Nucleophilic Substitution Reactions for Positron Emission Tomography; Factors Influencing the Reactivity of [^{18}F]Fluoride

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“Medical radiochemistry and radiopharmacy were — and still are — treated as orchid areas within their scientific home faculties; it needs special interest and freakish dedication in the preparation of drugs on a sub-nanomolar scale.”

—Wolfgang Wadsak, 2010

CONTENTS

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Anders Svadberg

ABBREVIATIONS AND SYMBOLS

AEC	Anion-exchange cartridge
β^+	Positron
CT	Computed tomography
EP	European Pharmacopoeia
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
FDA	Food and Drug Administration
[^{18}F]FDG	2-deoxy-2-[^{18}F]fluoro-D-glucose
[^{18}F]FACBC	*Anti*-1-amino-3-[^{18}F]fluorocyclobutyl-1-carboxylic acid
ICP-OES	Inductively coupled plasma optical emission spectrometry
K_{222}	4, 7, 13, 16, 21, 24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane
keV	Kiloelectron volt
MeCN	Acetonitrile
MRI	Magnetic resonance imaging
n.c.a.	Non-carrier added
PET	Positron Emission Tomography
PTC	Phase transfer catalyst
QMA	Quaternary methyl ammonium
RCY	Radiochemical yield
TLC	Thin layer chromatography
Tracer	Radiolabeled compound that has a biological target
USP	United States Pharmacopeia

LIST OF PUBLICATIONS

The present thesis is based on the following publications and manuscript and will be referred to in the text by their Roman numerals.*

Paper I

Hjelstuen, O. K., Svadberg A., Olberg, D. E., Rosser, M., 2011. Standardization of fluorine-18 manufacturing processes: New scientific challenges for PET. *Eur. J. Pharm. Biopharm.* 78, 307-313.

Paper II

Svadberg, A., Clarke, A., Dyrstad, K., Martinsen, I., Hjelstuen, O. K., 2011. A critical study on borosilicate glassware and silica-based QMA's in nucleophilic substitution with [^{18}F]fluoride: influence of aluminum, boron and silicon on the reactivity of [^{18}F]fluoride. *Appl. Radiat. Isot*. 69, 289-294

Paper III

Svadberg, A., Wickstrøm, T., Hjelstuen, O. K., 2012. Degradation of acetonitrile in eluent solutions for [^{18}F]fluoride PET chemistry: impact on radiosynthesis of [^{18}F]FACBC and [^{18}F]FDG. *J. Labelled Compd. Radiopharm.* DOI: 10.1002/jlcr.1956 (article available online in advance of print)

Paper IV

Svadberg, A., Dyrstad, K., Hjelstuen, O. K. (2011). Cationic contaminants in irradiated [^{18}O]H_2O and their effect on [^{18}F]F^- reactivity. *Appl. Radiat. Isot.* Submitted.

ABSTRACT

Background

Clinical use of positron emission tomography (PET) is expanding from use of [^{18}F]FDG to a wider spectrum of PET-tracers and proprietary PET radiopharmaceuticals. The development of new PET-tracers is however far from trivial and the radiochemistry is a major limiting factor. Nucleophilic substitution reactions with [^{18}F]fluoride generally suffer from inconsistent labeling yields. There is a need to investigate the generic impurities causing such inconsistency in order to build more robust syntheses that also are in line with regulatory demands for approval of new PET radiopharmaceuticals.

Objectives

The aim of this thesis was to investigate how pharmaceutical-chemical impurities from a typical synthesis setup may influence labeling yields in nucleophilic substitution reactions with [^{18}F]fluoride. Following areas were studied: Borosilicate glassware, Kryptofix based eluent solutions, anion-exchange cartridges, and irradiated [^{18}O]water.

Materials and methods

The new commercial synthesizer, GE FASTlab™, was used as an investigational platform using three different benchmark reactions: [^{18}F]FACBC, [^{18}F]FDG and [^{18}F]Flutemetamol. The importance of identified impurities was investigated with radiochemical yield as the measured response.

Main findings and conclusions

Eluent solutions of K_{222}, K_2CO_3 in aqueous acetonitrile extracted ppm levels of silicon, boron and aluminum if stored in borosilicate glass at room temperature (days). It was revealed that relevant levels of aluminum present in the eluent solution (0.4–2 ppm) could pass a polymer-based anion-exchange cartridge and cause detrimental effects on the RCY.

Calcium, magnesium, zinc and aluminum were identified as potential impurities in irradiated [^{18}O]water. Aluminum, if present in the [^{18}O]water, was fully adsorbed on the anion-exchange cartridge and to a variable degree co-eluted with [^{18}F]fluoride, causing detrimental effect on

the RCY. Type of anion-exchange cartridge was a major determining factor for the amount of eluted aluminum.

Eluent solutions containing K_{222} and K_2CO_3 in aqueous acetonitrile degraded relatively quickly when stored at room temperature or above. Acetate, one of the degradation products would negatively affect RCY if generated at sufficient levels (hundreds of ppm). A methanol-based eluent solution was successfully developed, showing no degradation after 6 months of storage at 50 ^{o}C.

1. INTRODUCTION

The term molecular imaging may be defined as the noninvasive visualization of *in vivo* biological processes at the molecular or cellular levels using specific imaging tracers (Weissleder and Mahmood, 2001; Ametamey et al., 2008). In contrast to conventional diagnostic imaging such as x-ray, molecular imaging observes the physiological changes of a disease at a cellular level rather than on the anatomical level. The novelty of observing both structure and function gives the unique opportunity to reveal pathways and mechanisms responsible for disease in living subjects (Massoud and Gambhir, 2007). Molecular imaging may be used for early detection, characterization, real time monitoring of disease as well as investigating the efficacy of therapeutic drugs (Ametamey et al., 2008).

Positron emission tomography (PET) and single photon emission tomography (SPECT) makes up the branch of molecular imaging known as nuclear medicine. Three other modalities in which molecular imaging may be utilized are magnetic resonance imaging (MRI), optical imaging and ultrasound. Although there are different advantages and disadvantages among these modalities, they are not in contest with each other. Instead, they are more like different tools for different tasks and the techniques complement each other in use for human health.

