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Abstract

Astatine is a rare radioelement belonging to the halogen group. Considering the trace amounts of astatine produced in cyclotrons, its chemistry cannot be evaluated by spectroscopic tools. Analytical tools, provided that they are coupled with a radioactive detection system, may be an alternative way to study its chemistry. In this research work, High Performance Anion Exchange Chromatography (HPAEC) coupled to a gamma detector (γ) was used to evaluate astatine species under reducing conditions. Also, to strengthen the reliability of the experiments, a quantitative analysis using a reactive transport model has been done. The results confirm the existence of one species bearing one negative charge in the pH range 2–7.5. With respect to the other halogens, its behavior indicates the existence of negative ion, astatide At^-. The methodology was successfully applied to the speciation of the astatine in human serum. Under fixed experimental conditions (pH 7.4–7.5 and redox potential of 250 mV) astatine exists mainly as astatide At^- and does not interact with the major serum components. Also, the method might be useful for the *in vitro* stability assessment of ^{211}At-labelled molecules potentially applicable in nuclear medicine.

1. INTRODUCTION

Astatine (At), element 85, is below iodine in the periodic table of elements. It is a rare element representing short half-life radioactive isotopes that have to be produced in cyclotrons [1].

^{211}At is of considerable interest as it is a promising radiotherapeutic agent for targeted alpha therapy (TAT) in nuclear medicine [2-4]. In this field, the astatination through diazo intermediates under non-oxidizing mild conditions has been suggested to react with astatine anionic or radical species [5, 6]. The general approach developed more recently has been the use of bifunctional reagents conjugated to the proteins and labelled, similarly to the radioiodination, under oxidizing conditions of Chloramine T, hydrogen peroxide or N-iodosuccinimide with astatine cationic reactive species [2-7].

Astatinated molecules as well the bio-conjugates are quite unstable in vivo relative to their radioiodinated analogues [7]. Due to the fact that released astatine localizes in thyroid in humans, as iodide, iodate, chlorate, or pertechnetate [5] the form of astatide (At^-) has been proposed. Furthermore, the distribution (in PBS) between plasma and blood cells in vitro has established that it is poorly entrapped within the erythrocytes, which render it available for the transport in the blood [8]. The mechanism of "deastatination" in vivo remains still unknown, Wilbur has only pointed out the complex character of probably enzymatic, biochemical and/or physical process [7]. The stability is generally assessed using in vitro studies with blood serum by thin layer chromatography. Although the method is rapid, it gives no indication about astatine speciation.

Astatine chemistry remains generally not well understood. It is an invisible element: the produced amount of astatine allows only ultra trace concentrations (typically 10^{-11} to 10^{-15} mol L^{-1}) and thus no spectroscopic tools can be used to investigate astatine chemistry at the molecular level. In reducing conditions, astatine presents some other similarities with respect to its homologues of the halogen group, especially iodine. For example, it coprecipitates with insoluble iodide compounds [9,10] and astatine forms the hydrogen astatide (HAt) alike the halogens form hydrogen halides. The identification of HAt^+ and HI^+ species in gas phase by mass spectrometry [11] may be an indirect proof of the presence of At^-. Astatide (At^-) is therefore an expected species in various media. Surprisingly, only a few people have endeavored to identify the astatide by means of analytical tools. The negative charge of astatine species has been deduced from its ability to be retained by anionic exchanger Aminex A27 [12-14], and from electromobility measurements [15]. Berei et al. [5], using the data of Roessler [14]from high-pressure liquid radiochromatography experiments, showed a linear correlation between the retention volume and the inverse ionic radius for halogens and concluded that the astatine species under study behave as halogenide At^-. However, as it will be discussed later, due to the proportionality of the retention volume to the selectivity coefficient, the correlation should show an exponential curve trend. This discrepancy makes the results of Roessler et al. questionable and new data are needed.

In this paper, the first objective is to acquire new data by high-performance anion-exchange chromatography under reducing conditions using simple media 0.01–0.10 mol L^{-1} H/NaCl (chloride as the exchange species) with pH values ranging from 2.0 to 7.5 for a better identification of astatine species. For comparison, experiments are also performed with other halogens anions (F^-, Br^-, I^-). To help the evaluation of experimental data, a quantitative analysis using the reactive transport code PHREEQC [16] is proposed. Then, the second objective is to apply the methodology for speciation of astatine in blood serum. At pH of 7.4

its potential varies between 200 and 300 mV versus NHE (Normal Hydrogen Electrode) [17] which coincide with the values related to the existence of thermodynamically stable anionic astatine species.

2. EXPERIMENTAL

2.1. Materials

Commercially available chemical products of analytical grade or superior were purchased from Sigma-Aldrich. Transferrin from human blood plasma (≥ 95%) and albumin from human blood plasma (≥ 99%) were also purchased from Sigma-Aldrich. Human serum was supplied by Lonza.

^{211}At was produced by the nuclear reaction ^{209}Bi(α, 2n)^{211}At at the CEMTHI cyclotron (Orléans, France). Bismuth (Bi) target was prepared by the evaporation of bismuth under vacuum onto nitride aluminium backing (shapal-M from Goodfellow). The ceramic backing has good thermal properties and is heat resistant under beam. An elliptical deposit with a surface area of 3 cm^2 and a homogeneous thickness between 22.3 and 30.0 µm was obtained within few hours. The homogeneity of the deposit was checked using a profilometric analysis. Run duration as well as beam intensity were adapted to reach needed ^{211}At activity. For the present work, duration run varied from 2 to 3 hours and beam intensity from 1.7 to 2.3 µA leading to production from 100 to 317 MBq at the end of bombardment.

Detailed information about the production can be found elsewhere [18,19]. Astatine from the target was recovered by dry distillation and captured in methanol (yield at about 80%) [19]. The radionuclide purity was monitored using γ–ray spectroscopy. In order to lower the X-ray contribution, a lead shielding was placed between the detector and the sample. Typically, the stock solutions were obtained with a specific activity close to 100 MBq mL^{-1}. The radiotracer ^{131}I was obtained diluted in water with a specific activity close to 0.1MBq mL^{-1} from the CHU Nuclear Medicine (Nantes, France).

