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How Radiolysis Impacts Astatine **Speciation**?

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4 **ABSTRACT**
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7 This work deals with the impact of chloroform radiolysis on astatine chemistry. Astatine-
8 211 is a promising radionuclide for the targeted alpha therapy of cancers. Yet, the
9 production of ^{211}At based radiopharmaceuticals is often complicated by radiolysis due to
10 its own decay, leading to solvent degradation and then impacting astatine chemical state
11 and radiolabeling efficiency. A better understanding of the radiolysis phenomenon is thus
12 necessary. Two experiments are performed: (1) pH measurement during the At extraction
13 in chloroform medium, (2) pH determination calculated from the HCl chemical yield which
14 is obtained during the radiolysis of chloroform medium by γ -ray.
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17 In both cases, the pH values are determined at 2.0 ± 0.5 . Then, it is possible to predict
18 the acidic conditions during the ^{211}At production by HCl radiolytic yield determination. This
19 work demonstrated that the radiolysis induced by At during its production in CHCl_3
20 medium should be taken into account as it has a significant effect on acid-basic
21 conditions.
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24 The work helps understanding the mechanisms of production of a major impurity
25 generated from ^{211}At radiolysis in chloroform which alters its radiochemistry, allowing for
26 the identification of solutions to counteract these unwanted effects.
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38 **INTRODUCTION**
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48 Astatine-211 is a promising alpha-emitter radionuclide with a high potential for
49 applications in targeted alpha therapy of cancers (Guérard et al., 2013). Its intermediate
50 half-life (7.2 h) in comparison with other alpha emitters makes it potentially compatible
51 with various carrier compounds, from small molecules and peptides to large proteins such
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4 as antibodies. Radiolabeling chemistry which is employed for ^{211}At in most cases is similar
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6 to other halogens, in particularly to radioiodine. Various strategies have been developed
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8 recently which provided biologically relevant molecules labelled with ^{211}At with high
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10 radiochemical yields (Navarro et al., 2019; Reilly et al., 2018; Watanabe et al., 2019). Yet,
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12 difficulties to obtain robust labelling procedures have been reported when using certain
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14 solvents particularly chloroform. Lack of robustness of radiolabeling approaches
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16 performed in chloroform, which is clearly evident at high radioactivity, has been attributed
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18 to solvent radiolysis, induced by high energy α -particles (5,9-7,4 MeV), which in turn
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20 comes from ^{211}At decay. Products of chloroform decomposition may be responsible for
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22 the reduction in RCYs due to their reactivity with the reaction precursors (Pozzi and
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24 Zalutsky, 2005b) and/or alteration of astatine species necessary for the radiolabelling
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26 reaction (Aneheim et al., 2019). Although other solvents (e.g. alcohols or acetonitrile) are
27
28 now preferred for radiolabeling (in order to reduce issues related to radiolysis), chloroform
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30 remains an interesting solvent for astatine recovery after separation from its irradiated
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32 bismuth target. The main reason being that it is the only known solvent allowing
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34 evaporation to dryness without co-evaporation of astatine activity. This properties is highly
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36 interesting since it allows the fractionation of a batch of astatine into several samples that
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38 can be evaporated to dryness and then dissolve in any solvent adapted for each
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40 experiment (Aneheim et al., 2019).
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50 Thus, the study of chloroform radiolysis appears essential to better counteract issues
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52 associated with the use of this solvent.
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55 A large number of reports have previously focused on chloroform radiolysis in various
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57 contexts and experimental conditions (Abadie, 1982; Bibler and Hyder, 1968; Chen et al.,
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4 1960; Johnston et al., 1961; Ottolenghi and Stein, 1961; Schulte et al., 1953; Werner and
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6 Firestone, 1965). These authors suggested that hydrogen chloride is the main product of
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8 chloroform radiolytic degradation. However, all these publications (from the 50's to the
9
10 80's) gave equivocal radiolytic yield (G-yield) of generated hydrogen chloride because of
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12 differences in the experimental conditions. Either chloroform solutions purity were
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14 different which implies discrepancies in G-yield of HCl (Abadie, 1982; Chen et al., 1960;
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16 Johnston et al., 1961; Ottolenghi and Stein, 1961) or experimental conditions, for example
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18 various temperature, pressure or radiation dose rate (Bibler and Hyder, 1968; Johnston
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20 et al., 1961; Schulte et al., 1953; Werner and Firestone, 1965). For these reasons, we
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22 planned to revisit the experimental measurements of HCl yield during γ -ray experiments,
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24 more easy to be measured and close to the α ones, in specifically well-defined conditions
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26 in order to establish the potential quantities of HCl in routine production of astatine-211
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28 solution during radiolabeling and production of [^{211}At]-astatinated radiopharmaceuticals.
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37 **MATERIALS AND METHODS**