1.1 Positron emission tomography

PET imaging is a highly sensitive imaging technique that utilizes positron-emitting radioisotopes (β^+-emitters). These β^+-emitters may be incorporated into biological active molecules from which the radioisotope works as a tag, allowing visualization of its distribution *in vivo* through use of gamma-cameras. A β^+-emitter decays by emitting a positron — a positively charged electron — which almost instantly annihilates with a nearby electron (Turkington, 2001; Sanchez-Crespo et al., 2004). This annihilation results in two identical gamma-rays of identical energy that simultaneously travel 180° apart (Beringer and Montgomery, 1942). These two gamma-rays (511-keV photons) are then electronically detected as a coincidence event when they simultaneously strike opposing gamma detectors within 12 milliseconds as illustrated in Figure 1. The figure illustrates one line of coincidence, but during a PET scan, several millions of these coincidences are recorded and useful images

can subsequently be made accordingly to how the biomolecules are distributed *in vivo* (Levin, 2005). E.g., cancer can be detected after the labeled biomolecules gets accumulated inside the cancer cells.

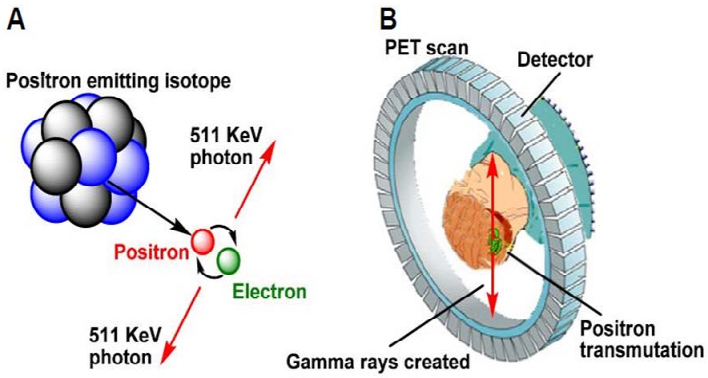


Figure 1. *(A) A positron and a negative electron annihilate, producing two 511 keV photons travelling in opposite directions. (B) The 511 keV photon was registered by the circular gamma ray detector array in the PET camera. Reprinted from (Li and Conti, 2010), Copyright © 2010, with permission from Elsevier.*

1.2 Revolution of PET: Introduction of PET/CT

PET as a medical application was invented in the early 1950s for localization of brain tumors (Wrenn et al., 1951). However, it had to wait half a century before it reached its breakthrough as a major diagnostic tool in the clinic. In spite of the superior sensitivity; PET has always suffered from low anatomical resolution, which in general is too low for accurate anatomic localization of pathology. Attempts to solve this problem were performed by scanning subjects a second time with an anatomical diagnostic technique like CT or MRI, and then fuse these images with the PET image. In the late 1980s, this was successfully achieved in head cancer detection (Pelizzari et al., 1989; Woods et al., 1993). While combining two different sets of images could work for rigid organs such as the brain, alignment of other parts of the body are problematic due to patient movement (especially caused by heart and lungs). In 1994, initiatives were started to design a combined PET/CT scanner in one unit. The device comprised a PET scanner and a CT scanner situated next to each other, enabling the possibility of fusing images taken nearly simultaneously (Beyer et al., 2000). The result can be exemplified with an image from this paper, the first published fused image from a combined scanner (Fig. 2.)

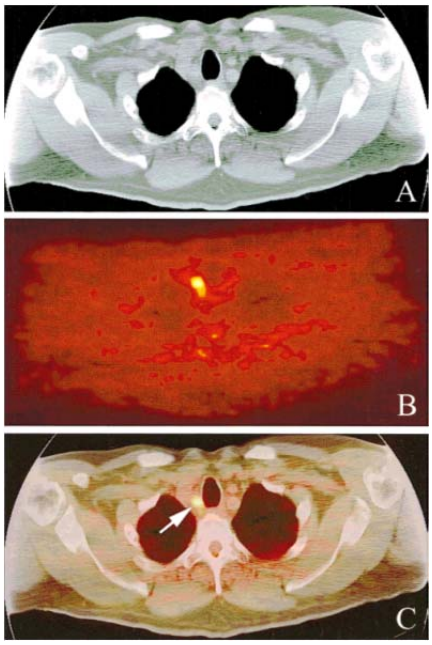


***Figure 2**. (A) CT image of thorax. (B) PET image of thorax showing higher [^{18}F]FDG uptake in the cancer tumor (yellow). (C) Fused PET/CT image localizes the cancer tumor (arrow). Reprinted from (Beyer et al., 2000), Copyright © 2000, with permission from the Society of Nuclear Medicine.*

The fused PET/CT scanner revolutionized the field of PET and already in 2001, the first commercial PET/CT scanner was introduced (Burger et al., 2002). Other manufactures followed and by 2007, five different companies offered commercial PET/CT scanners. In 2006, all PET scanners sold were combined PET/CT systems (Townsend, 2008). The commercial introduction of PET/CT has boosted the number of scans dramatically as illustrated by Figure 3. In 2010, it was estimated that there were over 650 biomedical cyclotrons serving 2200 PET/CT scanners in the world (IAEA, 2010). Combined PET/CT scanners represent an important evolution in technology that has helped bringing molecular imaging to the forefront in cancer diagnosis, staging and therapy monitoring. Notably, the growth of PET/CT was based on the single radiopharmaceutical [^{18}F]FDG, a sugar analogue for imaging of glucose metabolism (Gallagher et al., 1977). This tracer has been, and still is, the workhorse of PET and ~90% of all scans performed are with [^{18}F]FDG (Coenen et al., 2010). The fact that all human cells utilize glucose creates a wide range of possible uses, but oncology is by far the major application (Fletcher et al., 2008).

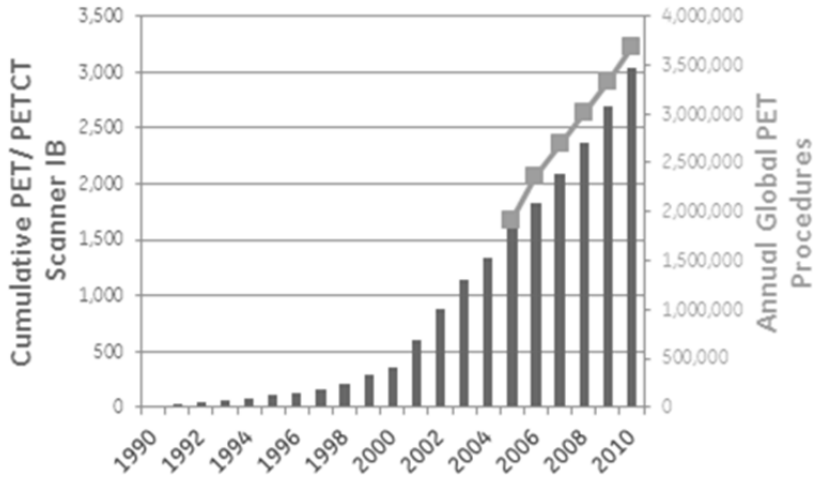


***Figure 3**. (Dark grey) Annual global sales of PET and PET/CT scanners (cumulative) from 1990 to 2010. (Light grey) Annual global PET procedures performed from 2006 to2010. Figure is reprinted with permission from Medical Options © 2012*