2.2. Analytical tools

The radionuclidic purity of ^{211}At was monitored by γ–ray spectrometry with a high purity germanium (HPGe) detector. The activity of the stock astatine solution was measured on both the X–rays from ^{211}Po and ^{211}At and γ–rays at 687.00 keV from ^{211}At, using a suitable geometry previously calibrated with standard gamma sources. ^{211}At recovery after the HPAEC–γ measurements was quantified by liquid scintillation counting using a Packard 2550 TR/AB liquid scintillation analyzer with the Ultima Gold LLT scintillation liquid.

A Fisherbrand type electrode freshly calibrated against dilute standard pH buffers (pH 1–10, Merck) was used to determine the pH. The potential (E) of aqueous solutions was measured using a Pt combined redox electrode (Metrohm type) calibrated against the redox buffer ($Fe(SCN)_6^{-3}/Fe(SCN)_6^{-4}$, 215 mV/Pt/SCE, Radiometer Analytical).

The HPLC device is a Dionex UltiMate3000 system consisting of a DGP-3600 MB pump, an AS3000 auto-sampler, a TCC-3200B column oven and a diode array DAD-3000 detector. The stationary phase is a Dionex AS20 anionic exchange column (0.2 cm diameter x 25 cm

length), with an AG20 guard column (0.2 cm diameter × 5 cm length). The AS20 consists of a hydrophilic polymer grafted with quaternary alkanol ammonium. The total capacity represented by both columns is 0.079 milliequivalents (meq). The degree of Cross-Linking (% of DiVinylBenzene, %DVB) amounts to 55%. The resin was designed for working in NaOH or KOH media. However, taking into account the context of the study, the sodium chloride medium was chosen (see 2.3. Experimental procedure). The γ–ray detector is a Raytest GabiStar, piloted by Gina Software. Count rate is 0–500.000 cps. The detection energy window was set between 50 and 1630 keV. Experimental data were acquired and processed by Chromeleon 6.80 Chromatograph Software.

The components in stock solutions and collected fractions were quantified by Inductively coupled plasma mass spectrometry (ICPMS) XSERIES2 Thermo Scientific for I^-, by Ion Chromatography (IC) with conductivity detection ICS 2500 from Dionex for F^- and Br^-, and by UV–VIS Spectrophotometer UV-1800 Shimadzu for albumin and transferrin by using the absorption coefficients of 9.30×10^4 and 3.53×10^4 cm^{-1} mol^{-1} L at 280 nm, respectively [20].

2.3. Experimental procedures

The solutions and eluents were freshly prepared using degassed (by ultrasonics) Milli–Q deionized water under argon atmosphere. Astatine is a redox-sensitive species [21]. In all eluents, the redox couple $SO_3^{2-}/S_2O_3^{2-}$ ($10^{-4}/10^{-3}$ mol L^{-1}) was used to maintain the potential at 250 mV versus NHE (Normal Hydrogen Electrode). It is a mean value characterizing the serum potential which varies between 200 and 300 mV versus NHE [17]. For astatine-in-blood serum assays, the eluent composition was close to the physiological solution (10^{-1} mol

163 L^{-1} NaCl, pH 7.4). The pH was buffered with 10^{-3} mol L^{-1} PBS that, as 10-fold concentrate,
164 contains 10^{-1} mol L^{-1} of sodium phosphate (Na_3PO_4) and 9% of NaCl. For the other
165 experiments, 10^{-1} mol L^{-1} NaCl (with or without 10^{-2} mol L^{-1} HCl) was used unless otherwise
166 stated. Eluents were kept under argon flow during proceedings.

167 The stock solutions were prepared by dissolving the appropriate mass of NaX (X = F, Cl,
168 Br, I) salts and each protein (not purified) in eluent under argon atmosphere. The samples (10^{-4}, 10^{-5} and 10^{-6} mol L^{-1} of NaX, 4.6 10^{-6} mol L^{-1} of transferrin and 7.9 10^{-5} mol L^{-1} of albumin)
170 were prepared by dilution in the appropriate eluent. pH was set to 2 by adding the appropriate
171 volume of HCl to some samples of NaX. The commercial serum was three times diluted in
172 the physiological-type eluent. The ^{211}At stock solution in methanol was added to the solutions
173 with varying concentrations from 6 10^{-14} to 1.5 10^{-11} mol L^{-1}. The mixtures were agitated for 2
174 h at 25 °C for equilibration.

175 The HPAEC method included a cleaning step by elution with HCl, the pH shifting
176 gradually from 5 to 2 during 20 min, followed by checking the resin stability. This was done
177 using I^- (10^{-5} mol L^{-1}) as internal standard giving a retention time of 11.2 min for a new AS20
178 column using 10^{-1} mol L^{-1} NaCl at 350 µL min^{-1}. Over time, the shift of 0.6 min towards
179 lower retention time was observed which is explained by a small decrease of the site capacity
180 of the resin and was taken into account in the quantitative modeling (see 3. Results and
181 discussion). Finally, the system was pre-equilibrated with the eluent of interest. The pre-
182 equilibration was controlled by measurement of pH and E at the inlet of the column and the
183 outlet of gamma detector. All experiments were performed under isocratic conditions at 25
184 °C. Samples were injected into the column through a 50 µL sample loop injector. I^- and Br^-
185 (both 10^{-5} mol L^{-1}) were detected online spectrophotometrically at 230, 214 nm, proteins and
186 blood serum at 280 nm. An online detection of astatine was done by γ–ray detector. F^- ($5x10^{-5}$
187 mol L^{-1}) was detected offline (from collected fractions) by IC with conductivity detection.