38 39 **Irradiation experiments**

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41 γ -Ray irradiations were performed on an gamma irradiator located at the ARRONAX
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43 cyclotron facility (Nantes – France). Irradiation cells made of PEEK (polyether ether
44
45 ketone) with an internal volume of 42 ml were mounted with a rotulex 19/9 glass tube.
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47 These cells were gas-tightened using a screwed joint in a glass-metallic valve. Specificity
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49 of each experiment is discussed thereafter. Irradiation of different 20 ml-samples of
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51 chloroform was performed from 1 to 7 days under aerated or oxygen free atmospheres
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53 under normal pressure (760 mmHg).
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7 **γ-Ray irradiation**
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9 γ-Ray irradiation was performed with a GSM D1 (Gamma-Service Medical) irradiator
10 containing a 123 TBq ^{137}Cs source. This radionuclide decays to $^{137\text{m}}\text{Ba}$, which delivers
11 661.7 keV γ-Ray. A dose cartography performed by Fricke dosimetry (Fricke and Hart,
12 1966) inside gamma irradiation chamber, indicated that the average deposited dose
13 inside the aqueous samples was between 7 and 9 Gy.min⁻¹. As γ-Ray is a penetrating
14 radiation, the dose can be considered as homogeneously deposited inside the whole
15 sample.
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26 **Analytical procedures**
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28 Solutions described in this section were prepared with commercial chemical products as
29 received with no further purification. All reactants were analytical grade and the aqueous
30 samples were prepared with ultrapure (MilliQ) water.
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36 **HCl analysis**
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38 To quantify HCl, produced by the irradiation of chloroform, it is necessary to extract HCl
39 molecules from chloroform bywater. Extraction experiments were performed to obtain the
40 percentage of HCl extracted from chloroform samples by water. Using 4 different
41 concentrations of HCl in 20 ml of chloroform, samples prepared ahead of time (0.1, 0.2,
42 0.5 and 1 mol.l⁻¹ by addition of 100 μl of aqueous HCl solution) and extraction protocol
43 was performed with a large volume of Ultrapure water (100 ml). After separation of layers,
44 titration of aqueous layer was performed using 0.1 mol.l⁻¹ NaOH to determine the
45 percentage of HCl extracted. Similarly, extraction and determination of HCl from irradiated
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4 samples of chloroform was done using a decanter followed by titration of acid with NaOH
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7 **(Figure 1).**
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10 The results show on average 75 ± 8 % of HCl from the HCl-chloroform samples was
11 extractable from chloroform to the aqueous phase. This ratio determined by the extraction
12 step that used in this work to establish the realistic HCl concentration obtained during the
13 radiolysis experiments. Therefore, we consider these uncertainties for all data plotted into
14 the figures.
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25 Radiolytic Yield Definition

26 The radiolytic yield is defined as the amount of a species that is formed or disappeared
27 per unit of deposited energy. It is expressed in the international system in $\mu\text{mol. J}^{-1}$ and
28 is calculated at a time t after transition of the ionizing irradiation according to the following
29 equation:
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$$37 G_t(X) = \frac{[X]_t}{\rho D}$$