There is a wide range of experimental tracers other than [^{18}F]FDG that are mainly used in the fields of oncology, neurology and cardiology (Mawlawi and Townsend, 2009). Many of these tracers have shown very promising results, but few of them are available to patients. It is therefore a considerable ambition to bring more of them into the clinic as readily available licensed radiopharmaceuticals (Coenen et al., 2010). Besides the clinical investigation of disease, PET/CT is increasingly used as tool for investigating new therapeutic drugs (Miller et al., 2008). More than 35% of new investigational drugs fail when entering clinical trials due to inappropriate pharmacokinetics (Salvadori, 2008). PET/CT has a unique possibility to investigate pharmacokinetic effects of drugs candidates early and thus a great potential to reduce such a high failure rate. It has also been suggested that PET/CT may be utilized as a mean to very early predict the efficacy of investigational lead drug candidates *in vivo* through "microdosing" studies. The idea is that reduced safety requirements could be claimed as the mass of the injectable drug is so low that it will not provoke any toxic or biochemical effects (Bergstrom et al., 2003).

1.3 [^{18}F]fluoride

There is a wide variety of different β^+-emitting isotopes utilized in PET and the most commonly used in diagnostic medicine are summarized in Table 1. The radionuclides carbon-11, nitrogen-13 and oxygen-15 are natural biological building blocks that make it possible to create PET tracers that are indistinguishable from its nonradioactive counterparts. This is of great advantage if the target of interest is very sensitive to the molecular structure. However, the short half-life of these three isotopes often limits their use.

Table 1. *Common positron-emitters used in PET*

Radionuclide	Half-life (min)	Maximum particle energy (MeV)	Decay product
^{11}C	20.4	0.96	^{11}B
^{13}N	10.0	1.19	^{13}C
^{15}O	2.07	1.723	^{15}N
^{18}F	109.8	0.635	^{18}O
^{64}Cu	768	0.656	^{64}Ni
^{68}Ga	67.6	1.899	^{68}Zn

Fluorine-18 is by far the most frequently used PET-isotope in nuclear medicine, mainly due to its half-life of 110 min, which is long enough to allow tracers synthesis, transportation, and imaging procedures to last over hours. Fluorine-18 has also a relatively low maximum energy (maximum 0.635 MeV), thus the emitted positron has a short mean range, leading to better resolution than β^+-emitters with higher energy (Snyder and Kilbourn, 2003). Although fluorine is not a natural constituent of most biomolecules, its substitution for hydrogen induces only a small steric difference due to similar van der Waals radii (fluorine = 1.35 Å, hydrogen = 1.20 Å). The differences in electronic character of the two elements, however, are very pronounced. For example, replacing hydrogen with fluorine in an aliphatic position will decrease the lipophilicity by a factor of five, while substitution in an aryl group increases the lipophilicity (Leo et al., 1971). Nevertheless, most PET tracers labeled with fluorine-18 are based on the analogy in steric demands of fluorine and hydrogen (Schubiger et al., 2007).

There are several nuclear reactions known for producing fluorine-18 (Nickles et al., 1986; Guillaume et al., 1991). Among these different pathways, the ^{18}O(p,n)^{18}F reaction is by far the most efficient method for producing high yielding fluorine-18 at large quantities (Ruth and

Wolf, 1979). This method applies the bombardment of protons onto oxygen-18, producing a free neutron and fluorine-18. The most commonly used oxygen-18 material is enriched [^{18}O]water, but [^{18}O]O_2 gas can be used for the same purpose. The use of the latter is however more complicated as it occurs by a two-step mechanism, producing fluorine-18 as a [^{18}F]F_2 gas available for electrophilic substitution (Nickles et al., 1984). This thesis will only address the use of [^{18}O]water targets, which produce aqueous anionic $^{18}F^-$ ([^{18}F]fluoride) (Kilbourn and Welch, 1983; Wieland and Wolf, 1983; Kilbourn et al., 1984). The proton irradiation of [^{18}O]water targets is a highly attractive method as it produces non-carrier added (n.c.a.) fluorine-18. This means that fluorine-18 can be obtained without the addition of carrier fluorine-19 and thereby achieve high specific activity. This is clearly advantageous and can be mandatory for investigations of low concentration binding-sites, e.g. neuronal receptors (Schubiger et al., 2007).

1.4 [^{18}F]fluoride chemistry

The use of the [^{18}F]fluoride ion as a nucleophile for labeling PET tracers was for a long time an unexploited field, but during the 1980s new and more efficient [^{18}O]water targets changed this (Kilbourn and Welch, 1983; Tewson et al., 1988). In 1986, the landmark paper by the group of Dr. Hamacher, achieving a high yielding synthesis of [^{18}F]FDG; more or less defined modern synthesis with [^{18}F]fluoride (Hamacher et al., 1986). The principals of this procedure still remain as the main route of making [^{18}F]FDG (Cai et al., 2008; Li and Conti, 2010).

The first step in all [^{18}F]fluoride chemistry is the removal of bulk [^{18}O]water. Fluoride is strongly solvated in water due to strong hydrogen bonding and therefore inactive as a nucleophile (Clark, 1980; Vlasov, 1993). Customarily, [^{18}F]fluoride is adsorbed onto an ion exchange resin followed by elution with an inorganic anion dissolved in an organic-aqueous solution (Schlyer et al., 1990). This procedure will recover the expensive [^{18}O]water which may be reused after purification (Asti et al., 2007; Moon et al., 2007). The adsorbed [^{18}F]fluoride is then eluted off the cartridge using an eluent that normally contains an aqueous acetonitrile solution with a carbonate salt (K_2CO_3, $KHCO_3$) accompanied by a cryptand like Kryptofix™ (K_{222}) or tetrabutyl ammonium (Hamacher et al., 1986; Brodack et al., 1988). The eluted [^{18}F]fluoride is then evaporated to dryness by heating the reaction vessel under reduced pressure. Aliquots of added acetonitrile during the evaporation achieves azeotropic conditions in the mixture, thus easing the drying cycle (Jewett et al., 1988). Degree of drying

highly depends on precursor to be labeled. The classical "rule" is that increased dryness will increase the reactivity of the [^{18}F]fluoride ion. There are however numerous reactions, especially aliphatic, that withstand relatively large amounts of water (µl) without influencing the radiochemical yield (RCY) (Block et al., 1986; Kilbourn et al., 1986). Nucleophilic attack from solvated fluoride has been described through computational model studies (Vincent and Hillier, 2005).

