

Development of [^{201}Tl](III) oxinate complex for *in vitro* cell labeling

A.R. Jalilian^{1*}, A. Hakimi², J. Garousi¹, F. Bolourinovin¹, M. Kamali-Dehghan¹, G. Aslani¹

¹ Nuclear Medicine Research Group (NMRG), Agricultural, Medical and Industrial Research School, Karaj, Iran,

² Islamic Azad University, Science and Research Unit, Tehran, Iran

Background: The incorporation of thallium-201 into 8-hydroxyquinoline was targeted for cell labeling due to interesting physical properties and wide availability of this nuclide as a single photon emission computed tomography (SPECT) radionuclide. **Materials and Methods:** Thallium-201 ($T_{1/2}=3.04$ d) in Tl^+ form was converted to Tl^{3+} cation in presence of $\text{O}_3/6\text{M HCl}$ and di-isopropyl ether, controlled by radio-thin layer chromatography (RTLC) /gel electrophoresis methods. The final evaporated activity reacted with ethanolic 8-hydroxy-quinoline (oxine) solution in normal saline to yield [^{201}Tl](III)oxinate at room temperature after 0.5 h, followed by solid phase extraction/purification using C_{18} Sep-Pak column and partition coefficient determination for water/lipid solubility. *In vitro* red blood cell (RBC) labeling was also performed. **Results:** A radiochemical yield of more than 95% was obtained. Radiochemical purity of 92% was obtained using RTLC (>90% using HPLC) with specific activity of about 820 GBq/mmol. The tracer was stable in the final product and in presence of human serum at 37°C up to 6h. The partition coefficient of $\text{logP}=5.5$ was obtained. The labeled compound was used in RBC labeling. The cell uptake ratio was 0.47 after 240 min. **Conclusion:** [^{201}Tl](III) oxinate used in this study is a widely available agent for use in RBC labeling studies in biology, medicine and various other research areas. *Iran. J. Radiat. Res.*, 2008; 6 (3): 145-150

Keywords: ^{201}Tl (III), complex, oxinate, cell labeling, HPLC, SPECT.

INTRODUCTION

Tl -201 labeled compounds are rare in the literature. In one study, a peptide-DTPA- ^{201}Tl conjugate was prepared for possible scintigraphy, but further biological evaluation of the conjugate was not reported⁽¹⁾. In another study, a DTPA-human polyclonal antibody conjugate was used in ^{201}Tl (III) labeling for infection diagnosis with no biological details⁽²⁾.

There are various cell labeling modalities using ^{111}In -oxinate, $^{99\text{m}}\text{Tc}$ -HMPAO etc. all have been reported in the literature, however due to In -111 price and Tc -99m limitations in reduction chemistry, alternatives such as [^{201}Tl](III) oxinate can be considered.

Recently we have reported the evaluation of some biologically active ^{201}Tl (III) compounds such as bleomycin for tumor imaging purposes with significant tumor uptake⁽³⁾ and radio labeled antibodies⁽⁴⁾. In continuation of our efforts to develop new ^{201}Tl (III)labeled compounds the focus has been on [^{201}Tl](III) oxinate for cell labeling, based on interesting complexation properties of metal oxinates and the background on the production and use of cell labeling tracers^(5, 6).

MATERIALS AND METHODS

Production of ^{201}Tl was performed in Agricultural, Medical and Industrial Research School, Karaj, Iran, using a 30 MeV cyclotron (Cyclone-30, IBA) based on the routine production of thallous chloride for national use. $^{203}\text{Tl}_2\text{O}_3$ with an enrichment of more than 95% was supplied by the Kurchatov Institute (Russia). Radio-chromatography was performed by counting of polymer-backed silica gel paper thin layer sheets using a thin layer chromatography

*Corresponding author:

Dr. Amir Reza Jalilian, Nuclear Medicine Research Group, Agricultural, Medical and Industrial Research School, P.O.Box:31485-498, Karaj, Iran.

Fax: +98 261 4411106

E-mail: ajalilian@nrcam.org

scanner, Bioscan AR2000, Paris, France. Analytical HPLC to determine the specific activity was performed by a Shimadzu LC-10AT, armed with two detector systems, flow scintillation analyzer (Packard-150 TR) and UV-visible (Shimadzu) using Whatman Partisphere C-18 column 250×4.6 mm, Whatman Co. NJ, USA. Eluent, $\text{H}_2\text{O}:\text{CH}_3\text{CN}$ (1:1), $\text{FR}=1$ ml/min. All calculations and RTLC counting were based on 167 keV peak. All values were expressed as mean $\pm$ standard deviation (Mean $\pm$ SD) and the data were compared using student *t*-test (<http://graphpad.com/quickcalcs/ttest1.cfm>).

