HURDLES FOR A BROADER USE OF ^{211}At AND FOR THE SYNTHESIS OF ^{211}At-LABELLED RADIOPHARMACEUTICALS AT HIGH ACTIVITIES FOR CLINICAL USE

O. R. Pozzi*, M. R.Zalutsky**
** National Atomic Energy Commission, Buenos Aires, Argentina*
*** Duke University Medical Center, Durham North Carolina, United States of America*

One of the key impediments to the use of ^{211}At is the very well known deleterious effect of high radiation fields caused by its alpha particles on the synthesis of ^{211}At-labelled radiopharmaceuticals. This is problematic because radiolysis-mediated effects can produce diminishing efficiency of electrophilic astatination reactions due to increasing deposition of radiation dose with increasing activities and with the passage of the time.

Astatine-211 has chemical properties that permit complex labelling strategies and a longer half-life than 2^{13}Bi that makes it more suitable when the targeting molecule does not gain immediate access to the tumour cells [1]. The first clinical evaluation was published in 2001 [2] in patients with brain tumour. Although this study circumvents many of the challenges to entering clinical studies with ^{211}At and many obstacles had to be surmounted before clinical studies could be initiated, several problems were encountered in maintaining efficient labelling with escalating radiation dose of α-particle even with fresh ^{211}At elution [3].

Astatine-211 also has an additional hurdle to overcome before to its clinical application in labelled radiopharmaceuticals related with its production and distribution. Among the potential group of promising α-emitter it is the only one produced by cyclotrons, but due to the scarcity of cyclotrons equipped with 25−30 MeV α-particle beams, it will of necessity be utilized in distant locations from the site of production. It presents a major chemical challenge because the diminishing efficiency of electrophilic astatination reactions with the passage of the time is well known, a problem likely related to the radiolysis produced by the high LET (linear energy transfer) meaning that large amounts of energy are deposited in a highly localized manner [2].

This problem has been most comprehensively investigated to understand and evaluate the role of the radiolysis effects of astatine alpha particles in the synthesis of therapeutic amounts of astatine labelled radiopharmaceuticals. The results of the study were published on three different papers [3−5].

Based on the contention that from all the molecules present during the astatination reaction the solvent molecules are at the highest molar concentration, therefore having the highest probability of interaction with the alpha particles, the study was made using 3 different solvent as model solvent, chloroform for chlorinated solvents, benzene for aromatic solvent and methanol for alcohols. Considering the high levels of radiolysis of the solvent to be expected due to the high levels of radiation dose deposited for alpha particles a logical assumption indicates that the high amounts of radicals, charged species and other very reactive reduced or oxidizing molecules produced by the radiolytic decomposition of the solvent might deeply affected the astatination reactions.
The results showed and confirmed that the type and extension of the problems that makes very difficult to do astatination chemistry at high activities were complete related with the type of solvent, the problems found in benzene are complete different from the ones found in chloroform, and different as well for the ones found in methanol. Briefly, on the first paper the solvent-related radiolytic effects over the astatination precursors in several solvents and pH were studied [4]. On the second paper the studies were extended to analyze the effect of the radiolysis-mediated process on the nature of the labelled product generated and the yields of SAB as a function of the radiation dose and pH [3]. The profound influence of the nature of the solvent in the ability to synthesize SAB found lead to the conclusion that radiolytic effects play an important role in the synthesis of therapeutic amounts of astatine-labelled radiopharmaceuticals. The third paper [5] explored the effect of radiation dose on the astatine species present before initiation of the labelling reaction based on the hypothesis that ^{211}At will react with highly reactive radiolytically generated species coming from the solvent, the studies were carried out only in methanol identified previously as the optimal solvent for astatodestannylation [3,5]. The study showed that astatination chemistry behaves completely different at the high activities, hence high radiation dose, required for the clinical and industrial scenario in comparison with the behaviour observed in research, where the activities

used normally below 185 MBq, produces radiation dose lower than 500−1000 Gy. In the three solvent the astatination yields for producing SAB decreases very fast as the radiation dose increases, making not possible to obtain high astatination yields at radiation dose higher than 1500−2000 Gy which makes impossible to make a labelling for the level of activity required to do chemistry for therapy, normally higher than 555 MBq, in order to get a final activity of the radiopharmaceutical for therapeutic purpose of at least 370 MBq or higher. But the reasons that explain the decreasing astatination yields are different for each solvent model [3].

The first goal of the comprehensive study was to investigate solvent-related radiolytic effects on the degradation of the radiohalogenation precursor used for the radiosynthesis of SAB [4]. This 211At-labeled molecule was selected not only because of the practical need for being able to efficiently produce it at high levels for clinical studies, but also because of its potential utility for labeling other proteins and peptides [6].