188 The recovery was determined from measurement of species' concentration before injection
189 and in the collected fractions.
190 For clarity in the data interpretation, the results are exploited as retention factors (k) with:
191 $$k = \frac{t_R - t_D}{t_D} \quad (1)$$
192 t_R and t_D being the retention time and the dead time (corresponding to the method of detection
193 used), respectively. When retention times are given, they refer to the position on the UV
194 chromatogram.
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197 *2.4. Quantitative analysis of experimental data*
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200 PHREEQC is a 1D transport code [16] built for transport processes modeling including
201 diffusion, advection and dispersion. All of these processes are combined with equilibrium and
202 kinetic chemical reactions. Initially developed for modeling the transport of contaminants in
203 soils, it is also well suited for the modeling of liquid chromatography data because the
204 processes in analytical and environmental fields are likely identical. The input file, divided
205 into three blocks, is briefly explained as follow.
206 The first block involves the aqueous speciation calculations. Using a chemical
207 composition of each solution (potential, pH, solutes and concentrations), it calculates the
208 distribution of aqueous species using implemented thermodynamic databases. In the current
209 study, the LLNL (Lawrence Livermore National Laboratory) thermodynamic database for F,
210 Cl, Br and I, and the recently reported data for At [21], were used for the modeling. All
211 equilibrium constants were extrapolated at zero ionic strength using the Truncated Davies
212 equation [22].

The second block involves the transport. As it will be experimentally shown latter, the transport is imposed by the high pressure pump and can be considered as an advection process. The diffusion process, which affects the width of the elution peaks, was not considered since the paper is interested in the retention properties of the exchanger.

The third block describes the reaction at the surface of the exchanger. Ion-exchange being an important mechanism occurring in soils, it is implemented in the code through the Gaines-Thomas convention [23] which was used in the study. The approach uses the law of mass action, based on half-reactions between an aqueous species and a fictive unoccupied exchange site for each exchanger. The reaction for the exchange species S-Cl is:

$$Cl^- + S^+ \rightleftarrows S - Cl \qquad (2)$$

where S^+ represents the exchange master species. Since all exchange sites are filled by exchange species (the concentration of master species is forced to zero), the master species is not included in the mole-balance equation for the exchanger. The activities of the exchange species are defined as equivalent fractions, being equal to the moles of sites occupied by an exchange species divided by the total number of exchange sites, in equivalents per L of solution (when density = 1). In NaCl medium, the exchange reaction of X^- (X = F, Br, I and At) can be written as:

$$X^- + S - Cl \rightleftarrows S - X + Cl^- \qquad (3)$$

The distribution of species is given by the law of mass action:

$$K_{X/Cl} = \frac{\{S-X\}\{Cl^-\}}{\{X^-\}\{S-Cl\}} = \frac{K_x}{K_{Cl}} \qquad (4)$$

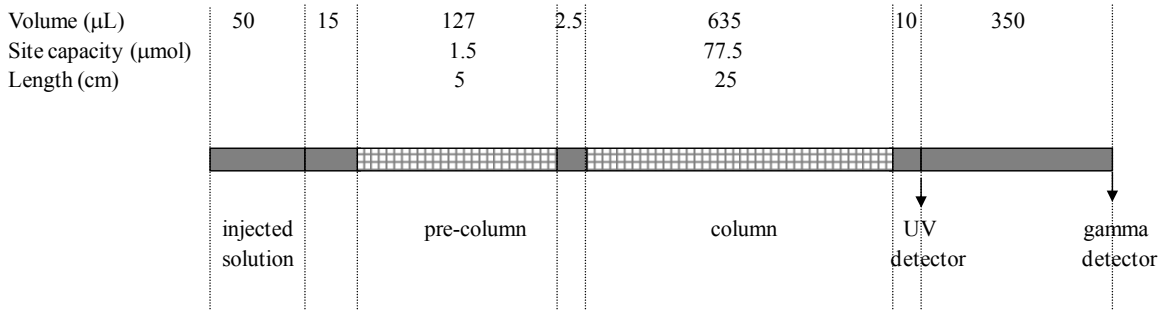
Curly brackets indicate the activities, and K_X and K_{Cl} the coefficients describing the interaction between the specified anion X^- and the exchange site S^+ according to the equation (2). The exchange or selectivity coefficients $K_{X/Cl}$ given in the paper are relative to Cl^-, i.e. $K_{Cl/Cl} = 1$. The activity coefficient for an exchange species is known to strongly depend on the

exchanger composition and the ionic strength [24, 25]. In the present study, the exchange species corresponds to S-Cl and the ionic strength is fixed to 10^{-1} mol L^{-1}. We then fix the activity coefficient constants equal to 1.

2.5. Model development

The system represented by the injection loop, tubing and columns is decomposed into a number of cells. Each cell is characterized by a given volume of water (whose composition is defined) and a given height. This simplistic representation of the system, the characteristics of which are given by DIONEX, is shown in Fig. 1. The model describes the void volume of a non-sorbing species, which corresponds to an UV peak at 2.4 min for a flux of 350 μL min^{-1}. The difference in volume (350 μL) between the two detectors was experimentally determined using both stable (^{127}I) and radioactive (^{131}I) iodide.

Fig. 1.



According to the ion-exchange reaction, sites (in mol) are added into the cells associated to the exchanger. For each cell, the initial conditions and the set of reactants can be defined individually, which provides flexibility to simulate a variety of chemical conditions throughout the column. It is then possible to differentiate between the pre-column, the AG20

guard column being packed with a resin of proportionally lower capacity, and the column AS20. Nevertheless, in the model, it was assumed that the selectivity coefficients are identical in both columns. Also, it was calculated that the guard column placed on-line prior to the analytical column increases retention time of about 4%. This value is in good agreement with supplier's information.

The infilling solution for the column is always solution number 0. Advection is modeled by "shifting" the solution 0 to cell 1, the solution in cell 1 to cell 2, and so on. At each shift, equilibrium is maintained in each cell.

