylic arm followed by reaction with hydrochloric acid to deprotect the amine, thus producing the hexadentate "$N_2S_2O_2/N_4O_2$" chelating agent.

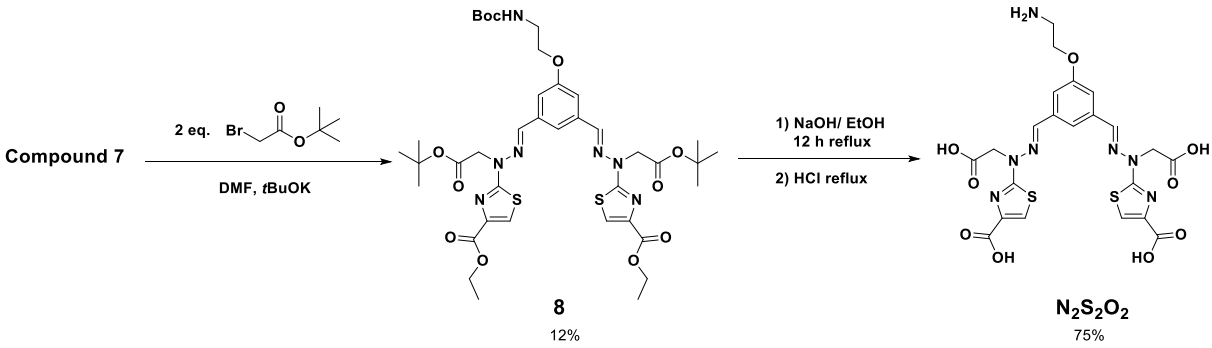


Figure. 4 Synthesis of "$N_2S_2O_2$/N_4O_2"

IV.3. Polonium – ligand complexation studies

a) Po(IV) reactivity with water

Po(IV) is highly reactive in aqueous solution. Mixed complexes of the form $Po(OH)_n(X)_p^{4-n-p}$ (X=Cl, Br, NO_3…) are generally produced when Po is mixed in aqueous media with inorganic ligands[38, 44, 45]. However, it has been shown that the hydrolysis species of the $Po(OH)_n^{4-n}$ form (with n=1-4) are formed in non-complexing perchlorate media[46]. The formation of such species has been evaluated theoretically[39-41]; the results suggest the probable occurrence of the clusters $Po(H_2O)_5(OH)_2^{2+}$, $Po(H_2O)_4(OH)_2^{2+}$ and $Po(H_2O)_3(OH)_3^{+}$. According to the experimental work of Ampelogova, $Po(OH)^{3+}$, $Po(OH)_2^{2+}$, $Po(OH)_3^{+}$ and $Po(OH)_4$ species exist in the pH ranges of 0.5-2, 2-2.8, 2.8-3.5 and 3.5-12, respectively. $Po(OH)_4$ is therefore the expected species at pH=7.4 [13, 46]. This strong reactivity with water may be compared to a certain extent with that of thorium (Th). Indeed, both Th and Po have the same charge and ionic radius, and these two parameters are directly related to cation reactivity. Using the hydrolysis constants of Th(IV) leads to a species distribution where $Th(OH)^{3+}$, $Th(OH)_2^{2+}$, $Th(OH)_3^{+}$ and $Th(OH)_4$ exist in the pH ranges 3-4, 4-5, 5-6 and 6-12, respectively. The distribution

diagram appears different from that proposed by Ampelogova, Po(IV) being *a priori* more reactive than Th(IV). This makes the actual data difficult to consider for the present work.

The change in speciation was studied in the pH range 1-8 in the biphasic liquid-liquid system in order to gain further insights into the hydrolysis reactions of Po(IV). The results are presented in Figure 5. Between pH 1 and pH 3, D values are almost constant (deviation below 10%). The expected change in speciation is therefore not experimentally observed. Above pH ~3.5, a change in speciation is seen. It is accompanied by a decrease in D from ~275 to ~18. According to the work of Ampelogova, this observation would correspond to the following reaction:

$$Po(OH)_3^+ + H_2O \Leftrightarrow Po(OH)_4 + H^+ \qquad \textbf{Eq. 7}$$

Using the same approach as the one presented in the modeling section, the following relation is obtained:

$$D = \frac{D_2 K[H^+]^{-1} + D_1}{(1 + K[H^+]^{-1})} \qquad \textbf{Eq. 8}$$

where D_2 and D_1 represent the distribution coefficients of $Po(OH)_4$ and $Po(OH)_3^+$ species, respectively, and K the constants associated with equilibrium 6. The exchange of one proton is in agreement with the slope of the curve and a thermodynamic constant of $8.14*10^{-5}$ can be calculated (solid line in Figure. 5).