38 where $[X]_t$ is the concentration of the species X at time of t (mol.l^{-1}), ρ is the density of the
39 irradiated solution (kg.l^{-1}) and D the absorbed dose (J.kg^{-1}).
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45 Thus, for the determination of the radiolytic yield of HCl during the irradiation of
46 chloroform, the equation is as following:
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$$50 G_t(\text{HCl}) = \frac{[\text{HCl}]}{\rho_{\text{CHCl}_3} \cdot D}$$

51 In which ρ_{CHCl_3} is equal to 1.48 kg.l^{-1} .
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4 **Production of astatine-211 solutions in chloroform**
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6 ^{211}At was produced at Arronax cyclotron facility using the $^{209}\text{Bi}(\alpha,2n)^{211}\text{At}$ reaction and
7 recovered from the irradiated target in chloroform using a dry-distillation protocol as
8 reported previously (Guérard et al., 2017). The radioactive sample was isolated and
9 shipped to CRCINA laboratory where radiolabelling chemistry is performed. On average
10 there is 300 to 400 MBq activity in a total volume of about 600 μl of chloroform with a
11 contact time of about 2 hours between the end of distillation and the HCl extraction
12 experiments, which corresponds to a dose range of 2.3 to 3.0 kGy received by chloroform
13 solution. Before radiolabeling, and approximately 2 h after recovering of ^{211}At activity in
14 chloroform, the chloroform ^{211}At solution was washed with 200 μl of water (TraceSelect
15 Grade, Fluka). After decantation, both layers were separated. The ^{211}At activity found in
16 the aqueous layer was negligible, nearly all activity being found in the chloroform layer.
17 The pH of the aqueous layer was measured by mean of a pH indicator strip (Merck, pH
18 range 0-6, pH gradation = 0.5). All assays indicated a pH of 1.5 ± 0.5 ($n = 4$). pH indicator
19 was used to compare it with HCl titration result not as the tool for HCl analysis.
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43 **RESULTS AND DISCUSSION**
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45 In this study, we investigated the amounts of HCl produced through irradiation of
46 chloroform by γ radiations in two different conditions (presence or absence of oxygen) at
47 low doses of irradiation. The latter one was the dose rate corresponding to the real
48 conditions of exposure of chloroform when performing [^{211}At]-astatinated
49 radiopharmaceuticals for pre-clinical development.
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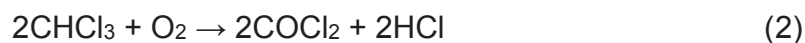
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4 **Determination of initial G- values of HCl during irradiation in the aerated and**
5 **oxygen free atmospheres**
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9 **Figure 2** presents HCl production as function of dose in aerated and oxygen free
10 atmospheres for CHCl₃ irradiated by γ-ray.
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15 According to the data derived from this figure, G-value for HCl in the dose range from
16 0 to 2580 Gy in the presence of air, is equal to $1.66 \pm 0.24 \mu\text{mol}\cdot\text{J}^{-1}$ whereas it is only
17 $1.14 \pm 0.17 \mu\text{mol}\cdot\text{J}^{-1}$ in the presence of Ar. We can see a clear difference between the
18 radiolytic yields of HCl production that can be explained by looking the possible reactions
19 in both conditions.
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22 For the Chloroform irradiation mechanisms, two different behaviors are possible:
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24 (i) One of them, when air is present above the sample, are the chemical reactions of
25 Chloroform with Oxygen in order to produce molecules of HCl, Cl₃COOH and COCl₂
26 which have been proposed in the primarily steps of chloroform irradiation (Schulte et al.,
27 1953):
28



44 However, reaction 2 can take place even without ionizing radiation, but it takes too long
45 (some days) (Clover, 1923). As a result, when measuring the radiolytic yield of HCl in
46 presence of air, we consider the HCl concentration zero at no absorbed radiation dose.
47
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49 HCl is produced through reaction (2) while Cl₃COOH is produced through reaction (1).
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51 (ii) The second one, in a free-oxygen environment, is the direct radiolysis of the CHCl₃
52 molecules to produce HCl via H° and Cl° radicals production (Chen et al., 1960):
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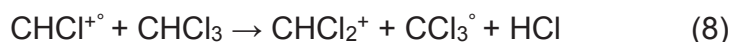
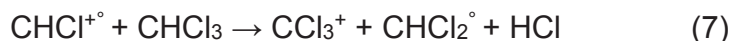
7 or



14 Radiolytic products such as dihydrogen (H_2) and dichloride (Cl_2) gases result from
15 recombination of H° and Cl° radicals, respectively (Lainé et al., 2017; Mostafa et al., 2018).