The model was first tested with trace amount of iodide (10^{-4} mol L^{-1}) in 3.5x10-^{1} mol L^{-1} NaOH medium. The experimental result has led to a retention factor k_I of 2.3. In such simple conditions (analyte occupies <1% of the column capacity, exchange between two monovalent anions), the exchange coefficient can be simply calculated according to:

$$K_{OH/I} = \frac{Q}{V_m \{OH^-\}} \frac{1}{k_I} \qquad (5)$$

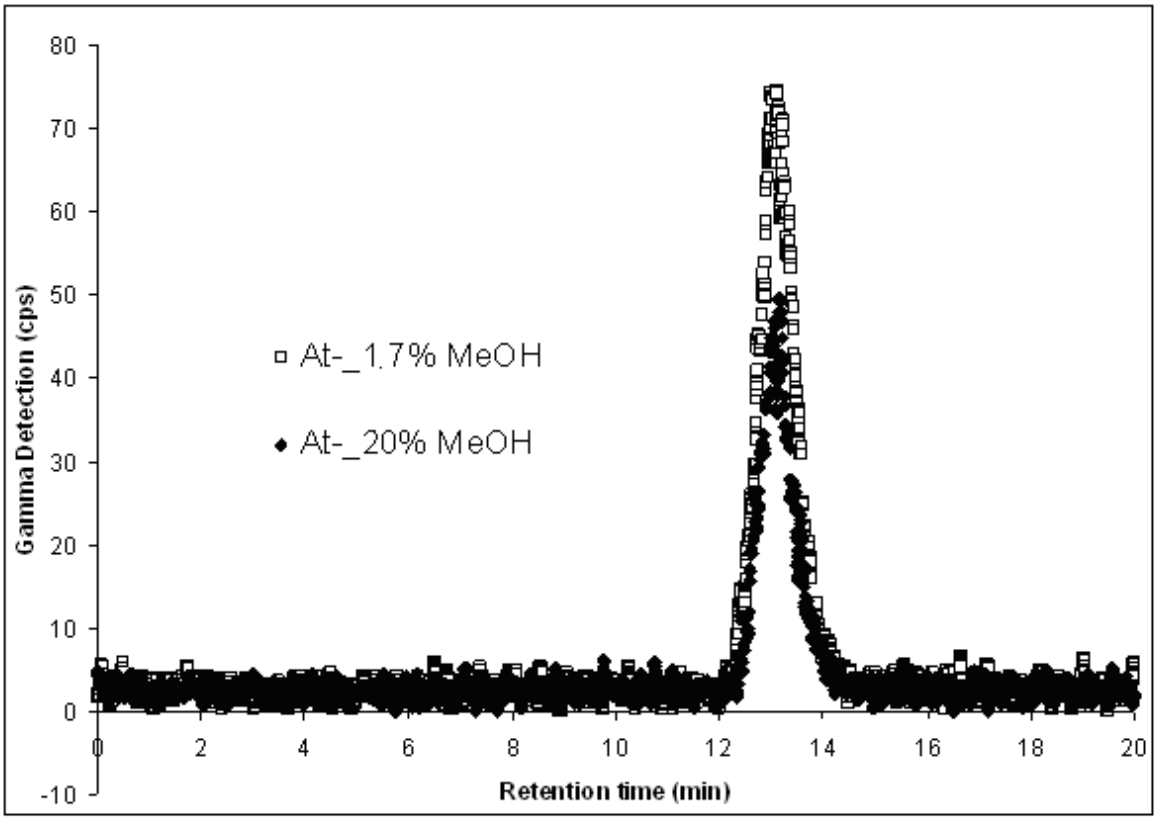
where Q is the capacity of the column in meq, and V_m is the dead volume of the column in mL. A value of 1.19 can be calculated (with $K_{OH/OH} = 1$) and agrees with the one derived from PHREEQC. The input file used for the calculation is given in the supplementary information.

3. RESULTS AND DISCUSSION

3.1. Characterization of At^-

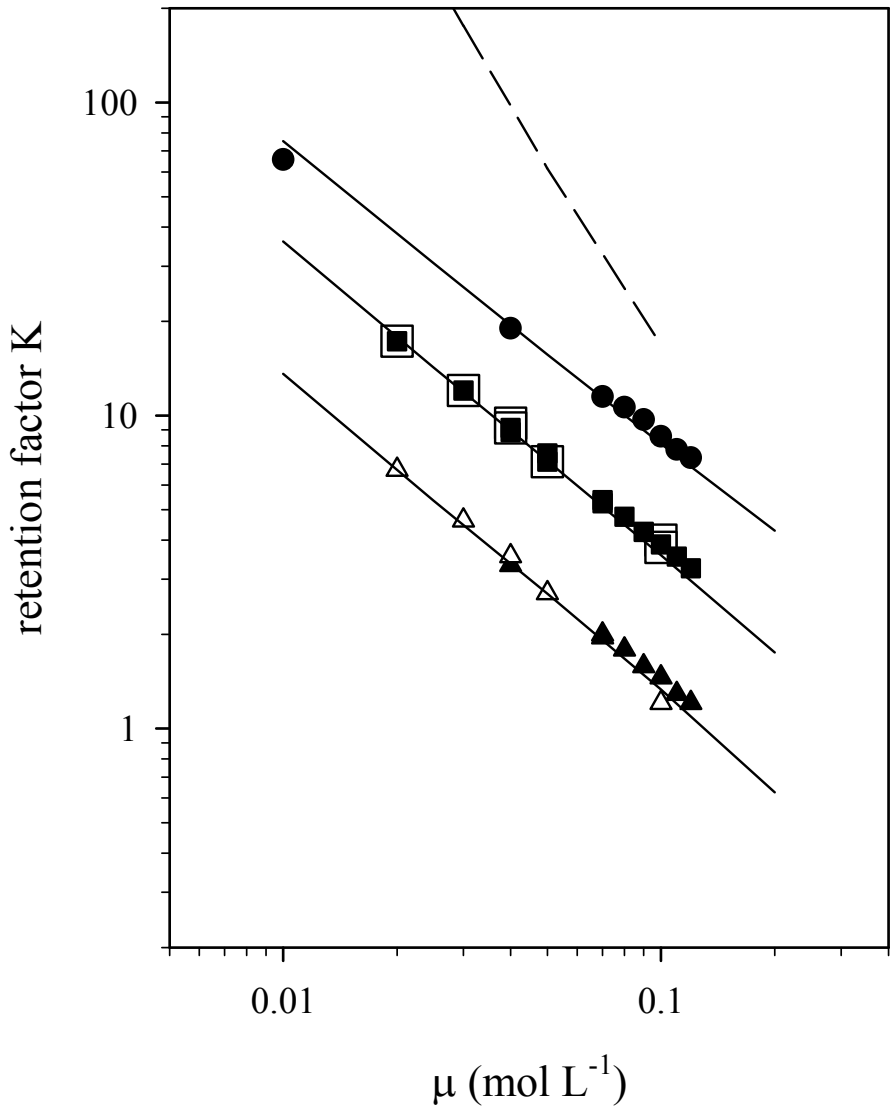
A typical gamma chromatogram is presented in Fig. 2. It shows a significant retention of astatine species on the anion exchanger with the retention time of 13 min and a recovery yield between 70 and 100%. It has been also settled that the retention of the astatine species is not affected by the presence of methanol coming from astatine stock solutions. In a typical experiment, the methanol content amounts to 2–5%. When varying the content of methanol from 1 even to 20% (in volume), no significant peak shift has been observed. This retention did not prove that an anionic species exists and the exchange reaction has occurred. In a recent work [26], some of us have shown that the cationic species of astatine existing under acidic oxidizing conditions can be adsorbed on both anionic and cationic exchangers. This peculiar behavior is related to the ultra-traces concentrations of astatine used in the experiments.