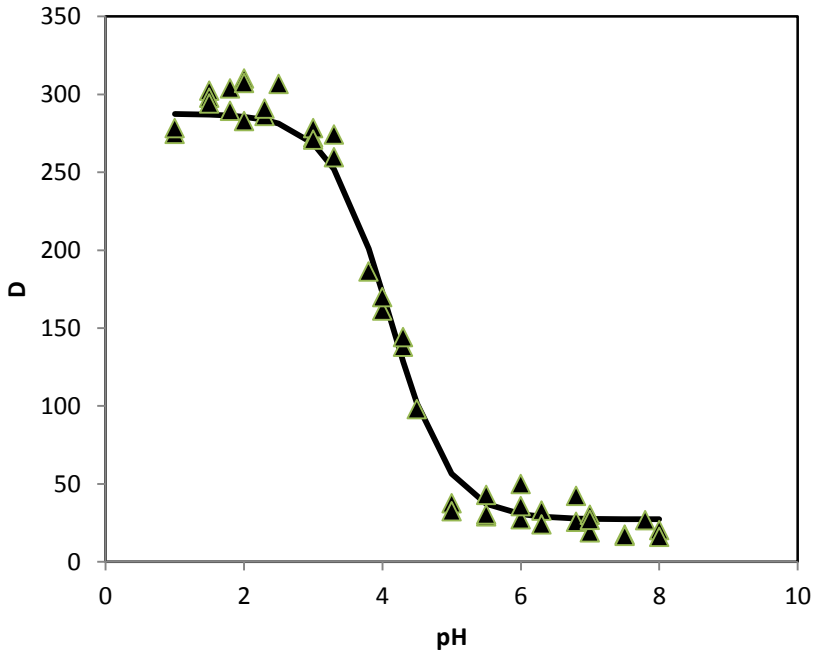


Figure. 5 Po(IV) complexation with H_2O and competition with Po extraction by HDz in dichloromethane; [HDZ] = 10^{-4} M, [Po] = 2.86 10^{-13} M. The lines correspond to the calculation made according to equilibrium 6 with the parameters given in the text.

The constant is however not sufficient to describe Po speciation in aqueous solution. One can only say that, there is *a priori* one given species at pH=7.4 which would correspond to $Po(OH)_4$. It therefore appears impossible to derive the thermodynamic complexation constants from the complexation tests carried out at pH 7.4. A simple model was thus used to derive conditional constants which can be compared from one ligand to another, the experimental data being measured in the same conditions (pH=7.4, 0.1 M $NaClO_4$).

b) Po(IV) reactivity with chelating agent

The experimental results are presented in Figure 6. Except for $N_2S_2O_2$, all the curves can

be well reproduced considering n=1 with the parameters given in Table 1. The interaction strength appears very strong for the three ligands: trace concentrations of the ligand ($<10^{-6}$ M) are sufficient to initiate Po complexation (and to lead to a decrease in D) and therefore to compete with the formation of the hydrolysis species $Po(OH)_4$. Among the ligands studied in the literature, DTPA appears much less efficient than BAL for Po complexation. There is a difference in magnitude of about 4 between the two conditional constants. This is totally in agreement with the results of the decorporation tests described in the literature which indicate that DTPA is not the best ligand for Po and that BAL is more appropriate [13].In spite of the presence of the soft heteroatoms, the ligand N_2S_2 does not appear well adapted for Po complexation; its complexation constant is even slightly lower than that of DTPA.

For $N_2S_2O_2$, there is no change in D value in the range of ligand concentration studied. However, as D was lower than the one measured without complexing agent, we can conclude that there was a change in speciation occurring in solution. One possibility to explain this result is that the complexation between $N_2S_2O_2$ is strong enough for 100% of Po to be complexed in the ligand concentration range studied. A conditional complexation constant above 10^{13} M^{-1} can thus be estimated. The difference in reactivity with respect to N_2S_2 shows that the introduction of the two binding arms in the platform strongly stabilizes Po complexation. Furthermore, the complexation strength appears even greater than for BAL. As expected, these results suggest that the pre-organization of the molecule is ideal for Po complexation.