Statistical significance was defined as $p < 0.05$. Radionuclidic purity was checked with the same detector. O_3 was produced by medicinal oxygen (Air Liquide, Belgium) using a conventional O_3 generator at a flow rate of 1 liter per minute. The oxidation to $^{201}\text{Tl}^{3+}$ was checked by cellulose acetate paper electrophoresis (Gellman) in 0.05N EDTA at 200 V for 10 min. The purification of final compound was performed by C_{18} Sep-Pak short columns (Waters).

Conversion of thallium-201 to $[\text{}^{201}\text{Tl}](\text{III})\text{oxinate}$ form

Thallous chloride solution (0.5 ml, 140 MBq) was treated with a mixture of *di*-isopropyl ether, hydrogen peroxide, (20%, 0.5 ml) and 6M HCl (1 ml) while ozone gas bubbled through the solution for 5 min. The organic layer was separated and the conversion of Tl^+ to Tl^{3+} cation was checked either by RTLC using two solvent systems (system A: 10% ammonium acetate:MeOH: 1:1, system B: acetone) or gel electrophoresis using Gellman papers. The organic layer was evaporated to dryness under a flow of N_2 at 30°C for labeling use.

Preparation of $[\text{}^{201}\text{Tl}](\text{III})\text{oxinate}$

$[\text{}^{201}\text{Tl}]\text{TlCl}_3$ (250-270 MBq) dissolved in the organic medium obtained above (0.5 ml) was immediately transferred to a 2 ml-borosilicate vial. The mixture was evaporated by slight warming (40°C) under

a nitrogen flow. A portion of 8-hydroxy-quinoline (0.2 mg, 560 nmol), dissolved in ethanol (0.2 ml), was added to the thallium residue and vortexed for 10 seconds and then normal saline (0.8 ml) was added to the mixture. The mixture was agitated for 1 min and incubated at 25°C for 30 min. In case of free Tl^+ and/or Tl^{3+} presence ($>10\%$ totally, checked by RTLC) in the final sample, the solution passed through a C_{18} Sep-Pak column pre-conditioned by ethanol (2 ml) and double distilled water (DDH_2O) (2 ml) subsequently, and purged with a flow of N_2 gas. The loaded column was eluted by DDH_2O (2 ml) followed by ethanol portions (5×1 ml) of and the fractions counted in a dose calibrator. The two fractions containing the maximum activity were mixed and concentrated to the volume of 0.1 ml under a flow of N_2 at $40\text{--}45^\circ\text{C}$. The vial mixture was diluted by the addition of 0.9% saline (2 ml) to bring the solution to $<5\%$ ethanol. pH was adjusted to 5.5-7 and the final solution was then passed through a $0.22\ \mu\text{m}$ filter.

Radiochemical purity of $[\text{}^{201}\text{Tl}](\text{III})\text{oxinate}$

Radio thin layer chromatography: Polymer-backed silica gel layer chromatography sheets were used as stationary phase and 10% aqueous ammonium acetate:MeOH (1:1, v/v) as the mobile phase.

High performance liquid chromatography: HPLC was performed on the final preparation using a mixture of water:acetonitrile 1:1 (v/v) as the eluent (flow rate: 1 ml/min, pressure: 120-140 kgF/cm²) for 20 min.

Stability of $[\text{}^{201}\text{Tl}](\text{III})\text{oxinate}$ complex in aqueous solution

Stability tests were based on previous studies performed for radiolabeled metal complexes ⁽⁸⁾. A sample of $[\text{}^{201}\text{Tl}](\text{III})\text{oxinate}$ (185 MBq) in aqueous solution was kept at room temperature for 6 hours while being checked by RTLC. Micro-samples (5 ml) taken from the shaken mixture were transferred onto the TLC papers and the ratio of free radiothallium to $[\text{}^{201}\text{Tl}](\text{III})\text{oxinate}$ was

checked (eluent: 10% ammonium acetate: MeOH (1:1)).

