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RAPID SEPARATION OF CARRIER-FREE INORGANIC AND ORGANIC COMPOUNDS OF RADIOIODINE AND ASTATINE BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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The radioisotopes ^{123}I (T = 13.3 h) and, potentially, ^{211}At (T = 7.2 h) find increasing interest for radiopharmaceutical applications in diagnosis and therapy. They were produced via the $^{122}Te(\alpha, 3n)^{123}Xe(\beta^+)^{123}I$ and the $^{209}Bi(\alpha, 2n)^{211}At$ processes. Fast and efficient separations of carrier-free species obtained from target processing, as well as from classical or decay-induced synthesis were archieved by means of high-pressure ion-exchange and partition chromatography. Inorganic forms (X^-, XO_3^-, At^+) could be identified and separated on pretreated Aminex A 27 and A 7 resins, and biomolecules such as 5-halodeoxyuridines and -uracils on Aminex A 25 resins and Merckosorb Si 60 silica. The chromatographic methods can also be used for stability tests of radiopharmaceuticals in biochemical mixtures, notably physiological fluids.

Introduction

Among the radioisotopes of iodine, ^{123}I (T = 13,3 h) has particularly favourable nuclear properties for radiopharmaceutical applications.[1-3] Due to its short half-life and apt γ-energy (159 keV) the relative dose absorbed by a patient, e.g. during the radioiodine test of the thyroid gland, is less by a factor of $\sim$100 than that of ^{131}I. The heavier homologue of iodine, the radioelement astatine, and in particular the relatively short lived α-emitter ^{211}At (T = 7.2 h), has potential importance for special problems in radiation therapy and biology.[4-8] ^{211}At lends itself as an effective internal radiation source since each decay leads to the emission of an α-particle which dissipates a mean energy of 6.8 MeV to a limited volume of tissue with a radius of 60 μ. Due to the high LET, the radiobiological efficiency of ^{211}At is superior to that of all iodine isotopes.[9]

For medical use the radiohalides must be administered in a chemical form suitable for selective incorporation. Similar to classical applications of radioiodide,

inorganic forms of At might also be of potential use for therapy, as well as for further synthesis of suitable biomolecules. Radioiodine-labelled 5-iododeoxyuridine (I UdR) is of great importance, e. g. for the determination of the death rate of cells after external irradiation, since it is incorporated into the DNA of proliferating cells like thymidine, a natural DNA precursor. The homologuous At UdR may therefore provide a selective introduction of ^{211}At into the DNA of tumor cells for an effective α-therapy.[8,10]

The carrier-free ^{123}I and ^{211}At species can be identified by a systematic study of their retention behaviour on chromatographic columns. In this context the method of high-speed liquid chromatography exhibits considerable advantages over classical column techniques. Its better resolution is important for effective purification and separation, as well as for the concentration of the desired product in a small volume of the eluate. Furthermore, the separation times for iodine and astatine compounds can be reduced to some minutes, which is particularly favourable in the case of the relatively short-lived radionuclides under consideration.

Experimental

Production of radioisotopes and preparation of compounds

^{123}I was produced by cyclotron irradiation of a thick, 95%-enriched ^{122}Te target with 42 MeV α-particles, via the ^{122}Te(α, 3n)^{123}Xe(β^+)^{123}I process.[2,11] The ^{123}Xe was swept off by a helium flow of 60 ml/min and adsorbed on charcoal at low temperatures for further applications. Yields of 50–200 μCi/μA · h with radionuclidic purity $\geqslant$ 99. 8% can be obtained.[22] Synthesis with ^{123}I can be carried out either via the intermediate formation of ICl on a classical basis,[11,12] or by a simple ^{123}Xe exposure technique via decay-induced reactions of the ^{123}I species. Yields of the exposure direct-labelling technique for the halogen substitution in α-iodofatty acids, iodityrosine, iodoinsuline and 5-halodeoxyuridines range from some few percent up to 60% depending on the substrate and its physical state.[13]