Fig. 2.



295 The quantitative analysis of further experimental data based on equation (3) has been
296 done. The competition effects for exchange sites were considered between chloride and
297 medium anions including astatine species. The competition with the hydroxide and the redox
298 couple $SO_3^{2-}/S_2O_3^{2-}$ anions was neglected. In the first case, the concentration of OH^- is too
299 weak for expecting a competition (pH ranging between 2.0 and 7.5). In the second case, this
300 was checked experimentally: as shown in Fig. 3, the retention times of I^- and Br^- are similar in
301 the presence or absence of the redox couple anions.

302 Fig. 3.



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The determination of selectivity (exchange) coefficients requires modeling of the equilibrium at all stages of the column. In our dynamic system, the principle of "local equilibrium" was assumed, i.e. the rate of reactions was much more rapid than the rate of solute transport. It was checked for astatine considering the ultra traces concentrations of solute injected (about 3.8×10^{8} atoms corresponding to a concentration at the outlet of the column of 10^{-13} mol L^{-1}). The conditions were considered as ideal when the flow rate was below 500 µL min^{-1}, no change of the astatine retention factor was observed with the flow rate (data not shown).

The influence of the ionic strength on the retention factor of Br^-, I^- and the astatine species is shown on Fig. 3. The log-log representation of the retention factor as a function of the ionic strength is linear. These experimental results are in agreement with the exchange process principle. Moreover, the modeling fairly reproduces the experimental data if we attribute one negative charge on astatine species. For illustration, the dashed line in Fig. 3 depicts the behaviour of a species with two negative charges. Hence, the experimental data confirm the existence of an anionic species with one negative charge.

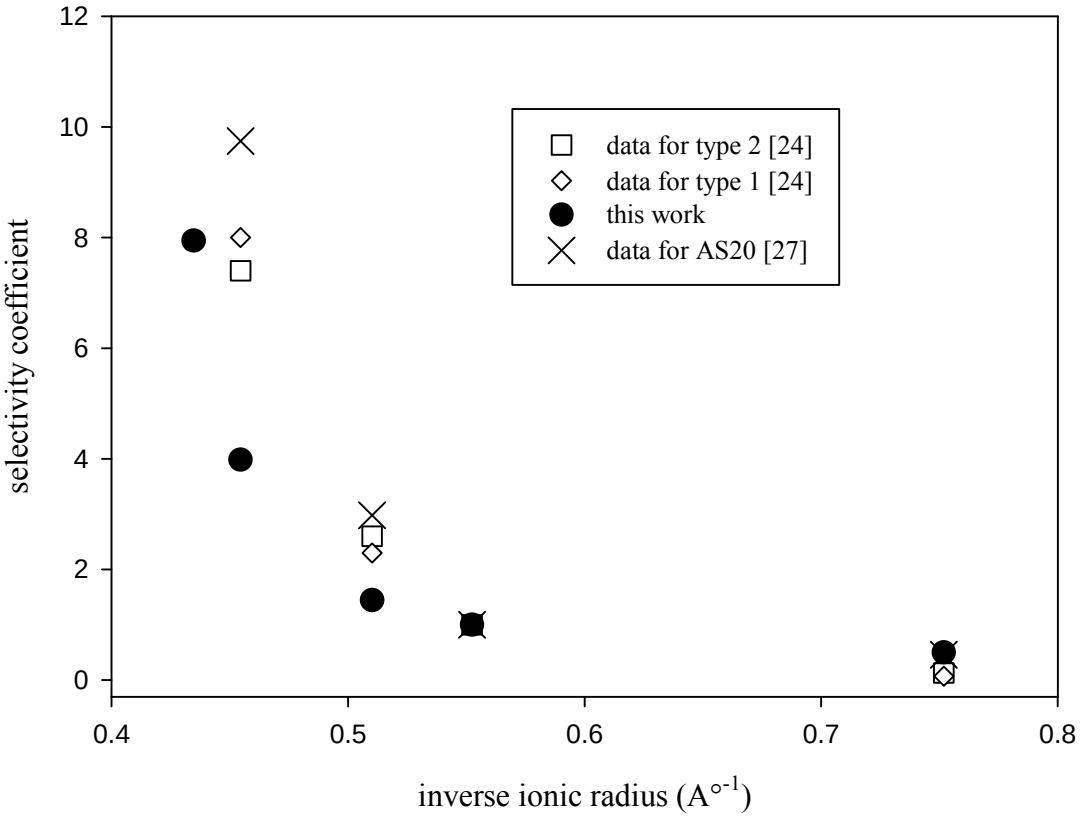
The tendency for the relative selectivity coefficient of this species with respect to the others halides was next questioned. Berei [5], using the data of Roessler [14], showed a linear correlation within the halides series by plotting the retention volume as a function of the inverse ionic radius and stated it as an indirect proof of the existence of At^-. The same trend should be found when plotting the relative selectivity coefficients as a function of the inverse ionic radius.