Stability studies in human serum

500 ml of freshly prepared human serum was added to 37 MBq of [²⁰¹Tl](III) oxinate solution (100 ml). The resulting mixture was incubated at 37°C for 6 h, and 1.5 ml aliquots were analyzed by RTLC up to 6 h of incubation to determine the complex stability.

Determination of partition coefficient

Partition coefficient (log *P*) of [²⁰¹Tl](III) oxinate was calculated followed by the determination of *P* (*P*= the ratio of specific activities of the organic and aqueous phases). A mixture of 1 ml of 1-octanol and 1 ml of isotonic acetate-buffered saline (pH=7) containing approximately 3.7 MBq of the radiolabeled thallium complex at 37°C was vortexed for 1 min and left for 5 min. Following centrifugation at >1200*g* for 5 min, the octanol and aqueous phases were sampled and counted in an automatic well-type counter. A 500 ml sample of the octanol phase from this experiment was shaken again two to three times with fresh buffer samples to ensure that traces of hydrophilic ²⁰¹Tl impurities had not altered the calculated *P* values. The reported log *P* values are the average of the second and third extractions from three to four independent measurements.

Cell labeling

In a typical run, healthy male volunteer blood samples (3 ml) were collected in sterile anticoagulant added polymer tubes. The samples were centrifuged at 3000 rpm for 5 minutes then the serum was discarded. The cell pellets were reconstituted in 1 ml of PBS followed by the addition of the [²⁰¹Tl] (III)oxinate final solution (3 MBq). The samples were kept at 4, 25 and 37°C for up to 3 hours. One -ml samples were taken at various time intervals (30, 60, 120 and 240 min) and

centrifuged at 3000 rpm for 5 min. The cell pellets were carefully washed with PBS and the washing solution was discarded. The activity of the cell pellet and the supernatants were counted in a dose calibrator and the ratio of cell/supernatants was determined (n=5).

RESULTS AND DISCUSSION

Since the complexes of oxinate with many trivalent metals are very stable, the idea was to prepare [²⁰¹Tl] (III) oxinate in high chemical purity. It has been recently shown that Tl³⁺ is a highly toxic cation but after complexation the toxicity decreases drastically ⁽⁹⁾. The toxicity has possibly been due to impaired glutathione metabolism ⁽¹⁰⁾ or major alterations in the rheology of the bi-layer, which could be partially responsible for the neurotoxic effects of this metal ⁽¹¹⁾. All of the above occurred when ²⁰¹Tl was present at micro molar amounts, while carrier-free ²⁰¹Tl was far below toxic levels (less than nano molar).

[²⁰¹Tl]Tl³⁺ production

In order to prepare thallic cation from thallos, according to our experiences, a mixture of H₂O₂ and 6M HCl in presence of O₃ and diisopropyl ether was used. The residue was re-dissolved in normal saline and checked for the results. The more polar Tl³⁺ cation remained at the origin (R_f=0.0) while less polar Tl⁺ showed a high R_f of about 0.9 using 10% ammonium acetate:methanol (1:1) as the eluent. In paper electrophoresis, 5 ml of the final sample was transferred to the paper and the migration of the Tl-EDTA⁻ to the cathode was compared with that of Tl⁺ migrating to the anode. The method showed a conversion of >99% for Tl⁺→Tl³⁺.

Radiolabeling of oxinate

Application of Tl (III) oxinate complex has already been reported for the determination of Tl(III) cation in the solutions using

colorimetric methods ⁽¹²⁾, although no direct application of this complex has been reported so far. The formation of the complex was tested at room temperature and though the radiolabeling was not completed within an hour, the reaction was not heated since it was shown to be a failure due to the reduction of Tl(I) to Tl(III) using higher temperatures ⁽¹³⁾. It was also shown that Tl(III)oxinate complex afforded a rather stable complex compared to Tl(I), suggesting that any failure in Tl(III) oxidation state retention would result in the dissociation of Tl(I) cation ⁽¹⁴⁾.

Because of the engagement of N and O atoms in the coordination of thallic cation, it was assumed that the polarity of radiolabeled complex was greatly different from

thallium cations and the final complex was possibly a lipophilic species. In Si-TLC studies, the free thallium(III) fraction corresponded to smaller R_f ($R_f=0.1$), while the oxinate complex migrates to the higher R_f ($R_f=0.9$) (figure 1). This property was employed to remove remaining portions of Tl cations using C18 Sep-Pak in order to increase the radiochemical purity (figure 2). Figure 1 demonstrates the radiochromatograms of the radiolabeling mixture before and after SPE purification.