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17 This implies that HCl and radiolytic gases compete for formation reactions, which needs
18 to be measured in further studies.
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21 In addition, recent studies by using pulse radiolysis shown that if surrounded chloroform
22 molecules participate with the $\text{CHCl}^{+\circ}$ the reaction (7) and (8) are more favorable (Bird et
23 al., 2020):
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38 At low doses, typically until 3000 Gy, in the first steps of chloroform irradiation, Cl_3COOH
39 (see reaction (1)) has been considered as a major product which produces quantitative
40 amount of peroxide before its reduction (Schulte et al., 1953). According to our results at
41 low doses (< 500 Gy) a difference is observed for the radiolytic yield of acids with and
42 without air. This difference was increased when the dose was increased which means
43 that reactions 2 and 4 are occurring in parallel. It can be concluded that the higher the
44 dose, the more important the effect of oxygen.
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Irradiation of chloroform at higher doses

The results obtained in this study was over a wide range of doses, from 0 to 93 kGy, (see **Figure 3**). This dose range corresponds to irradiation times of chloroform in our gamma-irradiator varying from 0 to 10 days.

Based on the results reported in Figure 3 and using the reactions (2-6), the radiolytic yield of HCl production in the dose range of 0-93294 Gy has been determined as $0.82 \pm 0.11 \mu\text{mol}\cdot\text{J}^{-1}$. Moreover, as already suggested in the previous part, two behaviors append during the HCl formation by chloroform irradiation: (i) in presence and absent of oxygen (ii) in the high dose range ($> 3 \text{ kGy}$) the radicals H° and Cl° production by the direct radiolysis of CHCl_3 (reactions (3-6)).

The mechanism of abstraction of H and Cl from CHCl_3 during dose enhancement, suggested in this work, is consistent with literature (Chen et al., 1960). Authors have proposed that the abstraction of H and Cl atoms from CHCl_3 molecules, by Cl° and H° radicals, can be considered as the major reactions producing HCl during the irradiation in the absence of oxygen by γ -rays (Chen et al., 1960).

Most of the reported results regarding irradiation of chloroform in the literature have been obtained in O_2 and water-free situations as in our conditions. However, the literature shows a large discrepancy (see **Table 1**).

All authors have underlined the discrepancy of the G-values (from 0.45 to $1.23 \mu\text{mol}\cdot\text{J}^{-1}$). Then, we can explain the difference by temperature, pressure conditions and HCl production behavior due to the impact of oxygen as already suggested. In addition, the range of dose studied in previous works may explain this discrepancy. For example in our study, a neat difference of G-value is observed as a function of the dose range: for low