^{211}At was produced by cyclotron irradiation of thin Bi targets ($\sim$100 mg/cm^2) by 29 MeV α-particles, via the ^{209}Bi(α, 2n)^{211}At process, with yields of 0. 3–1 mCi/μA · h. The purity of ^{211}At amounted to more than 99% with respect to ^{210}At, which decays to the radiotoxic ^{210}Po. $CHCl_3$ solutions of At^o were prepared from the target by conventional dissolution and extraction techniques. Inorganic species such as At^-, At^+, AtO_3^-[6,14] could be formed with almost 100% yield via distillation of the $CHCl_3$ through a thin layer of water containing reducing (SO_3^{2-}) or oxidizing ($HNO_3/Cr_2O_7^{2-}$, HClO, $S_2O_8^{2-}$) agents, as shown in Fig. 1. This method allowed the transfer of At to a very small volume with specific activities of up to 5 mCi/ml. Labelling of biomolecules such as deoxyuridine (UdR) and uracil (U) could be achieved by electrophilic reaction of AtCl, however, only with yields of a few percent.

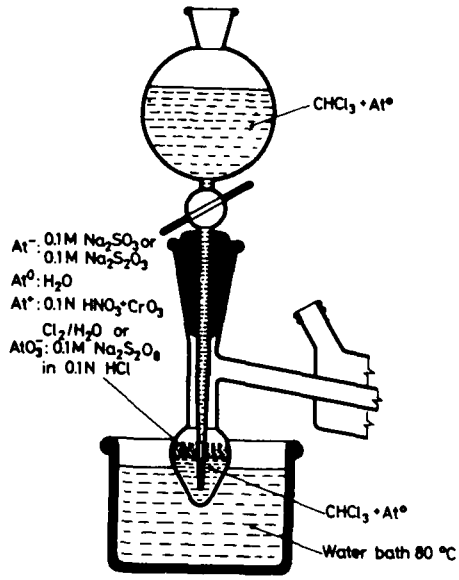

Fig. 1. Arrangement for quantitative extraction of astatine into water solutions by distillation of $CHCl_3$

High-pressure liquid radiochromatography

The conventional apparatus used for high-pressure liquid chromatography with the modifications for combined radioactivity and adsorbance measurement is schematically shown in Fig. 2. In Table 1 the conditions for five different chromatographic columns used in this work are reported. The separations were carried out via ion-exchange on strongly basic Aminex A 27 and A 25 and on strongly acidic Aminex A 7 resins from Bio-Rad Laboratories, Richmond, (columns A to D) and via partition chromatography on Merckosorb Si 60 from Merck, Darmstadt, (column E). In order to avoid irreversible adsorption and redox processes of the weightless amounts – in particular in the case of astatine – some of the exchange resins were pretreated with Cl_2-water; heating of eluent and column to a temperature between 23 and 80 °C generally increased the yield; the solvent must be passed through membrane filters and boiled prior to use; for stabilization of inorganic forms addition of reducing or oxidizing agents to the eluent was necessary; the losses of At-compounds due to adsorption during the chromatographic analysis may be reduced by addition of the corresponding I-compounds to the solvent; in the analysis of physiological fluids such as blood the admixture of 5% n-butanol to the solvent inhibits the precipitation of serum albumins.