There are alternative means of activating the [^{18}F]fluoride, like conversion into the intermediate [^{18}F]fluorotrimethylsiliane (Hutchins et al., 1985; Rosenthal et al., 1985), or via electrochemical procedures (Hamacher et al., 2002; Reischl et al., 2002). However, these methods are cumbersome and seldom implemented (Cai et al., 2008).

Nucleophilic substitution with [^{18}F]fluoride normally occurs by heating and re-dissolving the dried residue of the K^+[^{18}F]fluoride-K_{222} complex with the precursor in a polar aprotic solvent such as acetonitrile, DMSO and DMF (Hamacher et al., 1986). Acetonitrile, in contrast to DMSO or DMF, has the advantage that it can easily be removed by evaporation. Choice of solvent will depend on the nature of the substrates, but it has been described that acetonitrile can provide higher RCY when comparing different solvents (Block et al., 1987). In recent years, the use of certain polar protic solvents has been explored and applied successfully in several examples (Kim et al., 2006; Lee et al., 2007; Kim et al., 2008).

Nucleophilic substitution with n.c.a. [^{18}F]fluoride is in general divided into aliphatic and aromatic displacement reactions. The aliphatic reactions proceeds according to a S_N2-mechanism, using common leaving groups such as sulfonic acid esters (e.g. triflates, tosylates or mesylates) or halides (Cl, Br or I) (Lasne et al., 2002). Nucleophilic aromatic substitution with n.c.a. [^{18}F]fluoride appears particularly well suited to the synthesis of aryl [^{18}F]fluorides (Kilbourn, 1990). A prerequisite, nonetheless, is that the aromatic ring needs to be activated by the presence of one or more electron-withdrawing groups positioned ortho- or para- to the leaving group. Examples of such activating groups are nitro-, cyano- and carbonyl-groups (Kilbourn, 1990; Dolle´ et al., 2008). There are several different alternatives for leaving groups, but nitro and trimethyl ammonium groups are the most widely used (Ding et al., 1990).

1.5 Pitfalls in [^{18}F]fluoride chemistry

The potential of PET/CT as a modality with a wide range of applications strongly depends on the availability of different tracers. Their development is however far from trivial and the radiochemistry involved has recently been described as a major limiting factor for the field of PET (Li and Conti, 2010).

Nucleophilic substitution reactions with [^{18}F]fluoride look at first glance relatively straight forward and the incorporation reaction is in principal the same as performed with non-radioactive [^{19}F]fluoride. There are, however, several fundamental differences that complicates matters compared to [^{19}F]fluoride chemistry. The greatest challenge is the low amount of [^{18}F]fluoride involved which is typically at the sub-nanomole level. Since the fluoride ion has a strong tendency for complex formation in the presence of Lewis acids or heavy metals, highly variable ^{18}F-labeling yields often results from inaccessible [^{18}F]fluoride complexes (Berridge and Tewson, 1986; Nickles et al., 1986; Tewson, 1989). Perhaps more than any other radionuclide, the ultimate success of and subsequent chemical application hinges on a number of factors that are determined upstream in the process at the moment of production (Nickles et al., 1986).

So far, the demand for purer [^{18}F]fluoride has to a large extent been satisfied through upgrading of cyclotrons and targetry systems. Improved cyclotron technology from only a few vendors has standardized the bombardment process, and continuous development of water targets over the last two decades has greatly improved the impurity profile of the aqueous [^{18}F]fluoride. The choice of target material has shifted from nickel-plated copper, titanium, silver and today niobium and tantalum are the most popular choices (Kilbourn et al., 1984; Huszar and Weinreich, 1985; Tewson et al., 1988; Berridge and Kjellstrom, 1999; Zeisler et al., 2000; Berridge et al., 2002; Satyamurthy et al., 2002). The target entrance foil, which is in direct contact with the enriched [^{18}O] water, can be made of materials like Ti, Ag, stainless steel, niobium and Havar (Kilbourn et al., 1984; Iwata et al., 1987; Nye et al., 2006). Havar is still the most common entrance foil material, but Havar sputtered with niobium or tantalum has recently been presented as promising improvements (Wilson et al., 2008; Gagnon et al., 2011). Nevertheless, [^{18}O]water targets still remains as a developing area despite all the major improvements. The proton beam is highly corrosive, causing radioactive and nonradioactive impurities in the irradiated [^{18}O]water as described in recent papers (Gillies et al., 2006; Avila-Rodriguez et al., 2008; Bowden et al., 2009; Ferguson et al., 2011). It is well known

that nonradioactive impurities in the irradiated [^{18}O]water can have a negative impact on the ^{18}F-labeling yields (Kilbourn et al., 1984; Tewson et al., 1988; Schlyer et al., 1993).

Subsequent to [^{18}O]water irradiation, the aqueous [^{18}F]fluoride solution is transferred from the target to a synthesizer unit where the labeling reaction occur. This transfer and flow involves several different surfaces that comes in direct contact with the aqueous [^{18}F]fluoride. First, the aqueous [^{18}F]fluoride is normally sent through several meters of tubing from the cyclotron target chamber to a hot cell located in a separate room. Type of tubing, length, rinsing routines and age will vary for each PET center. Stainless steel and plastic such as polypropylene, polyethylene, PEEK and Teflon are all examples of tubing material used (Heselius et al., 1989). A common challenge is that transfer lines deteriorate over time and replacement with new ones are consequently needed (Harris et al., 1989). These deteriorating effects could introduce leachables into the aqueous [^{18}F]fluoride that may influence the ^{18}F-labeling reaction. Such effects have however, not been studied to any extensive degree. The importance of these factors can be exemplified by recent work showing how radiolysis of Teflon-tubing leach off relatively large amounts of cold [^{19}F]fluoride compared to other plastic types (Fuchtner et al., 2008; Berridge et al., 2009).

When the aqueous [^{18}F]fluoride enters a shielded hot cell, it can be introduced directly to the synthesizer unit or typically, be collected in a borosilicate v-vial before transfer to the synthesizer. The latter approach allows the operator to measure the amount of activity that is received in the hot cell and also works as visual check of the aqueous [^{18}F]fluoride. As with the tubing, little systematic research has been conducted on how extractables and leachables from borosilicate glass may affect the [^{18}F]fluoride reactivity. It is well known however, that [^{18}F]fluoride may bind to the surfaces of borosilicate glass. (Beg and Brown, 1963; Mudrova and Svoboda, 1972; Gnade et al., 1981; Coenen et al., 1985; Brodack et al., 1986; Nickles et al., 1986). There is an extensive use of borosilicate glass in most synthesis setups. The container for non-irradiated [^{18}O]water, reagent vials, collection vials and reaction vessels are often made of borosilicate glass. There seems to be a general impression in the field that problems with “sticking” of [^{18}F]fluoride may be solved by practical means that varies from site to site (e.g. type of equipment, washing routines, siliconizing the borosilicate surface) (Harris et al., 1989; Heselius et al., 1989).