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4 range (< 3 kGy) G-value = 1.66 $\mu\text{mol}\cdot\text{J}^{-1}$ while at high range (< 93 kGy) G-value = 0.66
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6 $\mu\text{mol}\cdot\text{J}^{-1}$. It should be noted that the G-value at higher doses was calculated from obtained
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8 data in dose range of 3-93 kGy (cf figure 3).
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11 That is the reason why, we have chosen to use the same dose range (< 3 kGy),
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13 determined in the experimental section, for the radiolysis and the ^{211}At extractions
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15 experiments in order to determine the realistic quantity of HCl produced by radiolysis
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17 which comes from ^{211}At .
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21 Therefore, in the next part, we compare the HCl production for the real dose deposited in
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23 chloroform solution defined in the ^{211}At extraction (between 2.3 and 3 kGy), which
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25 corresponds to a contact time between At and chloroform solutions of 2 hours, with our γ
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27 irradiation conditions in a close range of dose rate in the both cases (27 $\text{Gy}\cdot\text{min}^{-1}$ for ^{211}At
28
29 extraction vs. 10 $\text{Gy}\cdot\text{min}^{-1}$ in the radiolysis ones). However, the possibility of differences
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31 in HCl production between γ and α radiolysis may be considered in the context of
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33 radiolysis arising from astatine-211. Nonetheless, according to reactions (3-6), HCl
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35 production depends on the radicals produced by radiolysis (H° , Cl°). As the linear energy
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37 transfer (LET) in α radiation is higher than γ one, therefore the produced radicals are
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39 combined faster in spurs therefore, the molecular yield is higher than radical yield in α
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41 radiation (Appleby and Schwarz, 1969). That is the main difference between the two types
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43 of radiation. Consequently, by considering this difference, the HCl production is, in theory,
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45 higher in the case of γ -ray radiolysis and the G-value determined in this work is
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47 overestimated in comparison with the one actually occurring during exposition of
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49 chloroform to ^{211}At . In order to determine this overestimation, we have measured the HCl
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51 production by extraction from batches of ^{211}At in chloroform.
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Impact/Consequences for Astatine Extraction/Complexation

Being a radioelement, with only short half-life radioisotopes, astatine chemistry is extremely complex to study. Most work with astatine has been performed with ^{211}At ($t_{1/2} = 7.2$ h), the most stable isotope, ^{210}At , exhibiting a $t_{1/2}$ of only 8.1 h being avoided for radioprotection concerns. Additionally, the weak amounts produced by accelerators (few pictograms at best), make it nearly impossible to use conventional spectroscopic techniques for precise determination of chemical properties of astatinated species. A major issue in studying astatine chemistry is the evolution over time of the astatinated species in solution, including their oxidation state, which affects the reproducibility of radiolabelling procedures. This phenomenon has been attributed to radiolysis of the solvent, which generates degradation products that may then react with astatine and modify its chemical nature. In the case of chloroform, previous reports have suggested that radicals formed during radiolysis could impact radiolabelling procedures (eg Cl^\bullet that may react with precursors instead of astatine and then decrease radiochemical yields (Pozzi and Zalutsky, 2005a), or that recombination of radicals can form species suspected to react with astatine, or adducts affecting its behavior (volatility, extractability, solubility) (Aneheim et al., 2019).

Here we focused our attention on HCl production upon chloroform radiolysis since acidity of the media appears as an essential parameter governing the astatine species in solution as shown in recent reports coupling experimental data with computer simulations to construct the Pourbaix diagram of astatine in water (Champion et al., 2013; Sergentu et al., 2016), and that may impact its use for analysis and radiolabeling chemistry. It is assumed that in acidic and oxidative medium, three species coexist (At^+ , AtO^+ , and

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4 AtOOH), the predominant species being in part determined by the pH of the solution.
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7 Considering this, and since concentration of HCl varies over time in chloroform it is clear
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9 that experiments conducted with astatine isolated in chloroform are likely to lack
10 consistency and reproducibility due to the dose deposited in stock solutions when activity
11 in solution and time before radiolabeling and use vary. The solution to this problem is
12 simple to set up, by adding an extraction step in water before use for radiolabeling.
13
14 Extraction experiments discussed above showed that most of HCl could be extracted from
15 chloroform to water, which was confirmed when applied to ^{211}At stock solutions. This
16 additional step in the purification process of ^{211}At is time-consuming (< 10 min) and
17 generates negligible loss of activity due to the extremely low transfer of astatine to
18 aqueous layer. From the G-yield value determined above ($1.66 \mu\text{mol}\cdot\text{J}^{-1}$ in the dose range
19 studied), we can estimate the HCl concentration for the dose applied to the chloroform
20 corresponding to the time between the isolation of astatine in chloroform, to the extraction
21 step (between 2.3 and 3 kGy). The calculated range of HCl concentration is between 3.8
22 and $5 \text{ mmol}\cdot\text{L}^{-1}$ (*i.e.* $\text{pH} = 2.3 \pm 0.1$) close to the real pH value measured in the Astatine
23 extraction experiments ($= 1.5 \pm 0.5$). The difference can be attributed to the G-values
24 difference between γ and α irradiations and the production of other radiolytic species,
25 which contribute to the acido-basic conditions and must be characterized such as
26 Cl_3COOH . Experiment performed in further studies would be the α irradiation of
27 chloroform solutions using a helium beam to measure the G-yield in these specific
28 conditions and compare them with astatine irradiations (α in the both cases) and the
29 analysis by Infrared Spectrometry of other radiolytic species produced during chloroform
30 irradiation.
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CONCLUSION

