CHAPTER 2

APPLICATION OF [\(^{18}\text{F}\)]FLUORIDE IN RADIOCHEMISTRY

In this Chapter several methods of producing fluorine-18 are reviewed, mainly related to the nucleophilic form. Some chemical characteristics of \([^{18}\text{F}]\)fluoride are discussed and the targets used for the production of \([^{18}\text{F}]\)fluoride are described.

2.1 Fluorine-18 production methods

Fluorine-18 can be produced in a nucleophilic as well as an electrophilic form by a wide variety of nuclear reactions (Table 2.1). \(^9\)\(^8\)\(^4\) Many production methods based on both chemical forms of \([^{18}\text{F}]\)fluorine have been published in the last decade.

<table>
<thead>
<tr>
<th>entry</th>
<th>reaction</th>
<th>target</th>
<th>beam energy (MeV)</th>
<th>product</th>
<th>production rate (MBq/(\mu)Ah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{5,6})</td>
<td>(^{18}\text{O} ,(p,n))</td>
<td>(\text{H}_2^{18}\text{O})</td>
<td>11</td>
<td>([^{18}\text{F}])</td>
<td>1500</td>
</tr>
<tr>
<td>2(^7)</td>
<td>(^{16}\text{O} ,(^3\text{He},p))</td>
<td>(\text{H}_2\text{O})</td>
<td>15</td>
<td>([^{18}\text{F}])</td>
<td>2200</td>
</tr>
<tr>
<td>3(^8)</td>
<td>(^{16}\text{O} ,(\alpha,pn))</td>
<td>(\text{H}_2\text{O})</td>
<td>22</td>
<td>([^{18}\text{F}])</td>
<td>400</td>
</tr>
<tr>
<td>4(^9)</td>
<td>(^{16}\text{O} ,(^3\text{H},n)^*)</td>
<td>(\text{Li}_2\text{CO}_3)</td>
<td>30</td>
<td>([^{18}\text{F}])</td>
<td>1100-3700(^b)</td>
</tr>
<tr>
<td>5(^{10})</td>
<td>(^{20}\text{Ne} ,(d,\alpha))</td>
<td>(\text{F}_2/\text{Ne})</td>
<td>11</td>
<td>([^{18}\text{F}]\text{F}_2)</td>
<td>400</td>
</tr>
<tr>
<td>6(^{10})</td>
<td>(^{20}\text{Ne} ,(d,\alpha))</td>
<td>(\text{H}_2/\text{Ne})</td>
<td>11</td>
<td>(\text{H}[^{18}\text{F}])</td>
<td>400</td>
</tr>
</tbody>
</table>

\(^a\) \(^3\text{H}\) is produced by the \(^6\text{Li}(n,\alpha)^3\text{H}\) reaction in a nuclear reactor; \(^b\) Batch yield.

Table 2.1 Production methods for fluorine-18.

Nowadays, mainly two target-systems are used to produce fluorine-18, the neon target (entries 5 and 6) and the \(^{18}\text{O}\)-enriched water target (entry 1). The use of an \(^{18}\text{O}\)water target is attractive because of its reliability and the high yield of the nuclear reaction. A disadvantage is the present world-wide shortage of \(^{18}\text{O}\)water and the matching high prices ($150/g). The production of \(\text{H}[^{18}\text{F}]\) using a neon target can therefore be a valuable alternative.

In a neon target, fluorine-18 can be produced as an electrophilic (\([^{18}\text{F}]\text{F}_2\)) or a nucleophilic (\([^{18}\text{F}]\)) reagent, depending on whether \(\text{F}_2^−\) or \(\text{H}_2^−\)-gas is added to the
neon target-gas. Blessing et al.\textsuperscript{90} reported yields of 400 MBq/μAh using a neon target, resulting in batches of 10 GBq (250 mCi) fluorine-18 after 1 h irradiation with a beam current of 25 μA.

The addition of hydrogen gas to the neon target leads to the \textit{in situ} formation of H[I\textsuperscript{18F}]. The [I\textsuperscript{18F}]fluoride can be isolated from the target as anhydrous H[I\textsuperscript{18F}] by a stream of hydrogen gas or as aqueous [I\textsuperscript{18F}] by rinsing the target with a small volume of water.