Another common denominator that represent a potential pitfall in [^{18}F]fluoride chemistry is the use of an anion-exchange cartridge (AEC) in the separation of [^{18}F]fluoride and bulk

[^{18}O]water (Schlyer et al., 1987; Hamacher et al., 1990; Schlyer et al., 1990). It was first introduced as a mean of recovering the expensive [^{18}O]water for later reuse, but it would also ease the subsequent evaporation as aqueous-organic mixtures could be used for elution of [^{18}F]fluoride (Jewett et al., 1988; Jewett et al., 1990). It has been speculated that the AEC could also function as a purification column as cationic impurities present in the irradiated [^{18}O]water would pass the cartridge during [^{18}F]fluoride adsorption (Kilbourn, 1990; Cai et al., 2008). At the same time, however, the AEC might introduce new impurities (Alexoff et al., 1992; Dence et al., 1995). It was discovered in 1997 that the commercially available Sep-Pak® QMA light Accell Plus (Waters) was applicable with [^{18}F]fluoride chemistry; rapidly making it the preferred choice as AEC across the PET community (Zhang et al., 1997).

1.6 Automation of [^{18}F]fluoride chemistry

The amount of radioactivity used in [^{18}F]fluoride chemistry intended for clinical use is too high (multi-GBq) for hands-on manipulation. It is therefore necessary to automate the chemistry so it can take place behind shielding away from the operator. The automated system is typically placed within a lead-shielded hot cell, which is controlled by an outside computer. In the 1970`s and 1980`s, automated systems were to a large degree semi-automated, self-constructed devices that were custom made for the individual hot cell (Barrio et al., 1981; Fowler et al., 1981). Today, the chemistry is fully automated and with pre-programmed software, but the basic concepts of hardware in conventional synthesis have not changed fundamentally (Alexoff, 2003; Li and Conti, 2010). Typically, the synthesizers include remote-controlled valves, solvent reservoirs, tubing and one or two reaction vessels. Much of the design is based on the successful procedure for [^{18}F]FDG synthesis presented by the group of Hamacher (Hamacher et al., 1986). This stereospecific, high yielding, one-pot synthesis has been recognized as a generic model of which other compounds could adapt to.

Modern commercial synthesizers are broadly divided into two categories. The first class is the stationary systems where all the components such as the tubing and valves are permanent and not changed in day-to-day operations. Preparation before a synthesis is accomplished by flushing the system with cleaning-solvents without removing the parts. This type of system requires significant validation to ensure no cross contamination and absence of cleaning solvents in the finished product. Examples of stationary systems include: Explora®FDG$_4$ (Siemens), Tracerlab™ FX$_{F-N}$ (GE Healthcare) and the Synchrom (Raytest). The second class

of synthesizers relies on disposable cassettes that are discarded after use. This system avoids the need of extensive cleaning between runs and will in general be a much better starting point for a GMP compliant process. Examples of cassette-type synthesizers include: FDG-Plus Synthesizer (Bioscan); Synthera® (IBA) and the FASTlab™ (GE Healthcare).

In recent years, microfluidics have been explored as a promising alternative to conventional vessel-based radiotracer synthesis. Miniaturization of synthesizers has the potential to deliver advantages such as saving precious hot cell space, reducing the reagent consumption and achieve better control over the reaction conditions. These benefits have been exemplified in several recent papers (Lee et al., 2005; Wester et al., 2009; Pascali et al., 2010; Bouvet et al., 2011; Ungersboeck et al., 2011). In spite of extensive research and development, microfluidics is still in a proof-of-concept status as there are yet certain limitations to microfluidic devices that must be solved before applying the technology to the commercial production of clinical PET tracers. The biggest challenge is perhaps how to handle high GBq levels of [^{18}F]fluoride. Another challenge is finding appropriate systems for integrating the pre-concentration of [^{18}F]fluoride from the bulk [^{18}O]water. Most of the devices described so far rely on activation of the [^{18}F]fluoride outside the microfluidic device. Recently, however, encouraging results on stand-alone systems with integrated pre-concentration of the [^{18}F]fluoride has been described (Saiki et al., 2010; Leonardis et al., 2011).

1.7 Regulatory aspects

By law, most countries define PET tracers as drugs (Wadsak and Mitterhauser, 2010). This involves a series of regulatory and legal aspects that are in accordance with good manufacturing practice (GMP). Since PET tracers are mostly administered intravenously, strict rules are applied for production and quality control; including special demands such as specific glass qualities, rubber materials for stoppers, sterility assessment etc.

There are certain aspects of PET tracer manufacture that differ distinctively from conventional drugs that consequently makes GMP compliance more challenging for PET tracers than conventional drugs (Langstrom and Hartvig, 2008; Coenen et al., 2010). For instance, the short half-life of frequently used PET isotopes makes it necessary to release the product before conventional sterility tests can be performed. Consequently, this requires that GMP must be built into the manufacturing process to assure a safe and robust product. The implementation of a thorough system for quality management is a given.

So far, most of the radiopharmaceutical regulatory demands have derived directly from industrial standards on conventional drugs (Salvadori, 2008). In recent years, however, several actions have been taken in order to change current regulatory paradigms. The Food and Drug Administration (FDA) has recently implemented a GMP guidance specifically for PET drugs (FDA, 2009, 2011), while in Europe the International Atomic Energy Agency (IAEA) and the European Association of Nuclear Medicine (EANM) have launched several guidelines (EANM, 2007; Verbruggen et al., 2008; IAEA, 2009; Elsinga et al., 2010).

Even though the regulatory paradigm of PET is under current change, the goal is nevertheless the same as with conventional drugs; to implement certain production standards and controls that can ensure the production of PET tracers are safe for patients regardless of what country the manufacturer is located. This implicates standardization of equipment, reagents, consumables and chemistry that operate within a fixed design space.

2. AIMS OF THE PROJECT

Automation and standardization of [^{18}F]fluoride chemistry is a prerequisite for clinical manufacture of ^{18}F-labeled PET tracers. Nucleophilic substitution reactions — being the preferred method for ^{18}F-labeling, generally suffer from unexplainable fluctuations in radiochemical yields. We wanted to seek a deeper understanding of [^{18}F]fluoride chemistry in order to build robust and reliable syntheses that are in line with pharmaceutical standards.