The gamma radiolysis of chloroform using a ^{137}Cs source was investigated in this study in order to determine the amount of hydrogen chloride (HCl) produced as a major product of chloroform radiolysis. Radiation doses ranged from 0 to 93 kGy, which had an impact on the chemistry of Astatine. According to our finding, during the accumulation of the dose, the amount of HCl acid follows a linear behavior at low dose with a G-value of ($1.66 \mu\text{mol}\cdot\text{J}^{-1}$) and a linear behavior at high dose with a G-value of ($0.82 \mu\text{mol}\cdot\text{J}^{-1}$). This different behavior at low and high dose may explain the large discrepancy found in the literature as a function of the dose range studied. Moreover, in the low dose range ($< 3\text{kGy}$), the trichlorohydroperoxymethane (Cl_3COOH) production by chloroform reaction with O_2 , must be taken into account for the mechanisms of HCl production.

These experimental results contribute in understanding of chloroform radiolysis mechanisms induced by astatine radioactivity during its extraction/complexation processes. In fact, with the G-value determined in this work we calculated the theoretical pH value in the Chloroform/Astatine solution ($\text{pH} = 2.3$) close to the one measured in the real Astatine complexation/extraction process ($\text{pH} = 1.5$). Then, as a conclusion, the radiolysis mechanism which can explain the acidic pH in the chloroform/astatine solution must be taken into account for this kind of radiochemical experiments as already suggested but not, until now, demonstrated in the literature (Aneheim et al., 2019; Pozzi and Zalutsky, 2005a, 2017). Moreover, synthesis of ^{211}At radiopharmaceuticals such as SAB (N-succinimidyl 3- ^{211}At -astatobenzoate) suffers from chemical decomposition (Pozzi and Zalutsky, 2005a) and formation of HCl by radiolysis may explain this problem.

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4 Our results may also explain why different oxidation states of ^{211}At exist in chloroform at
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6 the same time (Aneheim et al., 2019). It is helpful to measure the radiolytic yield of H_2 and
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8 Cl_2 in order to better understand the competition of HCl formation reaction with those
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10 gases, as H° and Cl° radicals are involved in these reactions. Moreover, based on this
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12 experiment, we would then recommend washing astatine solution with water in order to
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14 improve consistency before using for radiolabeling.
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4 **FIGURE LEGENDS**
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7 **Figure 1** : Amount of HCl extracted in water from chloroform

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10 **Figure 2** : Experimental G-yield values of HCl), produced during irradiation of CHCl₃ in
11 aerated (red spots) and oxygen free (blue ones) atmospheres by ¹³⁷Cs-source irradiator
12 E_γ = 0.667 MeV, DR = 7-9 Gy.min⁻¹, Total Dose = 2580 Gy. The obtained data are shown
13 the oxygen in the air can participate in HCl production. When measuring the radiolytic
14 yield of HCl in the presence of air, the HCl concentration is considered zero at no
15 absorbed radiation dose, as explained in results.
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25 **Figure 3** : HCl concentration, produced during irradiation of CHCl₃ in aerated atmosphere
26 by ¹³⁷Cs-source irradiator E_γ = 0.667 MeV, DR = 7-9 Gy.min⁻¹, Total Dose = 93300 Gy.
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38 **TABLE and FIGURES**
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42 **Table 1**: G-yield values of HCl from chloroform irradiation in literature and this work
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Dose(kGy)	0.005	320	4.6 – 11.8	26 – 210	16 – 420	93.3
G(HCl) (μmol.J⁻¹)	0.45	1.18	0.59	1.23	0.55	0.82
Ref.	(Abadie, 1982)	(Chen et al., 1960)	(Ottolenghi and Stein, 1961)	(Johnston et al., 1961)	(Werner and Firestone , 1965)	This Work