Table 1
Columns and conditions used for high-pressure liquid radiochromatography

Column packing	A Aminex A27 anion-exchanger	B Aminex A27 anion-exchanger, Cl_2-pretreated	C Aminex A7 cation-exchanger, Cl_2-pretreated	D Aminex A25 anion-exchanger	E Merckosorb Si 60
Grain size, μ	12 to 15	12 to 15	7 to 11	~18	~20
Column length, mm	110	110	110	400	1,000
Int. diameter, mm	1.8	1.8	1.8	1.8	2.0
Void volume, ml	0.25	0.25	0.25	0.65	1.25
Pressure, atm	20 to 50	20 to 50	20 to 50	20 to 50	20 to 70
Flow rate, ml/min	0.2 to 1.0	0.4 to 1.0	0.2 to 0.8	0.2 to 0.5	0.12 to 0.3
Typ. sample vol., μl	5 to 10	5 to 10	10	5 to 10	2 to 5
Counter ion	NO_3^-	NO_3^-	H^+	$HCOO^-$	-
Eluent	$NaNO_3$-solution	$NaNO_3$-solution	0.1N HNO_3 + $5 \cdot 10^{-3}$M $Cr_2O_7^{2-}$	2M HCOOH	n-butanol with H_2O
Temperature, °C	23	35 to 80	80	23 to 70	23

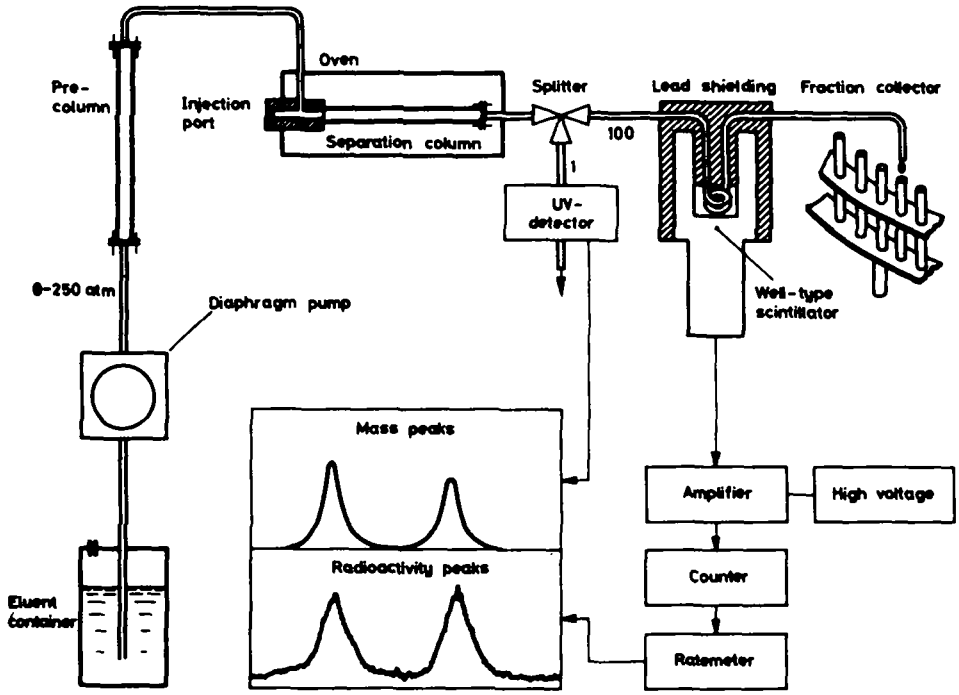

Fig. 2. Schematic apparatus for high-pressure liquid radiochromatography

Results and discussion

Halides, halates and astatine cation

Carrier-free $^{123}I^-$ and $^{211}At^-$ ions are eluted quantitatively from Aminex A 27 anion-exchange resins (column B) when the above-mentioned precautions are applied. As seen in Fig. 3, the radioactivity peaks of the halides show the typical sequence with increasing intervals as already observed for the mass peaks of macroscopic amounts.[16] In this series the new value for At^- indicates more clearly that the retention is a non-linear function of the ionic radii or volumes even if the evaluated ionic radius for At^- of 2.3 Å (cf. Ref.[6]) might be too low. It is rather the increasing polarisability in the sequence from Cl to At which results in stronger homopolar bonding and, thus, in increased affinity to the exchange resin.[17]