The overall aim of the thesis was to investigate how common pharmaceutical-chemical impurities may influence labeling yields in nucleophilic substitution reactions with [^{18}F]fluoride.

Specific aims were:

- Identify from literature general pitfalls in nucleophilic substitution reactions with [^{18}F]fluoride (paper I)
- Study how potential leachables and extractables from borosilicate glassware may influence ^{18}F-labeling yields (paper II)
- Study how potential hydrolysis of acetonitrile in eluent solutions may influence ^{18}F-labeling yields (paper III)
- Study how commercially available AECs may remove or introduce impurities during adsorption of [^{18}F]fluoride (paper II and IV)
- Identify and study how impurities in the irradiated [^{18}O]water product may influence ^{18}F-labeling yields (paper II and IV)

Where possible, we also aimed to provide proposed solutions to observed issues.

3 SUMMARY OF PUBLICATIONS

3.1 PAPER I

A review — addressing new scientific challenges in modern manufacture of [^{18}F]fluoride PET pharmaceuticals. The field of PET has changed from being a useful research modality to become a major clinical tool. This transition demands more robust [^{18}F]fluoride chemistry and automated systems that are in accordance with quality standards for drug manufacture. Although there has been a significant development in [^{18}F]fluoride chemistry over the last 30 years, there is still a lack of basic knowledge in some areas which makes the chemistry semi-mature. The aim of this review was to describe the scientific pitfalls connected to either the chemistry itself, or the pharmaceutical components necessary to build robust radiochemical processes ready for multi-center manufacture. The whole process from irradiation of [^{18}O]water in a cyclotron, to the finished injectable end-product was evaluated in a chronological, stepwise manner. The [^{18}O]water itself, irradiation and transfer of [^{18}O]water, anion-exchange cartridges and use of borosilicate glass were some of the areas in which potential pitfalls may exist.

3.2 PAPER II

The use of borosilicate glassware and the commonly used Sep-Pak® QMA light Accell Plus cartridge (Waters) were investigated as sources of impurities that could influence the reactivity of the [^{18}F]fluoride ion. Aluminum, boron and silicon, all constituents of borosilicate glass, were found as water-soluble impurities in a typical PET setup conducting ^{18}F-labeling of an aliphatic PET tracer.

It was discovered that the borosilicate glass v-vial used for receiving the irradiated [^{18}O]water as it enters the hot cell, released silicon (8.2–14 ppm) and boron (3–11 ppm) into the irradiated [^{18}O]water during normal usage. When a typical K_{222}/K_2CO_3 eluent mixture was stored in a borosilicate glass vial, which is highly relevant for automated systems, extractables of soluble silicon, boron and aluminum increased with storage time (days). The Sep-Pak® QMA light Accell Plus cartridge, preconditioned with carbonate (K_2CO_3), released relatively

large amounts of water-soluble silica when treated with water. The amount of released silica increased with storage time (days).

An experimental design study with multivariate analysis was performed in order to study how impurities of silicon, boron and aluminum could affect the reactivity of the [^{18}F]fluoride ion during labeling. Specific salts of each element were tested at relevant levels in the radiolabeling of [^{18}F]FACBC. It was observed that only aluminum had a significant negative effect on ^{18}F-labeling yield; however, the effect was strong. In addition, an interaction effect between boron and aluminum was observed, as the negative effect from aluminum was reduced when boron was present.

3.3 PAPER III

It was discovered that eluent solutions of K_{222}, K_2CO_3 in aqueous acetonitrile, degrades upon storage. The aim of this study was to investigate the development and extent of degradation and how it could influence the ^{18}F-labeling yield in aliphatic substitution reactions.

Acetonitrile will at alkaline pH hydrolyze to acetamide and ammonium acetate. The hypothesis was that acetate may function as a competing nucleophile to [^{18}F]fluoride and thereby cause reduced ^{18}F-labeling yields. Two similar eluent solutions, optimized for the synthesis of [^{18}F]FDG and [^{18}F]FACBC, generated mg/ml levels of both acetamide and ammonium acetate after only weeks of storage at room temperature or above. The degradation of eluent led to a gradual decrease in RCY for the synthesis of [^{18}F]FDG and [^{18}F]FACBC, but the effect was much stronger with [^{18}F]FACBC.

Spiking studies with ammonium acetate revealed that coincidental factors like smaller volume of eluent and larger volume of labeling solvent made the synthesis of [^{18}F]FACBC more prone towards eluent degradation compared to the [^{18}F]FDG reaction. It was disclosed that the formation of acetate was the major cause of reduced yields, while the pH-shift in the eluent resulting from ammonium acetate formation only made a minor contribution.

After the cause of the degradation and reduced ^{18}F-labeling yields were identified, an alternative eluent with no acetonitrile was developed. A methanol based eluent was

successfully made in the synthesis of [^{18}F]FACBC, showing no degradation or change in ^{18}F-labeling yields after six months of storage at 50 ^{o}C.

3.4 PAPER IV

In this paper, the aim was two-fold. First, identify typical cationic contaminants present in irradiated [^{18}O]water after arrival into the hot cell. Secondly, investigate how these cationic contaminants interact with commonly used AECs, and if released from the AEC during [^{18}F]fluoride elution; investigate how these cations would affect the reactivity of the [^{18}F]fluoride during labeling.

Samples of irradiated [^{18}O]water were collected from seven different PET sites and screened for cationic impurities. Al, Ca, Mg and Zn were detected as significant impurities (0.02–5.34 ppm). A silica-based and a polymer-based AEC, was tested to study how these four cations were adsorbed and subsequently released from the AEC in an analogous fashion to [^{18}F]fluoride.

Aluminum demonstrated strong adsorption in both AECs tested, while zinc had variable degree of adsorption. Calcium and magnesium passed both AECs with no significant adsorption. In the following elution step, substantial levels of aluminum was released from the polymer based AEC (38–86%), while only to a limited degree from the silica-based AEC (1–10%). In either case, released aluminum had significant detrimental effect on the ^{18}F-labeling yield of the two benchmark reactions: [^{18}F]Flutemetamol and [^{18}F]FACBC.

4 EXPERIMENTAL CONSIDERATIONS

The GE FASTlab was used as an investigational platform for all radiochemistry performed. The software allows for pre-programmed synthesis with advanced and reproducible control over parameters such as temperature, volume of reagents and gas flow (Figure 4). In addition, the cassette-based module does not rely on manual cleaning between each radioactive run. The cassettes were only used once and thereby avoiding fluctuating results due to variable degree of cleaning between runs.