The use of a 1% fluorine/neon gas mixture in the target leads to the formation of [I\textsuperscript{18F}]F\textsubscript{2}. The [I\textsuperscript{18F}]F\textsubscript{2} that is formed in the target is highly reactive and interacts strongly with the target wall. The target has to be treated in advance with F\textsubscript{2} in order to coat the target wall with F\textsubscript{2}: the target is "passivated". The fluorine acts as a kind of "carrier gas" by facilitating the transport of the radioactivity from the target to the laboratory. The addition of non-radioactive fluorine to the target results in a lower specific activity (section 2.2) of the produced fluorine-18, so this production method is not applicable for the synthesis of receptor binding ligands which require a high specific activity. [I\textsuperscript{18F}]F\textsubscript{2} cannot be prepared as a carrier added (n.c.a.) reagent. The use of NO or Cl\textsubscript{2} as a carrier gas has also been suggested for the production of aqueous [I\textsuperscript{18F}]fluoride,\textsuperscript{91} but has hardly been applied in radiochemical synthesis.

In an [O\textsuperscript{18}]water target, which was used in this thesis, [I\textsuperscript{18F}]fluoride is produced in high yields. This type of target will be discussed in section 2.3.

2.2 Specific activity

The extent to which a compound labelled with a radionuclide is diluted with non-radioactive compound, is referred to as the \textit{specific activity}. The specific activity is calculated from the ratio of the amount of radioactivity (Becquerel, Bq or Curie, Ci) and the total mass of the compound (mol) and is usually expressed in GBq/μmol or Ci/mmol. The maximum specific activity of fluorine-18, with a half-life of 109.7 min, is 63,000 GBq/μmol (1.7 x 10\textsuperscript{6} Ci/mmol).

The specific activity of a product is of importance, because many applications of PET require the administration of only a small amount of drug to the patient. Especially receptor studies require products with a high specific activity, as the concentration of receptors in the human body is generally low. For steroid receptor studies, a minimum specific activity of 40 GBq/μmol (1000 Ci/mmol) is required.\textsuperscript{14} The presence of non-radioactive, "cold" fluorine-19 in the reaction mixture must therefore be prevented and n.c.a. [I\textsuperscript{18F}]fluoride is to be applied in the synthesis.
The specific activity of a labelled compound can be determined by several methods. During HPLC-purification, the UV-absorption can be measured and compared to the absorption of a known standard and thus the amount of mass coeluting with the radioactive peak can be estimated. Neutron activation,\textsuperscript{92} in \textit{vitro} receptor assays\textsuperscript{92} and GC-MS\textsuperscript{93} have also been proposed for the determination of the specific activity. In this thesis, the specific activity of the products is determined with UV-spectroscopy. The specific activity of the n.c.a. \textsuperscript{[\textit{18}F]}fluorinated products discussed in this thesis ranged from 10-100 GBq/\textmu mol ($10^2$-$10^4$ Ci/mmol) and was sufficiently high for the execution of receptor-related studies.

2.3 Fluorine-18 targets

Two nuclear reactions have been used for the production of \textsuperscript{[\textit{18}F]}fluoride: \textit{\textsuperscript{18}O}(\alpha,pn)\textsuperscript{18}F and \textit{\textsuperscript{18}O}(p,n)\textsuperscript{18}F, respectively. The first nuclear reaction was attractive because inexpensive (H\textsubscript{2}\textsuperscript{16}O) water can be used in the irradiation.

The \textit{\textsuperscript{18}O}(\alpha,pn)\textsuperscript{18}F nuclear reaction was performed in an 8 ml water target. The beam energy of 120 MeV was degraded to 60 MeV by the 2 mm titanium front of the target and was completely stopped in the water layer. The target chamber was made of stainless steel. With this target, a production yield of 550-750 MBq/\textmu Ah (15-20 mCi/\textmu Ah) \text-superscript{[\textit{18}F]}fluoride was found. However, the use of this target appeared to be impractical for several reasons. The relatively large volume of the target required the time consuming evaporation of a large amount of water. A second reason was the low beam current of 0.5-1 \textmu A available at the cyclotron of the Kernfysisch Versneller Instituut (KVI). In conclusion, this production method offered amounts of \text-superscript{[\textit{18}F]}fluoride too small to be of practical use in chemical synthesis.