The results also give an indication of the hydrophobic character of the complex which

varies in the order DTPA<N_2S_2~BAL<$N_2S_2O_2$. This makes the molecule particularly interesting as a decorporation agent which can a priori target both aqueous and cellular media.

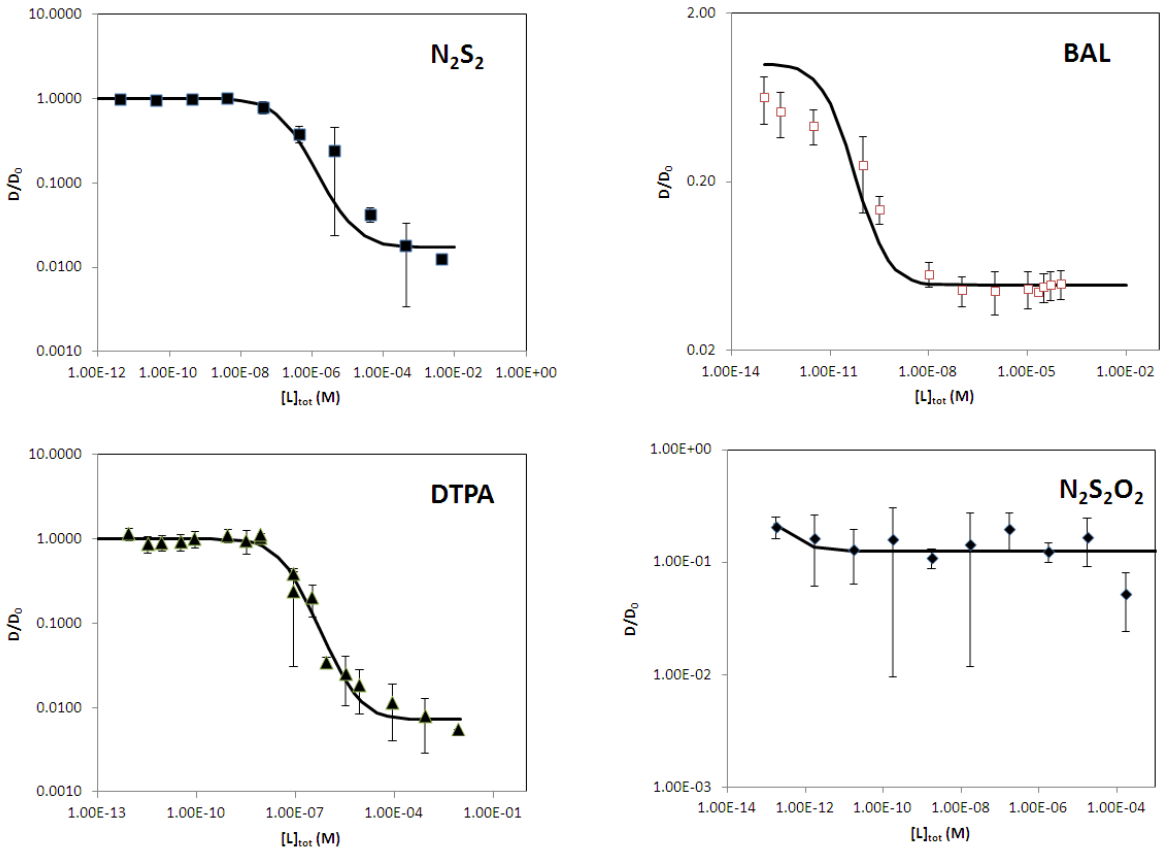


Figure. 6 Po(IV) complexation with the different ligands and competition with Po extraction by HDZ in dichloromethane; [HDz] = 10^{-5} M, pH=7.4, [Po] = 4 10^{-13} M. The lines correspond to the calculation made according to equilibrium 3 with the parameters given in Table 1.

Table 2. Parameters used to describe Po/ligand interaction

	N_2S_2	BAL	DTPA	$N_2S_2O_2$
K (M)	$5.39\ 10^6$	$8.00\ 10^{10}$	$2.17\ 10^7$	$\geq 5.00\ 10^{13}$
D_0	17.36	7.24	20.87	12.00
D_1	0.30	0.35	0.15	1.50

V. Conclusion

An *a priori* ideal ligand is proposed for Po complexation considering the available data that polonium is a soft metal with a coordination sphere of six. A synthetic method is described to provide a multidentate complexing agent. As expected, the complexation with Po is very strong at pH=7.4. The importance of the two binding arms is shown indirectly: there is a difference in the conditional stability constant higher than 10^7 orders of magnitude between N_2S_2/Po and $N_2S_2O_2$/Po complexes. Thermodynamic constants could not be determined because of the lack of information about Po(IV) reactivity with water. However, a thermodynamic constant of $8.14*10^{-5}$ for $Po(OH)_3^+/Po(OH)_4$ reaction is given. Finally, it is shown that the interaction is stronger than for BAL, the reference molecule to date. This makes $N_2S_2O_2$ an interesting potential chelating agent for decorporation. The next step is to assess the ability of the ligand to complex Po selectively in *in-vitro* conditions.