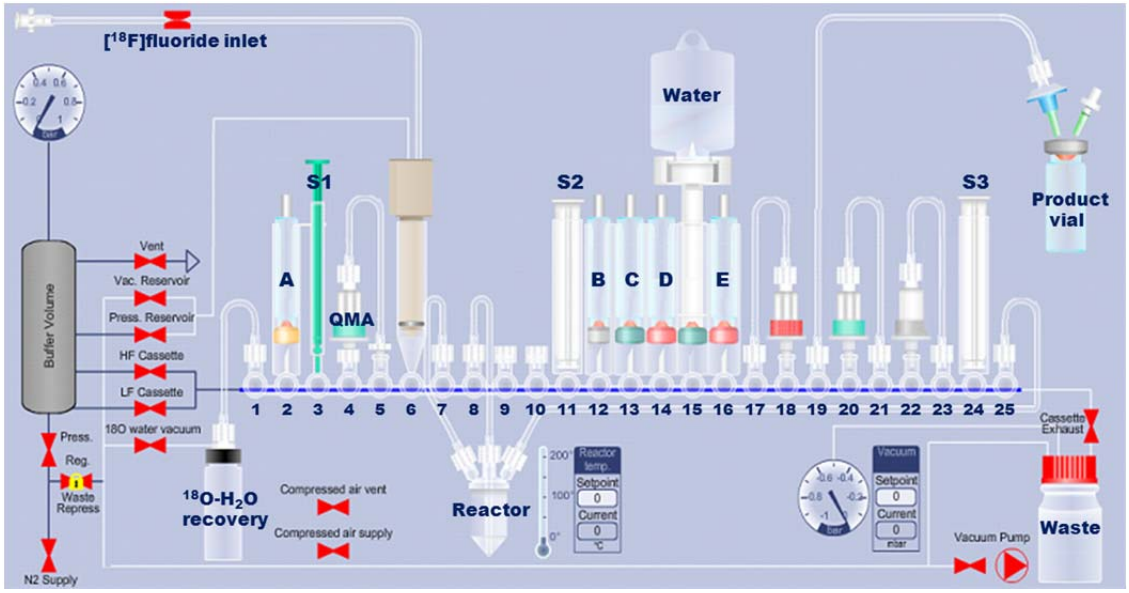


Figure 4. *General diagram of the FASTlab synthesizer. The cassette is built around a one-piece-moulded manifold with 25 three-way stopcocks, all made of polypropylene. Briefly, the cassette includes a 5 ml reactor (cyclic olefin copolymer), one 1 ml syringe (S1) and two 5 ml syringes (S2 and S3), spikes for connection with five prefilled vials (A-E), one water bag (100 ml) as well as various solid phase extraction (SPE) cartridges and filters. Fluid paths are controlled with nitrogen purging, vacuum and the three syringes. (This Figure is reprinted from paper III).*

The radiosyntheses of [^{18}F]FACBC, [^{18}F]FDG and [^{18}F]Flutemetamol were chosen as benchmark reactions in this work (Figure 5). These syntheses were suitable as each reaction offered high and consistent ^{18}F-labeling yields (> 65%± 1–2.3 standard deviations) (papers II–IV). Syntheses with high and consistent ^{18}F-labeling yield were beneficial in order to measure the effect of influential impurities with satisfying sensitivity. We used the [^{18}F]FACBC and

the [^{18}F]FDG reaction as both substances contains a similar S_N2 reaction with a typical triflate leaving group. The [^{18}F]Flutemetamol reaction was included as it represented a typical aromatic nucleophilic substitution reaction with a NO_2 leaving group.

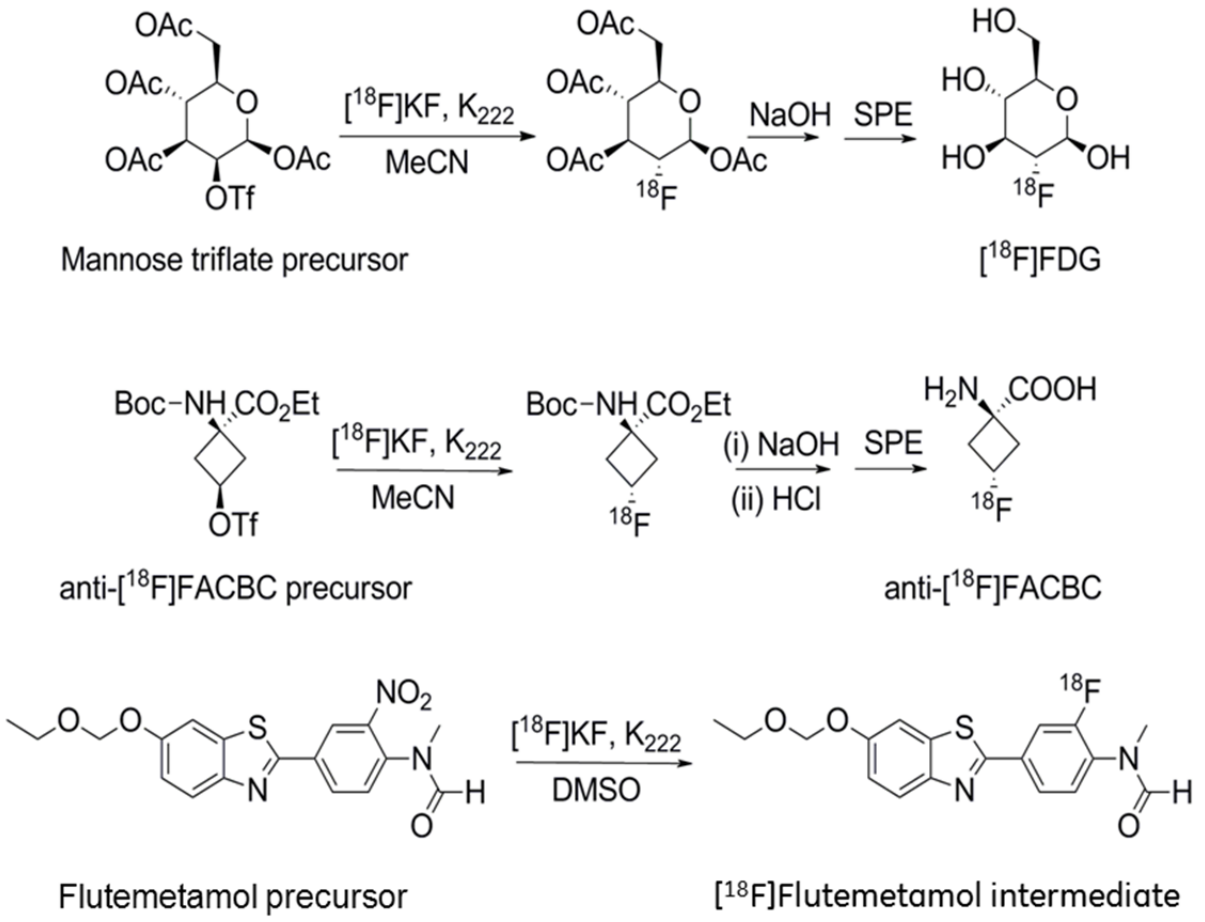
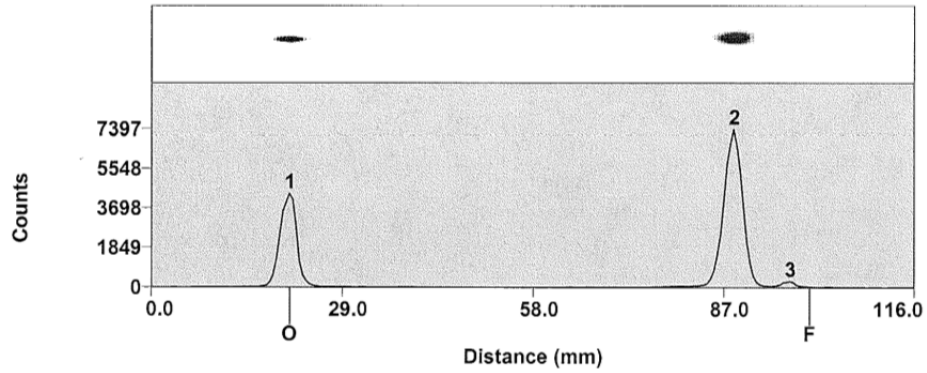