Although the peaks in Fig. 3 exhibit some tailing, more than 75% of the $^{123}I^-$ and $^{211}At^-$ can be recovered in 1 and 2 ml of the eluent, respectively. It can be seen from Fig. 4 that the retention volumes of halides and halates show a similar and almost linear dependence on the $NaNO_3$ concentration of the solvent. Fig. 4

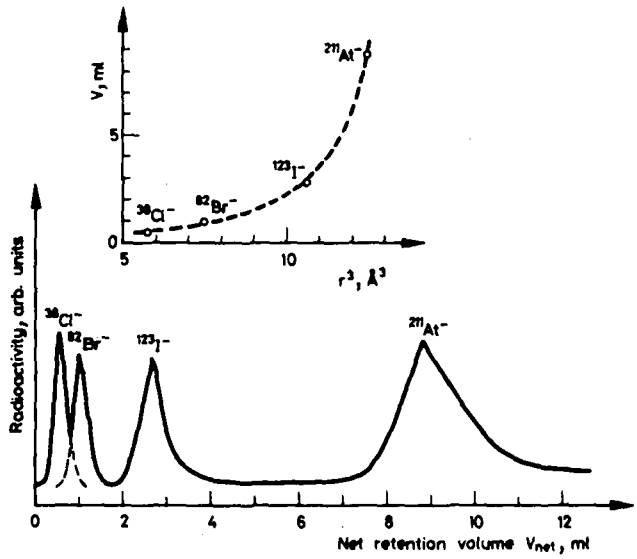

Fig. 3. Separation of carrier-free radiohalides on Aminex A 27 (column B) at 80 °C, solvent: 1N $NaNO_3$ with 0.1N Na_2SO_3

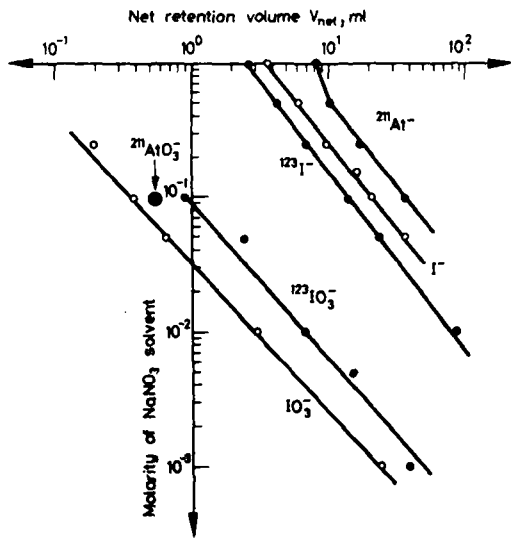

Fig. 4. Retention of radiohalides and -halates on Aminex A 27 (columns A and B) at various $NaNO_3$ solvent concentrations: ● carrier-free on column B, 80 °C, ○ 1 μmole samples on column A, 23 °C

Table 2
Retentions of monovalent cations on Aminex A7 cation-exchange resin
(column C at 80 °C, 1 to 10 μmole samples)

	Na^+	K^+	Rb^+	Cs^+	Tl^+	At^+
Ionic radius, Å (crystal)	0.98	1.33	1.47	1.67	1.47	?
Radioisotope measured	^{24}Na	^{42}K	^{86}Rb	^{134m}Cs	^{204}Tl	^{211}At
V_{net}, ml	4.0	6.3	6.8	10.3	11.8	11.0

thus allows the extrapolation of the retention for given separation conditions, or the selection of the conditions for a desired retention time. It also demonstrates that an effective concentration of carrier-free radiohalogens out of great volumes of solution can be achieved via multiple injections when using a weak solvent first, followed by a final elution with a strong solvent.