Acknowledgement

The authors thank the French National Center of Scientific Research (CNRS) and the Pays de Loire Region for the doctoral grant allocated to Ali Younes. We also thank Sebastien Gouin, Mamadou Barry for their help.

Bibliographie de l'Article IV

1. Ramsay, W., Polonium as medicine. *New York times* **1913**.
2. Harrison, J. R. L. a. D. L. a. A. P. a. B., Scott, Polonium-210 as a poison. *Journal of Radiological Protection* **2007,** 27, (1), 17.
3. Stannard, J. *Radioactivity and Health a History*; Pacific Northwest Laboratory, US Department of Energy. Springfield, VA: Office of Scientific and Technical Information: 1988.
4. Ladinskaya, L. A.; Parfenov, Y. D.; Popov, D. K.; Fedorova, A. V., *Arch. Environ. Health* **1973,** 27, 254.
5. Scott, B. R., Health risks evaluations for ingestion exposure of humans to polonium-210. *Dose-Response* **2007,** 5, 94-122.
6. Cohen, N.; Fellman, A. L.; Hickman, D. P.; Ralston, L. G.; Ayres, L. S., *Primate polonium metabolic models and their use in estimation of systemic radiation doses from bioassay data*. Mound Laboratory: 1989.
7. Argonne National Laboratory Environmental Science Division., Polonium, Human Health Fact Sheet. 2005.
8. Jefferson, R. D.; Goans, R. E.; Blain, P. G.; Thomas, S. H. L., Diagnosis and treatment of polonium poisoning. *Clinical Toxicology* **2009,** 47, (5), 379-392.
9. ICRP Publication 30, P., Ann. ICRP 2, (3/4), Pergamon Press, Oxford, England., *International Commission on Radiological Protection, Limits on Intakes of Radionuclides for Workers*. Publication 30, Pt. 1, Ann. ICRP 2, (3/4), Pergamon Press, Oxford, England.: 1979.
10. Hunt, G. J.; Rumney, H. S., *J. Radiol. Prot.* **2007,** 27, 405.
11. ICRP, *International Commission on Radiological Protection, Human Alimentary Tract Model for Radiological Protection, ICRP*. Publication 100, Ann. ICRP, 36, (1−2), Pergamon Press, Oxford, England.: 2006.
12. Stannard, J. N., *Radiat. Res.* **1964,** 5, 49.
13. Ansoborlo, E.; Berard, P.; Den Auwer, C.; Leggett, R.; Menetrier, F.; Younes, A.; Montavon, G.; Moisy, P., Review of Chemical and Radiotoxicological Properties of Polonium for Internal Contamination Purposes. *Chemical Research in Toxicology* 25, (8), 1551.
14. Ampelogova, N., *Radiokhim* **1974,** 16, (1), 52.
15. Koch, H.; Schmidt, H., *Z. Naturforsch* **1963,** 18b, 936.
16. Starik, I.; Ampelogova, N.; Kuznetsov, B., *Radiokhim* **1964,** 6, 507.
17. Smith, F. A.; Morrow, P. E.; Gibb, F. R., *Am. Ind. Hyg. Assoc. J.* **1961,** 22, 201.
18. Silberstein, H. E.; Valentine, W. N.; Minto, W. L.; Lawrence, J. S.; Fink, R. M.; Fink, R. M., *Biological studies with polonium, radium, and plutonium*. 1950; p 122.
19. Thomas, R. G., *Radiat. Res.* **1964,** 5, 29.
20. Lanzola, E. E.; Allegrini, M. E.; Taylor, D. M., *Radiat. Res.* **1973,** 56, 370.
21. Aposhian, H. V.; Bruce, D. C., *Radiat. Res.* **1991,** 126, 379.
22. Pearson, R. G., Hard and Soft Acids and Bases. *Journal of the American Chemical Society* **1963,** 85, (22), 3533.
23. Volf, V.; Rencova, J.; Jones, M. M.; Singh, P. K., *Int. J. Radiat. Biol.* **1995,** 68,