In HPLC studies reverse phase column was used in water/acetonitrile mixture as an eluent. The fast eluting components (1.14 and 2.85 minutes) were shown to be free $^{201}\text{Tl}^+$ and $^{201}\text{Tl}^{3+}$. Both compounds are ionic, so they are eluted faster than any complex

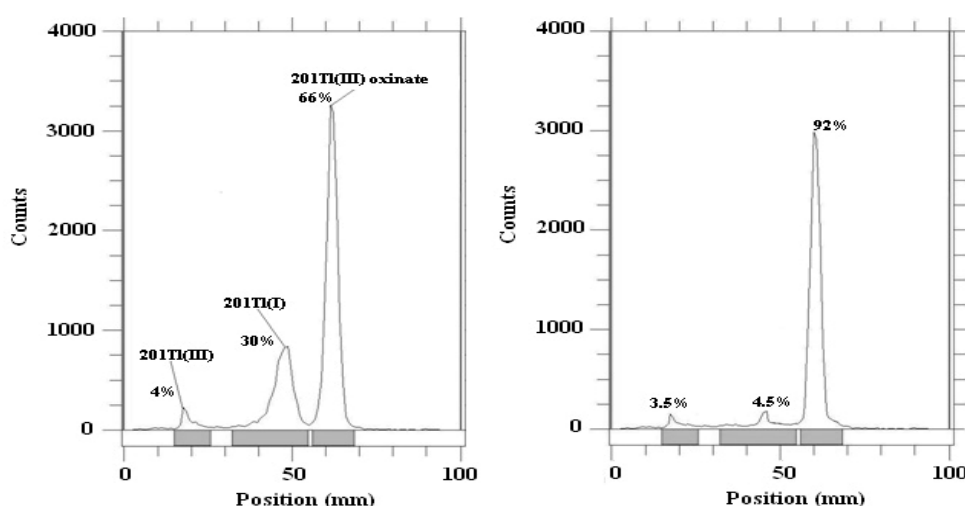


Figure 1. RTLC of final $[^{201}\text{Tl}](\text{III})\text{oxinate}$ solution before (left) and after (right) SPE purification ($n=5$).

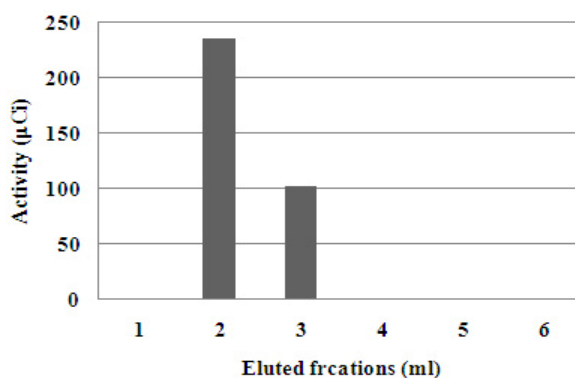


Figure 2. Radioactivity of eluted radiolabeled compound fractions from C₁₈ Sep-Pak.

species. The radiolabeled compound was finally washed out at 6.4 minutes however the radiopeak was not sharp in our methods (figure 3).

The final radiolabeled complex diluted with normal saline, and then was passed through a 0.22 micron (Cativex) filter for sterilization to avoid possible thermal instability of the complex at autoclave temperatures. The chemical stability of [²⁰¹Tl] (III) oxinate was high enough to perform further studies. RTLC of the final product showed no change in stability and the pattern for [²⁰¹Tl] (III)oxinate in aqueous solutions at pH=5.5-7 was not changed during 24 h at room temperature.

Serum stability studies

[²⁰¹Tl](III) oxinate was incubated in freshly prepared human serum for 24 h at 37°C. The aliquots of the resulting mixtures were analyzed to determine the kinetic stability of the radiolabeled conjugate. No decomposition of ²⁰¹Tl from the complex was observed during the course of the studies, and the radiochemical purity of the complex remained >99% for 6 h under physiological conditions.

Partition coefficient of the [²⁰¹Tl] (III)oxinate

As expected, the lipophilicity of the compound was rather high. The measured octanol/water partition coefficients, P, for

the ²⁰¹Tl-complex were found to be 5.6 at pH= 7 ⁽¹⁵⁾.