In contrast to the former nuclear reaction where "normal water" (H\textsubscript{2}\textsuperscript{16}O) is used, the \textit{\textsuperscript{18}O}(p,n)\textsuperscript{18}F reaction requires H\textsubscript{2}\textsuperscript{18}O. The high costs of \textsuperscript{18}O-enriched water necessitates either the efficient recovery of the \text-superscript{[\textit{18}O]}water or the minimisation of the target volume. Solin \textit{et al.}\textsuperscript{94} designed a target with a volume of 0.3 ml. We encountered problems with the cooling of this target at higher beam currents (>4 \textmu A).\textsuperscript{95} The consequent loss of water impeded the use of this target for the routine production of \text-superscript{[\textit{18}F]}fluoride.
The target we used at the KVI for the production of $[^{18}\text{F}]$fluoride by the $^{18}\text{O}(p,n)^{18}\text{F}$ nuclear reaction is shown in Figure 2.1. The initial beam energy of 24 MeV is degraded to 11.2 MeV by a 2 mm thick aluminium foil. The core of the target was made of nickel. The $[^{18}\text{O}]$water was retained in a 2 ml cavity in the center of the target-holder. The cavity was cooled at the back of the target with water and the target foil at the front was cooled with a flow of helium. Obviously, cooling of a target is important because a large amount of energy is dumped in a small volume of water (approximately 10 W/µA). Routinely, yields of 2 GBq/µAh (60 mCi/µAh) were obtained with this target, with beam currents as high as 7 µA. The target chamber is not closed during the irradiation, nevertheless the loss of $[^{18}\text{O}]$water is very low. Including the distillation procedure required for the isolation of the $[^{18}\text{F}]$fluoride, only 20-25% of the $[^{18}\text{O}]$water is lost.
With the establishment of the PET-Center at the University Hospital in Groningen, a silver target with a volume of 0.8 ml was used for the production of \[^{18}\text{F}]\text{fluoride. Silver is an attractive metal for the construction of \[^{18}\text{F}]\text{fluorine targets due to the high thermal conductivity and the low energy of the silver-fluorine bond (354 kJ/mol).\textsuperscript{96} Any silver contamination of the target water should therefore not hamper the reactivity of the \[^{18}\text{F}]\text{fluoride.\textsuperscript{97} During the irradiation the target chamber was pressurised with helium. By using an external pressure the boiling point of the \[^{18}\text{O}]\text{water in the target is increased and beam currents up to 20 } \mu\text{A can be applied, thus increasing the average yield of \[^{18}\text{F}]\text{fluoride per batch.}}\text{}}

2.4 Chemistry of \[^{18}\text{F}]\text{fluoride}

The short half-life of fluorine-18 puts constraints on the performance of the chemical reactions. Reactions that take more than two half-lives (for \[^{18}\text{F}]\text{fluoride a time span of 4 h) are not efficient, because the maximum chemical yield will not be higher than 25\%. Apart from the radioactivity involved with \[^{18}\text{F}]\text{fluoride, two other factors play an important role in the short nucleophilic life of \[^{18}\text{F}]\text{fluoride: quantity and solubility.}}\text{}}

2.4.1 No carrier added \[^{18}\text{F}]\text{fluoride}

Most fluorinating organic reactions make use of a large excess of fluoride with respect to the substrate.\textsuperscript{98} An example of this phenomenon is the opening of an epoxide in a solution of 70\% HF,\textsuperscript{99} resulting in a fluoride/substrate ratio of 30.

\[
\begin{array}{|c|c|c|c|}
\hline
\text{Reaction}^a & \text{Fluoride/substrate ratio} & \text{maximum} & \\
& & \text{yield}^b (\%) & \text{spec. activity (GBq}/\mu\text{mol}) \\
\hline
\text{S}_{\text{n}2} (\text{n.c.a.}) & 10^{-6}-10^{-4} & 100 & 630-63000 \\
70\% \text{HF} & 30 & 3 & 0.002 \\
\text{Balz-Schiemann} & 4 & 25 & 0.019 \\
\text{BF}_3 & 3 & 33 & 0.025 \\
\hline
\end{array}
\]

* 50 \mu\text{mol substrate + 3.7 GBq (100 mCi) \[^{18}\text{F}]\text{fluoride; b radiochemical yield, corrected for decay to end of bombardment (EOB).}}\text{}}

\textit{Table 2.2 \[^{18}\text{F}]\text{Fluoride/substrate ratio of selected fluorination reactions.}}
An aromatic fluorination reaction as the Balz-Schiemann reaction makes use of the decomposition of a BF₄-salt, with a fluoride/substrate ratio of 4. Even a simple nucleophilic substitution with fluoride usually requires the use of excess fluoride. However, a reaction with [¹⁸F]fluoride results in the reverse situation. A summary of these effects is shown in Table 2.2.