395.
24. Aposhian, H. V.; Dart, R. C.; Aposhian, M. M.; Dawson, B. V., *Res. Commun. Chem. Pathol. Pharmacol.* **1987,** 58, 157.
25. Bogdan, G. M.; Aposhian, H. V., *Biol. Met.* **1990,** 3, 232.
26. Rencova, J.; Volf, V.; Jones, M. M.; Singh, P. K., *Radiat. Prot. Dosim.* **1994,** 53, 311.
27. Rencova, J.; Svoboda, V.; Holusa, R.; Volf, V.; Jones, M. M.; Singh, P. K., *Int. J. Radiat. Biol.* **1997,** 72, 341.
28. Finch, R.; Liu, M.; Cory, A.; Cory, J.; Sartorelli, A., Triapine (3- aminopyridine-2-carboxaldehyde thiosemicarbazone; 3-AP): an inhibitor of ribonucleotide reductase with antineoplastic activity. *Adv. Enzyme Regul* **1999,** 39, 3-12.
29. Antholine, W.; Knight, J.; Whelan, H.; Petering, D., Studies of the reaction of 2-formylpyridine thiosemicarbazone and its iron and copper complexes with biological systems. *Mol Pharmacol* **1977,** 13, 89-98.
30. Laforest, R.; Dehdashti, F.; Lewis, J.; Schwarz, S., Dosimetry of 60/61/62/64Cu-ATSM: a hypoxia imaging agent for PET. *Eur. J. Nucl. Med. Mol. Imaging* **2005,** 32, 764-70.
31. Dietz, D.; Dehdashti, F.; Grigsby, P., Tumor hypoxia detected by positron emission tomography with 60Cu-ATSM as a predictor of response and survival in patients undergoing Neoadjuvant chemoradiotherapy for rectal carcinoma: a pilot study. *Dis. Colon. Rectum* **2008,** 51, 1641-1648.
32. Lewis, J.; Laforest, R.; Dehdashti, F.; Grigsby, P.; Welch, M.; Siegel, B., An imaging comparison of 64Cu-ATSM and 60Cu-ATSM in cancer of the uterine cervix. *J. Nucl. Med.* **2008,** 49, 1177-1182.
33. Jalilian, A.; Rostampour, N.; Rowshanfarzad, P.; Shafaii, K.; Kamali-Dehghan, M.; Akhlaghi, M., Preclinical studies of [61Cu]ATSM as a PET radiopharmaceutical for fibrosarcoma imaging. *Acta Pharm* **2009,** 59, 45-55.
34. Grigsby, P.; Malyapa, R.; Higashikubo, R., Comparison of molecular markers of hypoxia and imaging with (60)Cu-ATSM in cancer of the uterine cervix. *Mol. Imaging Biol.* **2007,** 9, 278-283.
35. Dehdashti, F.; Grigsby, P.; Lewis, J.; Laforest, R.; Siegel, B.; Welch, M., Assessing tumor hypoxia in cervical cancer by PET with ^{60}Cu-labeled diacetyl-bis(N4-methylthiosemicarbazone). *J. Nucl. Med.* **2008,** 49, 201-205.
36. Matarrese, M.; Bedeschi, P.; Scardaoni, R., Automated production of copper radioisotopes and preparation of high specific activity [(64)Cu]Cu-ATSM for PET studies. *Appl Radiat Isot* **2010,** 68, 5-13.
37. Younes, A.; Montavon, G.; Alliot, C.; Mokili, M.; Haddad, F.; Deniaud, D.; Champion, J., A route for polonium 210 production from alpha irradiated bismuth-209 target. *Journal of Radiochemical Acta* (to be published).
38. Hataye, I.; Suganuma, H.; Sakata, M.; Nagame, Y., Solvent extraction study on the hydrolysis of tracer concentration of polonium(IV) in perchlorate solutions. *Journal of Inorganic and Nuclear Chemistry* **1981,** 43, (9), 2101-2104.
39. Ayala, R.; Martinez, J. M.; Pappalardo, R. R.; Munoz-Paez, A.; Marcos, E. S., Po(IV) Hydration: A Quantum Chemical Study. *The Journal of Physical Chemistry B*