Cell labeling

Since it has been shown that the [²⁰¹Tl] (III)oxinate complex has lipophilic properties, it could easily pass through bi-layer phospholipids of cell membrane. In many cases the complexes stay intact in the intracellular space, resulting in easy leakage back to the outer cell space. Reduction of Tl (III) in the intracellular space was possible due to pH changes as well as reaction with cytoplasmic thiol-containing molecules. This can be an advantage of the [²⁰¹Tl](III)oxinate as a cell labeling agent, since the tracer is trapped in the cytoplasmic space. In case of reduction, Tl(I)oxinate complex has shown to be unstable ⁽¹²⁾ and may result in the release of Tl⁺ in the cytoplasmic space. Even in thallous form the leakage to extracellular space has not been reported. [²⁰¹Tl](III)oxinate can be used in blood cell labeling if added directly to human blood cell fractions. Various temperatures could be chosen for the cell labeling. According to our experiences, reproducible and linear data were obtained at 37°C. This might be explained by the fact that at this temperature natural existing enzymes or mechanism in cells are working properly so that the trapping of the tracer in the cell occurs more efficiently. The linearity

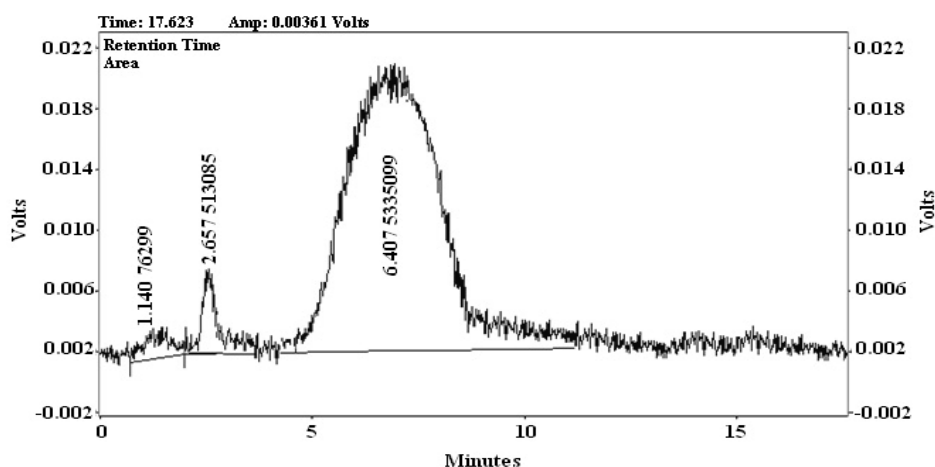


Figure 3. HPLC of final [²⁰¹Tl] (III)oxinate solution in 1mMDTPA as mobile phase using reverse stationary phase.

of the plot also may indicate that the penetration of the tracer into the cells was performed by simple diffusion mechanism, with no active transport mechanism involved (figure 4).

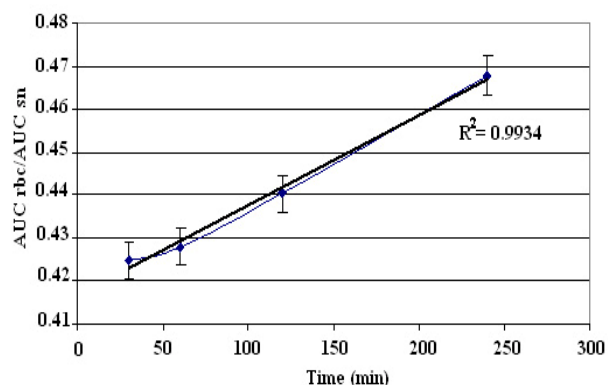


Figure 4. The rate of red blood cell labeling using $[^{201}\text{Tl}](\text{III})$ oxinate at 37°C ; $n=5$, ($R^2 = 0.993$).

CONCLUSION

The method used in this research for the production and chemical separation of $[^{201}\text{Tl}](\text{III})$ oxinate was quite simple and cost effective. Neither of the previous studies on cell labeling agents has used Tl-201, with constant stability of the Tl-oxinate complex. Total labeling and formation of $[^{201}\text{Tl}](\text{III})$ oxinate took about 40 minutes. A significantly high specific activity ($\gg 820 \text{ GBq/mmole}$) was obtained *via* insertion of the $[^{201}\text{Tl}]$ thallium (III) cation. The radiolabeled complex was stable in aqueous solutions as well as in human serum at 37°C for at least 6h, and no significant amount of other radioactive species was detected by RTLC.