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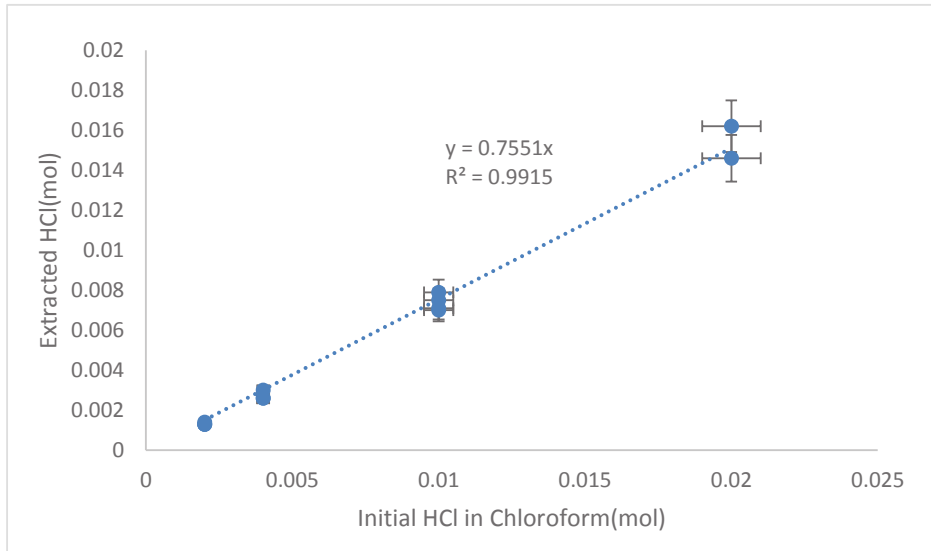