To relate the results of these experiments to conditions potentially encountered in higher-dose ^{211}At labelling, consider a situation in which a 370 MBq of ^{211}At-labeled mAb is required. Using experimental average yields previously published for preparation of ^{211}At-labelled mAb for clinical use of around 55% for the SAB synthesis and a mAb coupling yield of 76% [2], and considering the synthesis and purification times involved, the starting activity of ^{211}At would need to be more than 1,500 MBq. For a prototypical reaction condition for SAB synthesis of 500 μL and a reaction time of 20 min, the minimum dose that would be encountered when the reaction is run in chloroform would be about 2,500 Gy. Thus, a dose of 3,000 Gy to the SAB chloroform reaction mixture is a reasonable benchmark for evaluative purposes. If one assumes that similar levels of radioactivity would be required if the reaction were run in other solvents, then the equivalent benchmark doses in methanol and benzene would be 4,700 and 4,250 Gy, respectively, The difference on the Gy value at the same initial condition are due to the different densities of these solvents [4].

Of the three solvents that were evaluated, benzene offered the lowest degree of radiolytic decomposition for both tin precursors, which is consistent with the fact that benzene is generally considered nearly radiation inert because of its stability. This contention is based on studies with low-LET radiation.

The most serious problems were found in chloroform due to the production of chlorine radicals, which will produce a high level of side-reaction over the astatination precursor. The most important conclusion from the study is that chloroform, the solvent utilized to prepare SAB for clinical ^{211}At-labeled mAb production, is not suitable for use at high radiation dose levels. Not only was extensive radiolytic decomposition of the tin precursor observed, with 50% degradation taking place even at doses below 500 Gy, which is a pretty low level of radiation dose reached even when doing labelling at low activities for research. But also radiolytically induced generation of a non-radioactive byproduct was observed, which efficiently competed with astatinated precursor on the conjugation reaction to the antibody. These phenomena could explain the lower SAB yields, lower mAb coupling efficiencies and immunoreactivities that were observed at high doses [2]. A greater degree of stability was seen in both methanol and benzene, were more than 85% of the precursor remaining at 3500 Gy. No cold byproducts were observed with either solvent. The main conclusion of this study was that the nature of the solvent has a profound influence on the ability to synthesize high activity levels of SAB and possibly other ^{211}At-labeled radiopharmaceuticals.

The problems encountered with chloroform at high radiation doses are consistent with the known sensitivity of aliphatic halides to radiation. Chloroform is known to undergo many reactions and has a complex radiation chemistry resulting in the generation of a variety of free radicals.

Because of the high rate of production and chemical properties of Cl, there is a high probability that this radical could account for the presence of the byproduct, and could also explain the extensive radiolytic decomposition of BuSTB. The concentration of chlorine radicals increases as the radiation dose increases, and at the typical radiation dose necessary to reach for doing labelling for therapeutic purpose (higher than 2000 Gy) its amount will be higher than the amount of labelling precursor normally used on this reactions.

The next goal on the systematic research was to study how the radiolysis produced by the alpha particles influences the astatination reactions using the same model compound SAB [3]. The results showed that in chloroform SAB production declined rapidly with increasing dose, consistent with the documented radiolytic decomposition of BuSTB and MeSTB in this solvent. Even though both tin precursors were not appreciably degraded in benzene, SAB could not be produced in this solvent; instead highly lipophilic ^{211}At-labeled species were generated in near quantitative yields. Although a dose dependent decline in SAB yield also were observed in methanol, both in the presence and absence of oxidant, the results were better than those obtained with other solvents. The main conclusion was that clearly radiolytic factors could play an important role in the synthesis of clinical level activities of ^{211}At-labeled radiopharmaceuticals, necessitating the development of different reaction conditions than those that work successfully at lower activity levels.

The most surprising result was the one obtained in benzene. Although from the perspective of the stability of the tin precursor at high radiation doses, the best of the three solvents investigated was benzene, SAB could not be synthesized in benzene at any radiation dose level, either in the presence or absence of oxidant. It suggests that other factors in addition to availability of the precursor must be considered. The study showed that reaction of ^{211}At with benzene at high radiation doses resulted in the formation of very lipophilic species in yields generally greater than 90%. We speculate that the product(s) could be [^{211}At]astatobenzene and possibly other ^{211}At-labelled multi-ring compounds.

In methanol, the study showed that SAB production declined rapidly with increasing radiation dose. At about 1500 Gy yields showed a 20% decreasing but for radiation higher than 4500 Gy SAB yields had declined from about 80%, making these reaction conditions not ideal for performing ^{211}At labelling at the high doses needed for targeted radionuclide therapy which required starting activities producing radiation dose higher than 4500 Gy as explained before. Considering that even at radiation doses higher 5000 Gy, more than 90% of the BuSTB remained intact, precursor degradation cannot account for the rapidly decreasing SAB yield with increasing radiation dose.