The reported coefficients (Fig. 4) were compared with $K_{X/Cl}$ recalculated from published retention volumes obtained with polystyrene-divinylbenzene anion exchange resins with similar reactive sites [24] and those from retention factors specific for AS20 given by the

Virtual Column Separation Simulator 2 [27]. Note that values are given with respect to OH^- and the relative selectivity coefficients $K_{X/Cl}$ were recalculated according to:

$$K_{X/Cl} = \frac{K_{X/OH}}{K_{Cl/OH}} \quad (5)$$

Fig. 4.



On the one hand, as shown in Fig. 4, the $K_{X/Cl}$ values determined in the present work in chloride medium are not identical with the $K_{X/Cl}$ derived from hydroxide medium for AS20 and polystyrene-divinylbenzene anion exchangers. A correction of the activity coefficients for the exchange species would be necessary, since the medium strongly influences the values of selectivity coefficients [25]. Also, the differences may be explained by means of hydrophilic character of resins: AS20 is highly hydrophilic while polystyrene-divinylbenzene resin is less hydrophilic [28].

On the other hand, all plots of the relative selectivity coefficients as a function of the inverse ionic radius show an exponential-type curve. The empirical law of Berei [5] appears therefore to be not valid and could not demonstrate the existence of At^-. One can only conclude that (i) there is a coherence in the trend, i.e. $K_{At/Cl} > K_{I/Cl} > K_{Br/Cl} > K_{F/Cl}$, (ii) the species holds one negative charge, and (iii) the existence of an oxyanion is unlikely as it would not explain the data published in our previous work [21]. All results and the behaviour of astatine discussed in the introduction, provide an indirect proof of the existence of At^-.

3.2. Astatine speciation in blood serum

The behaviour of astatine in serum has been then investigated. The usual techniques of speciation of trace elements in biological environment were reviewed by Lobinski [29]. In the present case, the speciation of astatine in serum has been performed using HPAE–γ system with the aims to simulate the equilibrium of astatine in blood serum and to monitor astatine species formed under physiological serum conditions.

In order to minimize external perturbation on the equilibrium in serum, the physiological-type mobile phase containing 10^{-1} mol L^{-1} of NaCl, 10^{-3} mol L^{-1} of PBS buffer and $10^{-4}/10^{-3}$ mol L^{-1} of $SO_3^{2-}/S_2O_3^{2-}$ redox buffer has been prepared. The effect of the major serum components (transferrin, albumin, monovalent carbonate anion HCO_3^- and citrate) on At^- elution was studied in model media before working with the biological medium. The investigated constituent is injected into the column and chloride competing agent is in mobile phase. The retention of a solute on the column is clearly related to the exchange reaction as described in equation (3) when X^- = albumin, transferrin, PBS. For mixture containing

365 astatine, the simple comparison of the retention with the γ–chromatograph from previous part
366 of this study without any exchange evaluation has been done.
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369 *3.2.1. UV-characterization of major components in serum*
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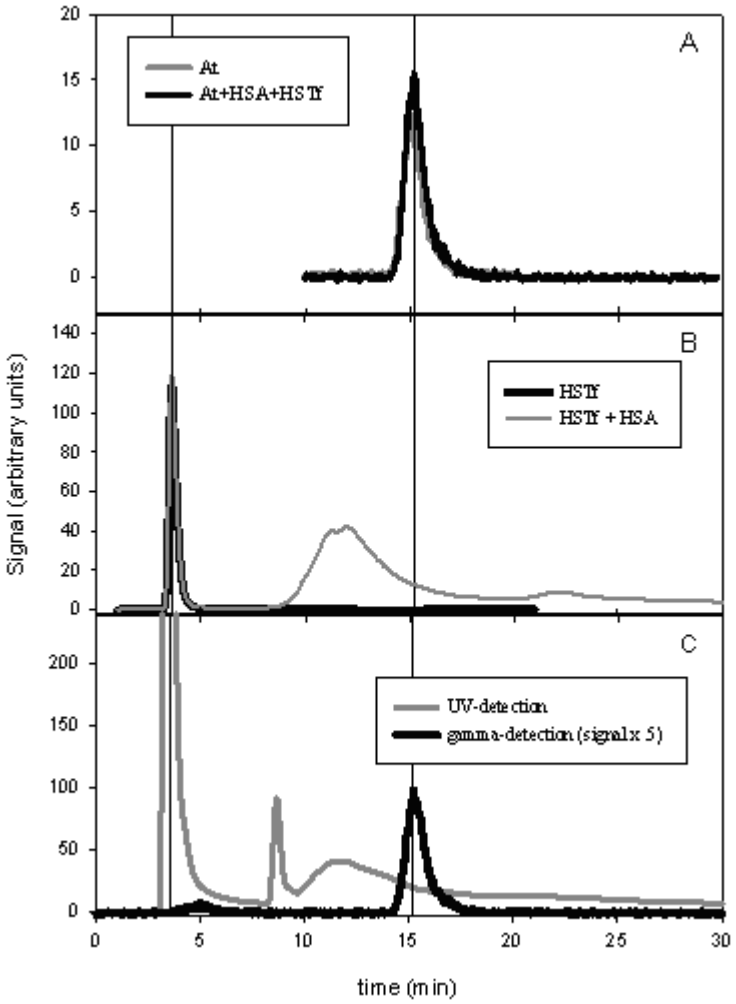
372 The UV–chromatogram of the blood serum is given in Fig. 5. Three peaks at 2.5, 7.7 and
373 10.5 min could be identified. The first peak eluted almost in the void volume (2.3 min) was
374 identified as the peak of transferrin (Fig. 5B). The protein, dissolved in the physiological-type
375 of eluent, was not retained on AS20 resin. Thus it is not expected to compete with astatine
376 species for sorption sites. The broad peak at 10.3–11.0 min coincides with the elution of
377 albumin which was dissolved in the synthetic physiological-type medium in the presence of
378 transferrin (grey line in Fig. 5). The large full width at half maximum of the peak is explained
379 by the presence of a mixture of the monomer and dimer of albumin. Whereas a better
380 separation of monomer and dimer was obtained at a flow rate of 500 μL min^{-1}, the resultant
381 pressure exceeded the limit of the column. In synthetic media, 75-80% of the proteins were
382 found at the exit of the column, while the restitution was lower (50-60 %) in the case of the
383 serum. Obviously, the AS20 resin allows the resolution of both important metallo-proteins in
384 "non-perturbing" conditions. Transferrin elutes nearly in the void volume while albumin is
385 significantly retained on stationary support. The albumin retention has been quantitatively
386 explained by an apparent selectivity coefficient $K_{Albumin/Cl} = 0.15$ for exchange of Cl^- and one
387 negative charge of albumin.
388 Fig. 5.