Figure 1 : Amount of HCl extracted in water from chloroform

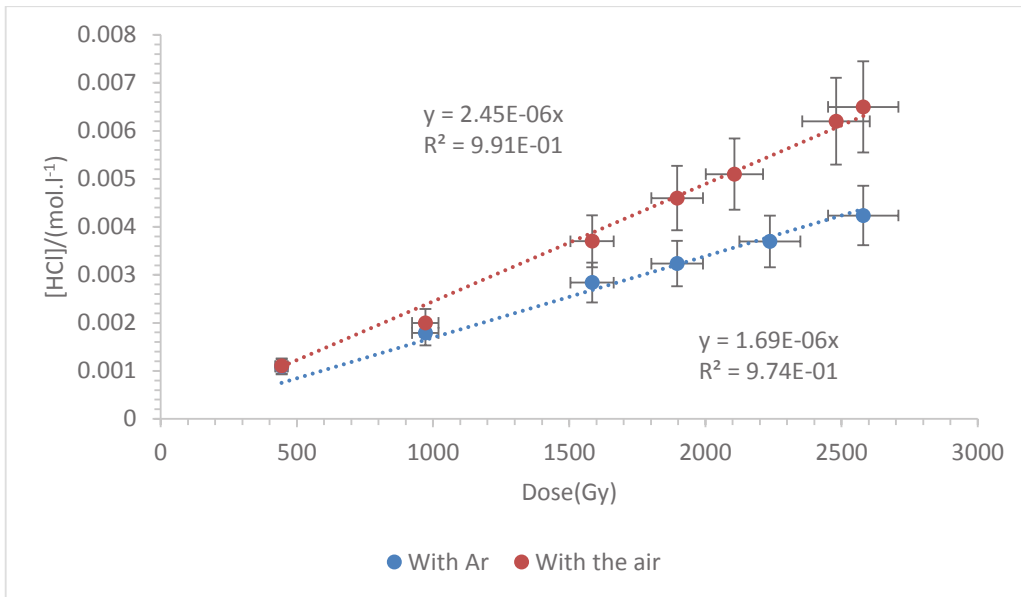


Figure 2 : Experimental G-yield values of HCl, produced during irradiation of CHCl_3 in aerated (red spots) and oxygen free (blue ones) atmospheres by ^{137}Cs -source irradiator $E_\gamma = 0.667 \text{ MeV}$, $\text{DR} = 7\text{-}9 \text{ Gy}\cdot\text{min}^{-1}$, Total Dose = 2580 Gy. The obtained data are shown the oxygen in the air can participate in HCl production. When measuring the radiolytic yield of HCl in the presence of air, the HCl concentration is considered zero at no absorbed radiation dose, as explained in results.

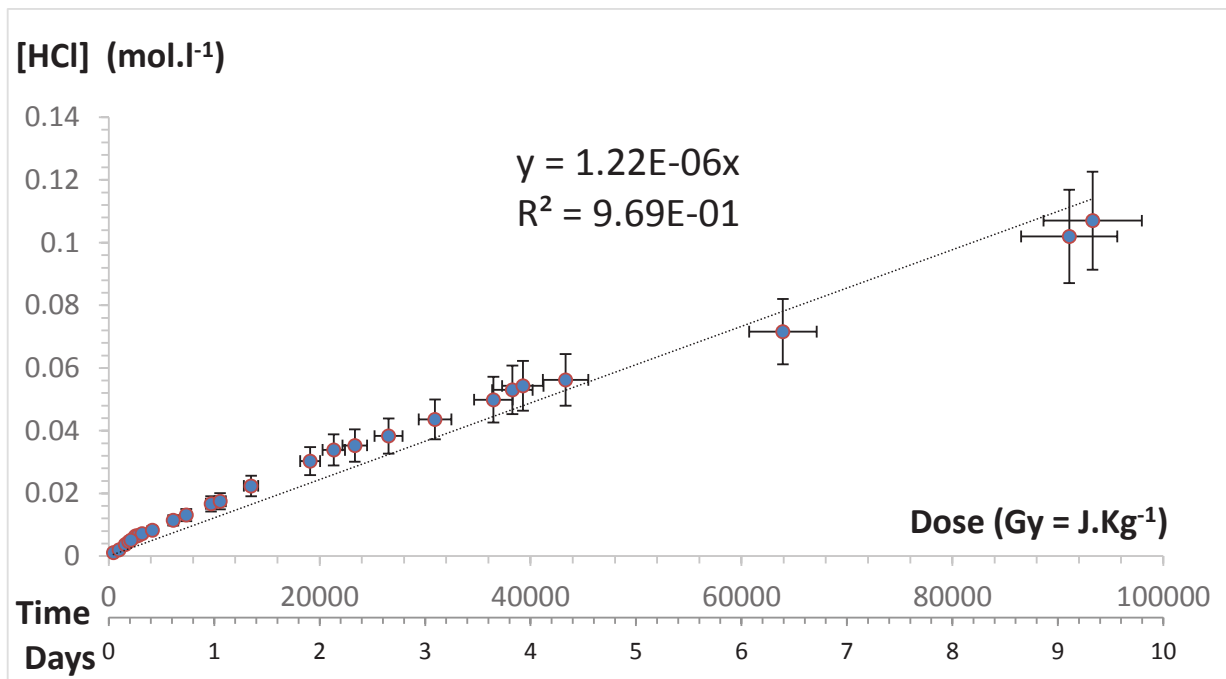


Figure 3 : HCl concentration, produced during irradiation of CHCl_3 in aerated atmosphere by ^{137}Cs -source irradiator $E_\gamma = 0.667 \text{ MeV}$, $\text{DR} = 7\text{-}9 \text{ Gy}\cdot\text{min}^{-1}$, Total Dose = 93300 Gy.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

- Parastoo Mahdi Khoshouei and Guillaume Blain performed the experiments.
- Johan Vandendorre and Mohammad Ghalei wrote the manuscript with contribution from all co-authors.
- All co-authors contributed to the discussion and interpretation of the data.