These results pointed out that more basic problems, not related with the astatination precursor, could be involved. One possible explanation would be radiation-induced changes in the chemical form of astatine itself departing from At^+, the species generally presumed to be required for efficient electrophilic astatodestannylation reactions. The hypothesis was that this scenario could occur due to the generation of reducing species during the radiolysis of methanol by high-LET ^{211}At alpha-particles. Calculation using the preferred G values recommended by the National Bureau of Standards for gamma radiolysis [7b] (G values for α-particles are not available) effectively showed that the amount of reduced species hydrogen and formaldehyde produced by the radiolysis of the solvent at the activities necessaries to do therapeutic labelling might become higher that the amount of oxidant itself used for the reaction.

In order to confirm this hypothesis the last goal set for the comprehensive research was the design of experiments to study the influence of the radiolysis produced by the alpha particles over the chemical behaviour of the astatine itself [5]. The study was done only in methanol because the previous studies showed that from the three types of solvents tested it was the only one that might we useful for doing labelling at high levels of activities.

The results obtained on the last study were consistent with this hypothesis. Briefly it showed that in methanol from all the chemical species involved on the reaction model used, the radioisotope itself was the most profoundly affected by the radiation dose deposited on the solvent. The result probed that the chemical form of the ^{211}At does become altered and converted to a reduced species with increasing radiation dose deposition into the solvent; solid experimental evidence lead us to hypothesize that the reduced astatine is most likely astatide. This reduction reaction of the radioisotope turns out to be a main deleterious effect over electrophilic astatination reactions at the high activities required for therapeutic purpose, making generation of electrophilic astatine more difficult.

The studies showed that at radiation doses below 1000 Gy, HPLC analysis indicated that > 90% of the ^{211}At was present in methanol as a single species, called At(1), while at higher doses, a second peak, called At(2), emerged. At(1) decreased and At(2) increased in a radiation dose dependent fashion, with At(2) becoming the predominant species at about 3000 Gy. At(2) was identified as a reduced form of astatine, presumably astatide, which could not be efficiently oxidized to a species suitable for electrophilic astatination. In methanol/acetic acid, > 95% of the astatine was present as At(2) even at doses below 1400 Gy. The emergence of a reduced form of astatine, At(2), at higher radiation doses is consistent with the decline in SAB yields observed under these conditions. Alteration of the chemical form of the astatine by radiolysis could account for the declining yields noted in the preparation of clinical-level ^{211}At-labeled radiopharmaceuticals as well as when the labelling chemistry is initiated hours after ^{211}At production. This suggests that At(2) is in a chemical form from which At+, the species generally considered to be required for electrophilic astatodestannylaton, cannot be efficiently generated. Unfortunately, definitive chemical identification of At(1) and At(2) could not be done because the lack of a stable astatine isotope limits the use of standard analytical techniques and prohibits the identification of several oxidation states. Clearly, due to its carrier free nature, when ^{211}At is isolated from the cyclotron target in the absence of oxidant, the number of moles of reducing species produced from methanol radiolysis could exceed those of astatine by several orders of magnitude, even at relatively low radiation doses. This could lead to the generation of reduced forms of astatine, which are not suitable for electrophilic astatination reactions. The results demonstrating an increasing fraction of At(2) with increasing radiation dose confirm this hypothesis. The shifting of activity from At(1) to At(2) in the presence of the reducing agent sodium sulfite is consistent with the possibility that the reduced species represented by At(2) is astatide. This conjecture is supported by the anionic behaviour showed by the electrophoresis and also supported by the fact that the retention time of At(2) was nearly identical to that of sodium [^{131}I]iodide. Thus, it appears that radiolysis of methanol could have two deleterious effects on electrophilic astatination reactions: consumption of the oxidant added to create At^+ and alteration of the ^{211}At added to reaction to a chemical form from which generation of a reactive electrophilic is more difficult.