For reasons discussed in Ref.[16] the halates are eluted much faster than the halides, and show an opposite retention sequence. Furthermore, in contrast to the halides, the carrier-free halates are retained more strongly on the pretreated and heated column B than the macroscopic amounts on column A. While $^{123}IO_3^-$ can be recovered quantitatively, the yield of AtO_3^- ranges only from 1 to 10% due to redox and adsorption processes, even if high concentrations of $S_2O_8^{2-}$ are present in the solvent. In this context it should be mentioned that the existence of a stable five-valent of At is yet not generally accepted (cf. Ref.[14]). A detailed study of the retention sequences of higher oxidized astatine forms may add a new positive argument to the astatate problem.

Another interesting problem in astatine chemistry is the existence of the At^+ cation. The strongest arguments for the At^+ state are drawn from experiments on electromigration[18] and chromatographic behaviour on cation-exchange resins of astatine species oxidized by dichromate ions.[19,20] Thus, it seemed promising to use high-pressure liquid radiochromatography on the strongly acidic Aminex A 7 cation-exchange resin (column C) to obtain further evidence on At^+ in HNO_3-dichromate solutions. Indeed, a new ^{211}At peak with a retention volume of about 11 ml and a yield of < 70% could be observed. For comparison, the retentions of some monovalent cations (1–10 μmole samples) have also been determined by radioactivity measurement. It can be seen from Table 2 that, as expected, the retention volumes increase with the radii and/or the mass of the ions, the assumed At^+ peak fitting this sequence. Since none of the other At forms, e.g. At^- or AtO_3^-, have comparable retention volumes, there is strong evidence that an astatine cation is present. The fact that At^+ is eluted before Tl^+ is in good agreement

with an ionic radius of less than 1.47 Å, which can be derived from its position in the periodic system. Thus, the existence of an AtO^+ species containing trivalent At (cf. Ref.[14]) does not seem probable.

5-Halodeoxyuridines and -uracils

5-Halodeoxyuridines (XUdR) and -uracils (XU) belong to the family of pyrimidine bases, which in general can be analyzed either by cation- or by anion-exchange (for a review cf. Ref.[21]). Separation can thus be achieved by anion-exchange on strongly basic resins such as Aminex A 25 (column C). The mass peaks of 1 μmole samples of XUdR and XU are represented in the upper and lower parts of Fig. 5, respectively. It can be seen from Fig. 5 that unlike the halides and halates the carrier-free ^{123}IUdR shows the same retention behaviour as macroscopic amounts of IUdR. The preparation of AtUdR generally results in two At fractions at V_{net} = 7 and 12 ml, respectively. A comparison with the retention volumes of the mass peaks suggests the identity of the first fraction with AtUdR and of the second with AtU. Obviously, the uracil had been formed during synthesis of AtUdR by splitting of the sugar moiety of the biomolecule. Besides, it has been proved that both At fractions cannot be ascribed to inorganic species such as At^- or AtO_3^-. The identity of the product can also be tested by the essentially undesired splitting of the sugar component: on heating the AtUdR in hydrochloric or nitric acid solutions at temperatures exceeding 80 °C a decrease of the deoxyuridine and a concomitant increase of the uracil fraction can be observed.

Further evidence on the identity and radiochemical purity of the organic species was obtained by using partition chromatography on column E instead of the ion-exchange column. Here the inverse retention behaviour is observed. The At compounds in both cases fit the sequence (cf. Table 3). The increasing retention

Table 3
Retention of 5-halodeoxyuridines and -uracils by anion-exchange on Aminex A25 (column D) and partition chromatography on Merckosorb Si 60 (column E) at 23 °C

Substance (1 μ mole)	Column	Net retention volume V_{net}, ml					
		X = H	F	Cl	Br	I	(At)
XUdR	D	0.6	1.1	1.8	2.4	4.0	7.3
	E	4.1	3.0	1.7	1.4	1.0	0.4
XU	D	0.7	2.4	–	3.2	5.9	12.1
	E	3.1	1.6	–	0.8	0.6	0.3