The performance of a reaction under n.c.a. conditions is, apart from course from the radioactive aspect, the main complication in the preparation of [¹⁸F]fluorinated compounds. The nanomolar scale on which the [¹⁸F]fluoride substitutions are carried out demands a high purity of the chemicals. Even the smallest impurity can reduce the reactivity of the [¹⁸F]fluoride and have a disastrous effect on the yield of the reaction. One source of impurities are traces of metal ions introduced into the [¹⁸O]water by the irradiation of the target. Some of these metals are claimed to form complexes with [¹⁸F]fluoride and render it unreactive towards nucleophilic substitution. During the work-up procedure these complexes are retained in the reaction vessel and they are not available for the reaction. The extent to which these complexes are formed is not clear. A significant portion of the produced [¹⁸F]fluoride, up to 30%, is lost during work-up, but this is also due to "sticking" of [¹⁸F]fluoride to the vessel wall. Many materials have been suggested to overcome this last problem, e.g. pyrolitic carbon (glassy carbon) or platinum. Nevertheless, with the use of a good phase transfer catalyst (PTC) most reactions can be carried out in ordinary borosilicate glassware.

With respect to the specific activity of the labelled products, we experienced that with the exchange of K₂CO₃ for K₃PO₄ in the substitution reactions with [¹⁸F]fluoride, the specific activity increased with one order of magnitude, from <40 GBq/μmol for K₂CO₃ to 40-400 GBq/μmol (1000-10,000 Ci/mmol) for K₃PO₄. This result was observed with several batches of K₂CO₃. However, other authors have used K₂CO₃ with success in the synthesis of [¹⁸F]fluorinated tracers of high specific activity. Therefore, our results do not suggest that K₂CO₃ has to be excluded, but one has to screen the reagents very thoroughly for unwanted and unexpected traces of fluoride.

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2.4.2 Recovery of $^{18}$FFluoride

Fluorine is the most electronegative element of the periodic table and it is one of the three elements (N, O, F) which can act as a H-bond acceptor. The fluoride ion is therefore strongly solvated in protic solvents such as water or methanol. The use of protic solvents or even the presence of water in the reaction mixture leads to a serious decrease in the reactivity of the $^{18}$Ffluoride. Therefore, it is necessary to manipulate the $^{18}$Ffluoride in such a way that it is obtained in an almost anhydrous form and in an aprotic solvent, such as acetonitrile, DMSO or DMF. The extent to which the $^{18}$Ffluoride should be anhydrous remains a point of discussion. Block et al. studied the effect of water on the reaction of $^{18}$Ffluoride with 1-bromoheptane in acetonitrile. They found that the addition of 3% water to the reaction mixture (resulting in a water/$^{18}$Ffluoride ratio of $10^6$) caused the yield of 1-$^{18}$Ffluoroheptane to drop only from 65 to 40%. However, we experienced that, with the synthesis of the 21-$^{18}$Ffluoro-steroids as described in Chapter 3, a small amount of water does inhibit the reaction completely.

In our labelling experiments we used the distillation technique for the recovery of the $^{18}$Owater and the isolation of $^{18}$Ffluoride. Lately, more sophisticated methods as electrolysis and ion-exchange resins have been suggested to recover the $^{18}$Ffluoride from the $^{18}$Owater. Using an electrochemical cell, Alexoff et al. succeeded in trapping and releasing 76% of the produced $^{18}$Ffluoride on a carbon electrode. The advantage of this method is the efficient recovery of the $^{18}$Owater (>98%) without isotopic dilution. However, the $^{18}$Ffluoride is recovered in an aqueous solution, so an additional work-up procedure is still required.

A promising method which has emerged during the last few years is the fixation of $^{18}$Ffluoride on an ion-exchange resin, thus facilitating the recovery of the $^{18}$Owater. The trapping of $^{18}$Ffluoride on a resin makes the time consuming evaporation of the $^{18}$Owater superfluous, and the $^{18}$Owater can be reused for the production of $^{18}$Ffluoride after distillation. The $^{18}$Ffluoride is recovered from the column by rinsing with an electrolyte solution, resulting in an aqueous or nearly anhydrous solution of $^{18}$Ffluoride. A point of interest in this method is the possible isotopic dilution of $^{18}$Owater with H$_2^{16}$O, but this can be avoided by previous hydration of the resin with an aliquot of $^{18}$Owater.