In contrast to other labeled oxinate complexes, $[^{201}\text{Tl}](\text{III})$ oxinate is a radiotracer with a suitable half life and potential use in the detection of cell migration in various studies such as inflammation imaging and stem cell fate studies. The high chemical stability of this radiopharmaceutical also makes it a very suitable candidate for cisternographic applications. Preliminary cell labeling showed that the best results are obtained at 37°C after 1-3 h.

ACKNOWLEDGEMENT

The authors wish to thank Dr A. Sattari and other colleagues in the radioisotope production team at NRCAM.

REFERENCES

- Chavatte K, Mertens J, Van Den Winkel P (2000) Method for effective ^{201}Tl (III) labeling of diethylenetriamine pentaacetic acid (DTPA)-functionalized peptides: radiosynthesis of $^{201}\text{Tl}(\text{III})$ DTPA-neurotensin (8-13). *J Labelled Compd and Radiopharm*, **43**: 1227-1234.
- Vranjes SD, Rtic TM, Fani M, Varvarigou AD, Archimandritis SD, Potamianos S, Bouziotis P, Xanthopoulos S, Comor JJ (2001) Development of Tl-HlgG for inflammation detection. *J Labelled Compd and Radiopharm*, **44**: 700-701.
- Jalilian AR, Akhlaghi M, Aboudzadeh R, Shirazi B, Raisali G, Salouti M, Babaii M (2006) $[^{201}\text{Tl}](\text{III})$ -Bleomycin for tumor imaging. *Radiochimica Acta*, **94**: 453-459.
- Jalilian AR, Khorrami A, Tavakoli MB, Kamali-Dehghan M, Yari Kamrani Y, Development of $[^{201}\text{Tl}](\text{III})$ -DTPA-Human Polyclonal Antibody Complex for Inflammation Detection (2007) *Radiochimica Acta*, **95**: 669-675.
- Jalilian AR, Rowshanfarzad P, A Rahiminejad, A.A. Rajamand (2006) Development of $[^{66}\text{Ga}]\text{Oxine}$ Complex: a Possible PET Tracer. *Nukleonika*, **51**: 155-159.
- Jalilian AR, Yari-Kamrani Y, Kamali-Dehghan M, Rajabifar S (2008) Preparation and Evaluation of $[^{201}\text{Tl}](\text{III})$ -DTPA Complex for Cell Labeling, *J Radioanal. Nucl Chem*, **275**: 109-114.
- Hnatowich DJ, Layne WW, Childs RL, Lanteigne D, Davis MA, Griffin TW, Doherty PW (1983) Radioactive labeling of antibody: a simple and efficient method. *Science* **220**: 613-619.
- Lewis MR, Wang M, Axworthy DB, Theodore LJ, Mallet RW, Fritzberg AR, Welch MJ, Anderson CJ (2003) *J Nucl Med*, **44**: 1284-1292.
- Lan CH and Lin TS (2005) Acute toxicity of trivalent thallium compounds to *Daphnia magna*. *Ecotoxicol Environ Saf*, **61**: 432-435.
- Hanzel CE, Villaverde MS, Verstraeten SV (2005) Glutathione metabolism is impaired *in-vitro* by thallium (III) hydroxide. *Toxicology*, **207**: 501-510.
- Villaverde MS and Verstraeten SV (2003) Effects of thallium (I) and thallium(III) on liposome membrane physical properties. *Arch Biochem Biophys*, **417**: 235-243.
- Moeler T and Cohen AJ (1950) *Anal Chem*, **22**: 686.
- Goto H, Kakita Y, Ichinose I (1967) Studies on the extraction and determination of metal salts with methyl isobutyl ketone. XII spectrophotometric determination of thallium as oxine salt by solvent extraction. *J Chem Soc Japan*, **88**: 640.
- Cornelis R, Caruso J, Heumann KG, Crews H (2003) Published by John Wiley and Sons *Handbook of elemental speciation: Techniques and Methodology* pp. 417.
- Coetzee PP, Fischer JL, Hu M (2003) Simultaneous separation and determination of Tl(I) and Tl (III) by IC-ICP-OES and IC-ICP-MS. *Water S.A.* **29**: 17-22.