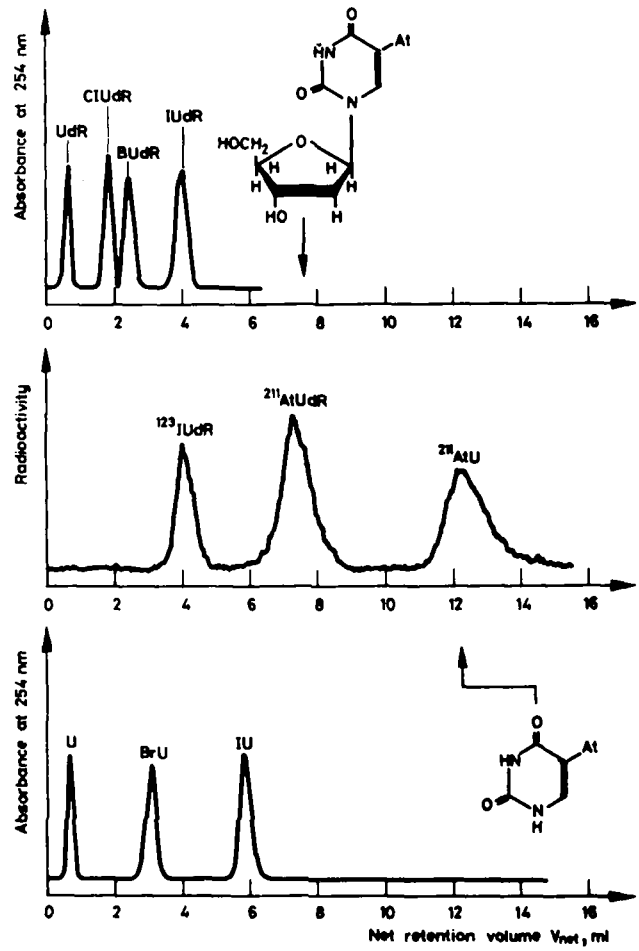

Fig. 5. Separation of 5-halodeoxyuridines and uracils on Aminex A 25 (column D) at 23 °C

volumes on the anion-exchange column when going from F to At substituents may reflect the acidic character of the molecule. The retention behaviour on the partition chromatography column, on the other hand, is governed by the size of the substituents, thus leading to a decrease in retention volume when going to the heavier halogens.

^{123}IUdR is recovered almost quantitatively from both columns. In the case of AtUdR and AtU, only a few percent of the total activity injected can be recovered from the ion-exchange column. Higher recoveries can generally be obtained

from the partition chromatographic column E. Since the latter method works under mild conditions at ambient temperature, and the water and butanol solvents used can easily be transferred into biologically compatible forms, this method is best suited for serial preparative separations, whereas due to its better resolution ion-exchange is more appropriate for identification studies.

Physiological fluids

The radiochromatographic technique lends itself to stability tests of radiopharmaceuticals, in particular for deiodination studies. The content of free radioiodine formed by decomposition or radiolysis of the originally pure pharmaceutical can easily be determined on column B. In a second step, the organic fractions may be separated on one of the columns mentioned in the preceding paragraph.

The examination of physiological systems can be carried out in an elegant way by means of high-pressure techniques. Injection of 20 µl samples of stabilized blood and serum on column B (45 °C) caused a maximum decrease of 15 and 30%, respectively, in the flow rate. In the case of blood injection the original flow rate was regained after 5–10 min, and thus a series of investigations can easily be performed. On the other hand, after 3–4 injections of serum the column is blocked. No irreversible adsorption of $^{131}I^-$ is observed during the tests, and the maximum elution of At^- is 80%. Preliminary studies on column D showed almost no blocking by blood or serum samples up to 20 µl and a satisfactory recovery of ^{123}IUdR. Thus, for in vivo studies of deiodination the samples of body fluids may be injected directly into the column, thereby avoiding the tedious and inexact extraction methods hitherto applied.

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