389

390 The experiments with the two main anionic low molecular weight components
391 (bicarbonate HCO_3^- and citrates $C_3H_5O(COO)_3^{3-}$) did not allow us to identify the peak eluted
392 at 7.7 min. Considering the complexity of the serum, this may be associated to the presence of
393 another low molecular weight molecule with high absorption extinction coefficient but
394 present in low amount. Therefore, it was not considered as a probable interfering species in a
395 first assumption. No further experiments were done for identifying it.

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398 *3.2.2. Characterization of astatine in serum*

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401 The At^- peak in the physiological-type medium was initially expected from modeling at
402 the retention time of 15.3 min with a retention factor of 8.6, considering the previously
403 determined selective coefficients and experimental conditions (electrolyte composition, μ, pH
404 and potential). The peak has appeared on the gamma chromatogram (Fig. 5) at the retention
405 time of 15.3 min with a retention factor of 5.4 and a restored yield of 100%.
406 The damage of the resin over time leading to a decrease in the number of sites was
407 therefore considered. The regular column tests done with iodide (see 2. Experimental section)
408 have given a retention factor of 3.3, instead of 3.7 for a new column. This shift quantitatively
409 explains 10% of decrease in retention sites number. This may result from a damage of the
410 resin through the irradiation of alpha particles which are highly energetic. The model
411 parameterization was changed accordingly for the further data evaluation.
412 However, the decrease of 10% sorption capacity of the resin can only partially explain the
413 astatine shift because the retention factor would lower from 8.6 to 7.8. Complementary
414 experiments with I^- under physiological type-conditions (in presence of PBS) were done. A
415 decrease in retention factor is observed as compared to the one determined with 0.1 mol L^{-1}
416 NaCl as eluent (2.6 instead of 3.3). This peak shift has evidenced that the PBS buffer compete
417 with X^- for sorption sites. In agreement with this assumption, the experimentally determined
418 retention factors of I^- (3.3) and At^- (5.4) can truly be predicted using the previously
419 determined coefficients $K_{At/Cl}$ and $K_{I/Cl}$ and including $logK_{PBS/Cl} = 1.7 \pm 0.1$.
420 Transferrin and especially albumin represent the main potential competing agents in the
421 blood medium. However, as Fig. 5A shows, no competition has occurred with proteins at the
422 concentration encountered in the blood serum: in the presence and absence of proteins, the
423 retention volumes of At^- are identical. This was also predicted using previously determined
424 selectivity coefficients $K_{Albumin/Cl}$, $K_{At/Cl}$ and $K_{PBS/Cl}$. A similar result was obtained when
425 carbonates and citrate ions were added to astatine solutions (data not shown). These results

show that Cl^- and phosphates present in the eluent exclusively govern the competition and that no interaction between At^- and major serum components occurs.

Astatine in presence of serum has given the peak at the same retention time (15.3 min) than the one observed in the physiological-type medium (Fig. 5A and Fig. 5C), and was restored more than 75%. This result can be seen as a genuine proof of the existence of At^- in the blood serum. The species was however restored at a yield slightly lower than those found with the physiological-type media. The presence of an additional cationic or neutral astatine species in weak amount strongly retained on the tubing of the HPAEC device cannot be excluded.

4. CONCLUSION

The usefulness of HPAEC–γ to get information regarding astatine speciation in simple and synthetic media, provided that a careful quantitative analysis is done, has been established. We report the first analytical result characterizing At^-: the species is anionic, holds one charge and its behavior is coherent in the halide series. A selectivity coefficient $K_{At/Cl}$ is given for the studied column. The methodology was successfully applied to biological medium. The resin used appears useful to separate the two important metallo proteins. Similar results with astatine were obtained in physiological-type and blood serum media; this indicates no interaction between astatine and serum components. In reducing conditions, astatine mainly exists as At^- in the blood serum. This is in agreement with in-vivo data found in the literature, i.e. astatide is attracted to the thyroid as I^- is [30]. The methodology is an alternative to the thin layer chromatography generally used for the in vitro

stability assessment of ^{211}At-labelled molecules. Based on our results, the appearance of a peak corresponding to a retention factor of 5.3 would indicate the presence of astatide released from radio-labelled molecule, i.e. the binding between ^{211}At and the carrier molecule in the sample incubated in the blood serum is not enough strong to compete with the formation of the thermodynamically stable astatide species in blood serum.

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FIGURE CAPTIONS

Fig. 1. Parameters used for the simulation (1D dimension).