The best approach would be to perform both the isolation of the $^{18}$Ffluoride and the subsequent nucleophilic reaction on the same column. Toorongian et al. have used this concept in the synthesis of the glucose derivative 2-
[^18F]fluorodeoxyglucose ([^18F]FDG 1.8). A 4-aminopyridinium anion-exchange resin was eluted with the [^18O]water containing [^18F]fluoride. The resin was dried with acetonitrile while increasing the temperature to 100 °C. A solution of the precursor of [^18F]FDG in acetonitrile was eluted over the resin at the same temperature in a time interval of 5-7 minutes. The [^18F]fluorinated intermediate was washed from the column with acetonitrile into a vial and hydrolysed, yielding the desired [^18F]FDG in 40% yield.

2.4.3 Solubility of [^18F]fluoride

Several phase transfer catalysts have been used to enhance the solubility of [^18F]fluoride in organic solvents, among them 18-crown-6 2.1, Kryptofix 222 (K222, 2.2) and tetrabutyl ammonium hydroxide 40.

![Figure 2.2 Phase transfer catalysts 18-Crown-6 (2.1) and Kryptofix 222 (2.2).](image)

The thermal instability of quaternary ammonium salts diminishes the applicability of tetrabutyl ammonium hydroxide as PTC in reactions with [^18F]fluoride. Hamacher et al. used 2.2 with great success in the preparation of [^18F]FDG. The cryptand 2.2 acts as a complexing agent for potassium and thus solubilises the [^18F]fluoride anion. The thermal stability of 2.2 is good and the resolubility, the efficiency of transferring the [^18F]fluoride into an organic solvent, of 2.2 is better than of 18-crown-6 2.1.

We used 2.2 in combination with K3PO4 throughout this thesis in the [^18F]fluoride substitution reactions. The work-up procedure of [^18F]fluoride is rather critical. The aqueous solution containing the [^18F]fluoride is concentrated under a stream of helium. A solution of 2.2 and K3PO4 in acetonitrile/water must be added to the radioactivity before the [^18O]water is distilled off completely, otherwise the resolubility of the [^18F]fluoride is very low. After carefully drying
the Kryptofix 222/[18F]fluoride complex (K\textsubscript{222}/[18F]) with anhydrous acetonitrile it is available for the actual substitution reaction. The use of 2.2 in combination with acetonitrile as solvent results in a good resolubility of the produced [18F]fluoride from the water phase to the organic phase. We found on a routine basis resolubilities of 70% and higher.

### 2.5 Concluding remarks

The development of efficient methods for producing no carrier added (n.c.a.) [18F]fluoride and its conversion into a chemically reactive form, has greatly contributed to the application of [18F]fluoride in the synthesis of tracers for PET. Especially the introduction of the phase transfer catalyst Kryptofix 222 in radiochemistry has broadened the scope of [18F]fluoride beyond its application as bone scanning agent.

### 2.6 Experimental part

**General.** - The nuclear reaction was either carried out at the KVI in a 2 ml nickel target, or at the University Hospital in a 0.8 ml silver target. The $^{18}$O-enriched water was supplied by Intersales BV, The Netherlands, in 50 or 98% enrichment. The $[^{18}\text{O}]$water was diluted to the appropriate concentration by addition of distilled water. After irradiation, $[^{18}\text{O}]$water was recovered and reused for the production of [18F]fluoride. Kryptofix 222 2.2, K$_3$PO$_4$.7H$_2$O and acetonitrile p.a. grade were supplied by Merck, Darmstadt, FRG.

**Work-up procedure of $[^{18}\text{F}]$fluoride.** - The $[^{18}\text{F}]$fluoride was produced by the $^{18}$O(p,n)$^{18}$F reaction. After careful evaporation of $[^{18}\text{O}]$water at a temperature of 120 °C, a solution of 10 mg (26 μmol) 2.2 and 3 mg (9 μmol) K$_3$PO$_4$.7H$_2$O dissolved in 1 ml acetonitrile/water 90/10 (v/v) was added to the $[^{18}\text{F}]$fluoride. The resulting K$_{222}$/[18F]-complex was dried by three successive evaporations of 0.3 ml acetonitrile at the same temperature. The dried K$_{222}$/[18F] was resolubilised in acetonitrile, sonicated for 1 min and used for the substitution reactions.