The reactions occurring during methanol radiolysis provide a rationale for the dose and pH dependent increase in At(2) production. In methanol, radiation renders the spur more acidic than the bulk due to the formation of $CH_3OH_2^+$ and acidic pH increases the production of reducing species in methanol [7]. Thus, as the radiation dose increases, the spur becomes more acidic, which in turn, increases generation of reduced species. We speculate that the sequence more acidic spur → more reducing species results in increased At (2) productions, which will continue incrementing as the spur overlap increases. Consistent with this speculation is the observation of increased production of At(2) at lower radiation doses in the presence compared with the absence of acetic acid. Our results presented here demonstrate for the first time another significant impediment to successful astatine chemistry: alteration in the chemical form of the ^{211}At with increasing radiation dose from one(s) suitable for electrophilic labelling reactions to one(s) that is not. We show that in methanol, the solvent identified to date as the only potential candidate for doing therapy-level astatodestannylation, astatine is present at low radiation doses in a form from which At^+ can readily be generated, perhaps stabilized in a complex with methanol. With increasing radiation dose deposition to the solvent, conversion to a reduced species, probably astatide, occurs, which would account for the decline in labelling yields at elevated radiation doses.

This comprehensive study pointed out the profound effect on the astatine chemistry of increasing radiation dose and dose rate. The study showed that from the radiochemistry perspective the deposited energy per distance (dE/dx), described by a typical Bragg curve, and the lower penetration depth of the alpha particles produces fundamental differences on the radiolysis behaviour in comparison with particles beta negative. With densely ionizing radiation (e.g. α-particles) the spur overlaps and forms a column of ions and excited species about the track. This behaviour underline the consequences of the track effects over the chemistry [8] because the radicals produced on the primary interaction have higher probabilities of interact with each other inside the track to give molecular products instead of diffusing away to the bulk in a more homogeneous reaction environment increasing the radical yields. The overlapping of the reactive radicals, because the overlapping of the spur, happens more on the track of high-LET particles; therefore the molecular yields should increase. The study showed solid experimental evidence of the consequence over the astatine chemistry at high activities coming from this mechanism and demonstrated and confirmed the working hypothesis that astatine chemistry at high activities critically depends on the solvent in which the reactions are being carried out and also that the radiolysis problem

is completely independent of the astatination precursor. It also showed that the direct translation of the optimal conditions found for the chemistry done at low activities can not always be applied for chemistry at high activities. Although the problems caused by radiolysis have been most comprehensively investigated with ^{211}At, similar difficulties probably exist with the other alpha particle emitters of interest for targeted radiotherapy.

Information recently released [9] showed a new approach to deal with the radiolysis problem, called stabilization strategy, which has been shown to overcome the main problems that have precluded the use of astatine at high radiation doses, making it possible to do chemistry in high radiation fields. This strategy allows obtaining high astatination yields working at high levels of radiation dose and high levels of radiation dose rate as well, similar to the yield obtained at low activities by regular astatination techniques. This technology could have a major impact on the field because it overcomes many of the current obstacles to the development of ^{211}At-labeled targeted therapeutics; it permits both possible scenarios - therapeutic applications and industrial applications, allowing distribution of astatine to locations distant from the production site.

REFERENCES

[1] WELCH M. Potential and pitfalls of therapy with α-particles. J Nucl Med. **46** (2005) 1254−1255.

[2] ZALUTSKY MR, ZHAO X-G, ALSTON KL, BIGNER DD. High-level production of α- particle-emitting 211At and preparation of 211At-labeled antibodies for clinical use. J Nucl Med. **42** (2001) 1508 –1515.

[3] POZZI OR, ZALUTSKY MR. Radiopharmaceutical chemistry of targeted radiotherapeutics, part 2: radiolytic effects of 211At α-particles influence N-succinimidyl 3-[211At]astatobenzoate synthesis. J Nucl Med. **46** (2005) 1393–1400.

[4] POZZI OR, ZALUTSKY MR. Radiopharmaceutical chemistry of targeted radiotherapeutics, part 1: effects of solvent on the degradation of radiohalogenation precursors by 211At α-particles. J Nucl Med. **46** (2005) 700–706.

[5] POZZI OR, ZALUTSKY MR. Radiopharmaceutical Chemistry of Targeted Radiotherapeutics, Part 3: α-Particle Induced Radiolytic Effects on the Chemical Behavior of 211At. J Nucl Med. **48** (2007) 1190-1196.

[6] VAIDYANATHAN G, ZALUTSKY MR. Astatine Radiopharmaceuticals: Prospects and Problems. Curr Radiopharm. **1** (2008) 177-219.

[7] BAXENDALE JH, WARDMAN P. The Radiolysis of Methanol: Product Yields, Rate Constants and Spectroscopic Parameters of Intermediates. Washington, DC: National Bureau of Standards; 1975:1–26, (b) Table 2.1.1. Report NSRDS-NBS 54.

[8] MOZUMDER, A., Fundamentals of Radiation Chemistry, Academic Press 1999, page 52.

[9] ZALUTSKY M.R., POZZI O.R. Stabilized Composition and Methods for Radiolabeling Pharmaceuticals with Alpha-particle Emitters US Patent 8337810 B2, 2012.