Fig. 2. Effect of the methanol content in the injected sample (in weigh percent) on the elution profile of astatine at 350 μL min^{-1}; eluent at pH 2 contains 0.1 mol L^{-1} of NaCl, 10^{-2} mol L^{-1} of HCl, and $10^{-4}/10^{-3}$ mol L^{-1} of $SO_3^{2-}/S_2O_3^{2-}$.

Fig. 3. HPAEC results for At^- (circles), I^- (squares) and, Br^- (triangles); the flow rate was fixed between 350 and 450 μL min^{-1}. Filled symbols: 0.1 mol L^{-1} NaCl; open symbols: 10^{-2} mol L^{-1} of HCl, $10^{-4}/10^{-3}$ mol L^{-1} of $SO_3^{2-}/S_2O_3^{2-}$ and 10^{-1} mol L^{-1} of NaCl. The lines are calculated with $logK_{X/Cl}$ values of 0.16, 0.58 and 0.90 for Br^-, I^- and At^-, respectively. The dashed line represents a prediction considering that astatine species holds two negative charge.

Fig. 4. Variation of relative exchange selectivity coefficients for quaternary ammonium ions exchange resins as a function of the inverse ionic radii for the halides series. The filled and open symbols depict the data measured in this work and the published one [27] found for a similar type of resin, respectively. The selectivity coefficient for F^- was deduced from an experiment giving an elution time of 8 min (eluent: $5x10^{-2}$ mol L^{-1} NaCl; flow rate: 200 μL min^{-1}).

Fig. 5. Speciation of astatine in human serum. (A) γ–chromatogram of astatine in the presence or absence of transferrin and albumin proteins (signal multiplied by a factor of 5 to

REFERENCES

1. D. R. Fisher, Curr. Radiopharm. 1 (2008) 127–134.
2. D. S. Wilbur, Curr. Radiopharm. 3 (2008) 144–176.
3. G. Vaidyanathan, M. R. Zalutsky, Curr. Radiopharm. 1 (2008) 177–196.
4. S. Lindegren, S. Frost, T. Back, E. Haglund, J. Elgqvist and H. Jensen, J. Nucl. Med. 49 (2008) 1537–1545.
5. K. Berei, L. Vasaros, Astatine Compounds, 8th ed., Springer-Verlag, Berlin, 1985.
6. K. Berei, L. Vasaros, in: S. Patai and Z. Rappoport (Eds.), The chemistry of halides, pseudo-halides and azides, John Wiley & Sons Ltd., New York, 1995, pp. 787–819.
7. D. S. Wilbur, M.-K. Chyan, D. K. Hamlin and M. A. Perry, Bioconjugate Chem. 20 (2009) 591–602.
8. T.C. Richardson, Int. J. Appl. Rad. Instrum. B 13 (1986) 583–584.
9. G. L. Johnson, R. F. Leininger and E. Segre, J. Chem. Phys. 17 (1949) 1–10.
10. V. D. Nefedov, Y. V. Norseev, M. A. Toropova and V. A. Khalkin, Russ. Chem. Rev. 2 (1968) 87–98.
11. E. H. Appelman, E. N. Sloth and M. H. Studier, Inorg. Chem. 5 (1966) 766–769.
12. A. Cavallero and K. Rossler, Radiochim. Acta 47 (1989) 113–117.
13. Meyer, Chromatographie trägerfreier anorganisher Formen von Jod-123 und Astat-211 und ihre Verwendung zur Halogenierung von Uracil and Desoxyuridin, Berichte Nr.1076, Kernforschungsanlage Jülich, Jülich, 1974.
14. K. Roessler, W. Tornau and G. Stoecklin, J. Radioanal. Chem. 21 (1974) 199–209.
15. R. Dreyer, I. Dreyer, F. Rosch and G. J. Beyer, Radiochem. Radioanal. Lett. 54 (1982) 165–175.
16. D. L. Parkhurst, C. A. J. Appelo, User's guide to phreeqc—a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations, USGS Report No. 99–4259,1999.
17. E. Ansoborlo, O. Prat, P. Moisy, C. Den Auwer, P. Guilbaud, M. VCarriere, B. Gouget, J. Duffield, D. Doizi, T. Vercouter, C. Moulin, V. Moulin, Biochimie 88 (2006) 1605–1618.
18. C. Alliot, M. Chérel, J. Barbet, T. Sauvage, G. Montavon, Radiochim. Acta 97 (2009) 161–165.
19. S. Lindegren, T. Back, H. J. Jensen, Appl. Radiat. Isot. 55 (2001) 157–160.
20. H. Sun, K. Y. Szeto, J. Inorg. Biochem. 94 (2003) 114–120.
21. J. Champion, C. Alliot, E. Renault, B. M. Mokili, M. Chérel, N. Galland and G. Montavon, J. Phys. Chem. A 114 (2010) 576–582.
22. C. W. Davies, Ion association, Butterworths, Washington D.C., 1962.
23. G. L. Gaines, H. C. Thomas, J. Chem. Phys. 21 (1953) 714–718.
24. F. de Dardel, Techniques de l'ingénieur, J2 783-782-716 (1998) 1–16.
25. B. Trémillon, Les séparations par les résines échangeuses d'ions, Gauthier-Villars, Paris, 1965.
26. J. Champion, C. Alliot, S. Huclier, D. Deniaud, Z. Asfari, G. Montavon, Inorg. Chim. Acta 362 (2009) 2654–2661.
27. J. E. Madden, M. J. Shaw, G. W. Dicinoski, N. Avdalovic, P. R. Haddad, Anal. Chem. 74 (2002) 6023–6230.
28. C. Liang, C. A. Lucy, J. Chromatogr. A 1217 (2010) 8154–8160.
29. R. Lobinski, C. Moulin, R. Ortega, Biochimie 88 (2006) 1591–1604.
30. D. S. Wilbur, Bioconjugate Chem. 19 (2008) 158–170.

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