2008, 112, (17), 5416-5422.
40. Ayala, R.; Martinez, J. M.; Pappalardo, R. R.; Munoz-Paez, A.; Sanchez Marcos, E., *J. Phys. Chem. B* **2009,** 113, 487.
41. Ayala, R.; Spezia, R.; Vuilleumier, R.; MartiÌnez, J. M.; Pappalardo, R. R.; SaÌnchez Marcos, E., An Ab Initio Molecular Dynamics Study on the Hydrolysis of the Po(IV) Aquaion in Water. *The Journal of Physical Chemistry B* **2010,** 114, (40), 12866-12874.
42. Star, A.; Liu, Y.; Grant, K.; Ridvan, L.; Stoddart, J. F.; Steuerman, D. W.; Diehl, M. R.; Boukai, A., *J. R. Macromolecules* **2003,** 36, 553-560.
43. Bennani, Y. L.; Marron, K. S.; Mais, D. E.; Flatten, K.; Nadzan, A. M.; Boehm, M. F., *The Journal of Organic Chemistry* **1998,** 63, 543-550.
44. Suganuma, H.; Hataye, I., Solvent extraction study on the hydrolysis of tracer concentration of Po(IV) in chloride solutions. *Journal of Inorganic and Nuclear Chemistry* **1981,** 43, (10), 2511-2515.
45. Hataye, I.; Suganuma, H.; Sakata, M., Solvent extraction study on the hydrolysis of tracer concentration of polonium(IV) in nitrate solutions. *Journal of Inorganic and Nuclear Chemistry* **1981,** 43, (10), 2575-2577.
46. Ampelogova, N., *Radiokhim* **1975,** 17, 69.

Conclusion générale et perspectives

Bien que l'existence de polonium a été envisagée par Mendeleïev en 1889 et sa découverte a été officialisée en juillet 1898 par les travaux de Pierre et Marie Curie, les propriétés chimiques de cet élément et ses complexes en milieu aqueux et biologiques sont encore peu connues. Cela est dû à sa disponibilité en quantité extrêmement faible dans l'environnement et l'absence d'isotopes stables. Cependant, le polonium-210 est considéré comme l'un des principaux composants de la radioactivité naturelle affectant les humains et son utilisation comme poison mortel a été démontrée dans le cas de Mr. A. Litvinenko en 2006. Par conséquent, la recherche fondamentale pour comprendre ses propriétés physico-chimiques est importante afin d'élaborer des protocoles et des stratégies pour minimiser et comprendre ses effets.

Le travail présenté s'inscrit dans ce contexte et porte sur l'exploration de la chimie de polonium, et plus particulièrement les propriétés de complexation du Po(IV) en solution aqueuse. Pour cela, plusieurs tâches ont été réalisées :

- une étude bibliographique approfondie sur la spéciation du Po(IV) et sa réactivité en solution aqueuse;
- le développement d'une nouvelle méthode de production et d'extraction du polonium-210 ;
- l'étude du comportement du polonium en milieu HCl ;
- la synthèse de nouveaux agents chélatants potentiellement intéressant pour la décorporation du polonium ;
- la mesure des constantes de complexation entre Po(IV) et les agents chélatants dans des conditions applicables à des études biologiques (pH =7,4).

Les résultats principaux qui ont été obtenus dans ce travail sont résumés ci-dessous.

Tout d'abord, une méthode de production du polonium-210 a été mise en place à partir de l'irradiation d'une cible de bismuth stable par des particules alpha à 37 MeV. En effet, ce bombardement mène à la production d'astate-210 (8.1 heures) qui se désintègre en ^{210}Po. Un procédé par voie humide a été développé pour la séparation des traces de ^{210}Po à partir d'une solution contenant des quantités macroscopiques de bismuth. Il repose sur un procédé d'extraction liquide-liquide avec du phosphate de tributyle (TBP) dans du para-xylene. Le procédé de purification retenue consiste en cinq étapes: (i) la dissolution de la cible de Bi dans l'acide nitrique 10 M suivie d'un reconditionnement dans HCl 7 M,(ii) l'extraction de ^{210}Po par le TBP dans du para-xylène (iii) le lavage de la phase organique avec HCl 7 M et (iv) l'extraction en retour de ^{210}Po dans de l'acide nitrique 9 M et (v) remise en solution dans un milieu approprié. Le processus optimisé conduit à une solution de ^{210}Po purifiée avec un taux de récupération global de 93%.

Une partie de ce travail a été approfondie pour comprendre la réactivité du Po en milieu HCl vis-à-vis du TBP, mais également de la trioctylamine (TOA). Pour une concentration acide chlorhydrique variant de 6 à 12 M, les résultats montrent l'existence d'une espèce de charge -2 et confirment la présence de $PoCl_6^{2-}$. Entre 0,2 et 6M HCl, une autre espèce neutre est présente, correspondant à $PoCl_4$. Pour des concentrations inférieurs à 0,2 M, un complexe hydroxo-chloro de polonium $Po(OH)_2Cl_2$ apparaît dans la phase aqueuse. Les données expérimentales ont été modélisées pour déterminer les espèces formées ainsi que les constantes de formations associées :

$PoCl_4 + 2Cl^- \rightleftharpoons PoCl_6^{2-}$ Log β=4,6± 0,2

$Po(OH)_2Cl_2 + 4Cl^- + 2H^+ \rightleftharpoons PoCl_6^{2-} + 2H_2O$ Log β=2,7± 0,8

$PoCl_6^{2-} + 2\overline{TBP} + 2H_2O \rightleftharpoons \overline{Po(OH)_2Cl_2(TBP)_2} + 2H^+ + 4Cl^-$ Log K=2,18± 0,06

$PoCl_6^{2-} + 4\overline{TBP} \rightleftharpoons \overline{PoCl_4(TBP)_4} + 2Cl^-$ Log K=3,35± 0,08

$PoCl_6^{2-} + 2\overline{TBP} + H^+ \rightleftharpoons \overline{HPoCl_5(TBP)_2} + Cl^-$ Log K=2,14± 0,07

Ce travail permet également de proposer un paramètre SIT pour $PoCl_6^{2-}$: ε=-0,01± 0,008

Enfin, sur la base des données disponibles dans la littérature sur les propriétés de coordination du Po(IV), un ligand à priori idéal de type «$N_2S_2O_2/N_4O_2$ » a été conçu et synthétisé : le polonium est connu pour être un métal « mou » selon le concept acide-base de Pearson avec une sphère de coordination probable de six.

La synthèse de la N_2S_2/N_4 est réalisée en 7 étapes avec un rendement global de 16% à partir de l'acide commercial 5-hydroxyisophthalique. Les étapes clés sont d'une part la réduction sélective des fonctions esters en aldéhydes correspondants et la cyclisation de la *bis*-thiosemicarbazone en *bis*-thiazole par action de bromopyruvate d'éthyle. Afin de disposer du ligand hydrosoluble $N_2S_2O_2$ nous sommes repartis du ligand protégé N_4/N_2S_2 et nous avons ajouté deux fonctions méthylesters par alkylation des amines secondaires. La réaction à lieu en milieu basique, en présence de *tertio*-butanolate de potassium, avec un rendement modeste de 12% mais non optimisé. La raison est due (i) à la difficulté de purification du produit final qui est pollué par du produit de départ et du composé mono

alkylé, les trois molécules étant très difficiles à séparer par chromatographie sur silice, (ii) à la présence des différents groupements protecteurs susceptibles de réagir avec la base lors de la réaction de couplage. Quoiqu'il en soit, après déprotections des esters et de l'amine, la molécule hexadentate hydrosoluble cible $N_2S_2O_2$ est obtenue avec un rendement de 75%.

La capacité du nouveau ligand de se complexer avec le polonium à pH = 7,4 a été vérifiée et comparée avec d'autres agents chélatants. Le travail réalisé nous a également permis de proposer une constante de $8{,}14*10^{-5}$ pour l'équilibre suivant :

$$Po(OH)_3^+ + H_2O \Leftrightarrow Po(OH)_4 + H^+$$

Le résultat de l'étude comparative indique l'ordre de stabilité suivant : N_2S_2/N_4 < DTPA << BAL < $N_2S_2O_2/N_4O_2$. Ces résultats indiquent que l'interaction de la molécule $N_2S_2O_2$ est la plus forte ce qui fait du ligand $N_2S_2O_2/N_4O_2$ un agent chélateur potentiellement intéressant comme agent décorporant.

Perspectives

Le travail lié au développement de la production du Po-210 a permis de générer des données qui n'ont pas été exploitées, comme c'est le cas pour celle obtenues en milieu nitrique. Il serait donc intéressant de poursuivre la démarche faite en milieu HCl afin d'identifier le diagramme de spéciation des espèces du Po(IV) en milieu nitrique.

Le ligand synthétisé $N_2S_2O_2/N_4O_2$ montre une forte complexation avec le polonium à pH=7,4 en solution aqueuse. Le travail indique également qu'il est aujourd'hui difficile d'évaluer les constantes thermodynamiques de formation des complexes, et ceci en l'absence de données quantitatives qui permettent de quantifier la formation des espèces hydrolysées de Po(IV). Cette étude de base apparaît donc nécessaire pour pouvoir avancer sur ces sujets. D'un point de vue plus appliqué, il apparait intéressant de poursuivre ce travail et de valider l'intérêt du ligand comme agent décorporant en travaillant dans des conditions *in vivo*. Il s'agit de l'étape à réaliser au préalable avant de passer sur d'éventuels tests sur des souris.

Exploration de la chimie du polonium

Le polonium (Po) est le premier élément radioactif découvert par Marie Curie en 1898. Po est un élément naturel hautement radioactif. Cependant, la chimie du polonium demeure jusqu'à présent peu connue, en raison de son instabilité (absence d'un isotope stable) et de sa faible disponibilité : peu d'informations sont exploitées de la chimie de Po (IV) en solution aqueuse. Dans ce projet, la chimie du polonium en solution aqueuse est étudiée afin de mieux comprendre sa chimie de coordination. Comprendre sa réactivité en solution permettrait d'établir un protocole de minimisation de ces effets mortels. Une nouvelle méthode a été développée pour produire le Po-210, en bombardant une cible de Bi-209 par un faisceau de rayonnement alpha de 37 MeV. La purification constituait en elle-même un défi considérable, pour justement obtenir des quantités d'ultra-trace de Po-210 ($2{,}6\ 10^{-13}$ mol). Pour la purification, la méthode d'extraction liquide-liquide est mise en place avec l'utilisation du TBP dans le p-xylène dans 7M d'HCl et 9M HNO_3. Des études expérimentales et théoriques ont montré la présence de deux espèces principales, le $PoCl_6^{2-}$, comme espèce majoritaire, et le $PoCl_4$. Ensuite, un agent de chélation macrocyclique a été synthétisé. Ce macrocyclique est caractérisé par la formation de quatre interactions autour de Po avec deux atomes mous (N/S) et deux groupes carboxyliques conduisant à une coordination adaptée de type octaédrique Po (IV). L'affinité de ce ligant, Po (IV), est aussi étudiée à pH = 7,4 montrant son potentiel comme agent de complexation, qui sera l'objet d'études plus approfondies à l'avenir.

Mots clés : *Polonium-210, Irradiation alpha, Extraction liquide-liquide, Production du radio-isotopes, Synthèse des ligands macrocycliques, Complexation, Spéciation, Chimie de coordination*

Exploration of the chemistry of polonium

Although polonium (Po) was discovered in 1898 by Pierre and Marie Curie, little is said about the complexation chemistry of Po(IV) in aqueous solution. Its chemistry in solution is not well understood due to the absence of a stable isotopes and it availability in extremely small quantities. Po is a highly toxic element and is considered as an important component of natural radiation affecting humans. The project intends to perform polonium's chemistry studies in aqueous solution in order to contribute to better understand its coordination chemistry. A new methodology for the production and purification of Po-210 was first developed by bombarding Bi-209 foil with a 37 MeV alpha beam. The main challenge was to purify a tracer amount of Po-210 ($2.6\ 10^{-13}$ mole) from macroscopic amount of Bi ($2.8\ 10^{-2}$ mole). Po-210 was purified by employing liquid–liquid extraction method using TBP in p-xylene from 7M HCl and back extracted into 9M HNO_3. Experimental and theoretical studies to specify the nature of Po species were performed showing the presence of $PoCl_6^{2-}$ as a major species and the extracted Po-TBP species were $Po(OH)_2Cl_2.(TBP)_2$, $PoCl_4.(TBP)_4$ and $HPoCl_5.(TBP)_2$. Finally, a novel water soluble "$N_2S_2O_2/N_4O_2$" macrocyclic complexing agent was designed and synthesized. It presents a priori the good characteristics for Po complexation, i.e. a platform presenting four soft heteroatoms (N/S) and additional two pendant carboxylic groups to complete the octahedral coordination shell suitable for Po(IV) complexation. Its strong affinity for Po(IV) was verified at pH=7,4 and its potential use as a decorporating agent worth to be studied in a future work.

Keywords: *Polonium-210, Alpha-irradiation, Liquid-liquid extraction, Production of radio-isotopes, Synthesis of macrocyclic ligands, Complexation, Speciation, Chemistry of coordination.*

Discipline: Chimie