Figure 5. *Radiosyntheses used as benchmark reactions in this work. (Top) Radiosynthesis of [^{18}F]FDG. (Middle) Radiosynthesis of [^{18}F]FACBC. (Bottom) Radiosynthesis of [^{18}F]Flutemetamol intermediate.*

The use of experimental design, rather than testing one variable at the time, was an efficient approach in which several parameters were tested simultaneously. Full factorial designs were used to determine both main effects and possible interaction effects induced by the tested salts. The term radiochemical yield (RCY) was defined as: The yield of the ^{18}F-labeled compound expressed as a fraction of the [^{18}F]fluoride activity originally present (decay corrected). Note that in the experimental designs, the ^{18}F-labeling yield rather than end-of-synthesis yield was the measured response (papers II and IV). The primary objective was to

measure how the added impurities would influence the [^{18}F]fluoride reactivity and thus the ^{18}F-labeling yield was a more precise measurement as the subsequent work-up (removal of protection groups and purification) adds variation to the measured response due to natural variation in the hydrolysis of protection groups. In paper III, the end-of-synthesis yield was the measured response as it was hypothesized that the large amounts of acetamide and ammonium acetate could not only influence the labeling step but also the subsequent work-up. Radio-TLC was used to find the fraction of ^{18}F-labeled compound in order to calculate RCY in all radiochemistry. A radio-TLC of the reaction mixture in the ^{18}F-labeling of Flutemetamol intermediate is depicted as an example (Figure 6).



ID	Gross Counts	Baseline Subtract	Net Counts	Net CPM	Net % Sum Regions	Net % Total	Lane Rf	Label
Lane #2	Origin=21.00 Front=100.00							
2-1	26,854	275	26,579	88,596.66	33.8	33.6	0.000	
2-2	50,848	315	50,533	168,443.33	64.2	63.9	0.854	
2-3	1,723	110	1,613	5,376.67	2.0	2.0	0.962	
Lane	80,302	1,160	79,142	263,806.66		100.0		
Unres	877	460	417	1,390.00		0.5		

Figure 6. *Radio-TLC of reaction mixture after ^{18}F-labeling of [^{18}F]Flutemetamol precursor. Peak 1 is [^{18}F]fluoride, peak 2 is ^{18}F-labeled [^{18}F]Flutemetamol intermediate, and peak 3 is an impurity (ethyl acetate was used as mobile phase).*

In the experimental designs, added salts were used rather than native impurities. This was done in order to have a fixed design-space with good control over the tested parameters. The use of added salts does however involve use of a certain counter-ion in addition to the impurity of interest. There were two important aspects to consider when deciding what counter-ion to use. First, the chosen counter-ion must give a salt with sufficient solubility. Secondly, the counter-ion should not influence the reactivity of [^{18}F]fluoride by itself.

We deliberately chose chloride and sulfate as counter-anions in this work as these anions give salts with satisfactory solubility and little or no influence on [^{18}F]fluoride reactivity. It has been shown that anions such as chloride and sulfate can act as competing nucleophiles to [^{18}F]fluoride, but decrease of RCY occurred due to precursor consumption rather than affecting the [^{18}F]fluoride reactivity (Gatley, 1981; Gatley and Shaughnessy, 1982; Block et al., 1986; Alexoff et al., 1992). In this work, the maximum amount of added sulfate (1.1 μmol) and chloride (0.42μmol) was by far exceeded by the amount of either precursor used (72–75 μmol). Furthermore, since the additions of Ca, Mg and Zn as sulfate salts did not have a significant impact on RCY, this indirectly shows that the added sulfate did not have a noticeable impact on RCY. Presumably, the impact of chloride would be even weaker than from sulfate as it has been shown that sulfate is a stronger competing nucleophile to [^{18}F]fluoride than chloride (Gatley and Shaughnessy, 1982). In addition it has been shown that presence of 1.8 ppm of chloride in the irradiated [^{18}O]water does not have a significant impact on RCY of [^{18}F]FDG (Asti et al., 2007).

5 RESULTS AND DISCUSSION

At the time this project was initiated, there were few concrete explanations on why [^{18}F]fluoride chemistry often suffer from inconsistent yields. Although possible sources have been described through empirical observations and speculations in several papers, few systematic studies were found. The pitfalls described in paper I was used as a starting-point to investigate factors that most likely could render the [^{18}F]fluoride unreactive.

The use of borosilicate glassware, commercial available AECs and the irradiated [^{18}O]water product, were emphasized as areas of incomplete knowledge in [^{18}F]fluoride chemistry and were thus investigated as potential pitfalls in this thesis.

5.1 Impurities from borosilicate glassware and anion-exchange cartridges

Nearly all laboratory glassware is made of borosilicate glass. Borosilicate glass is one of the most durable materials in common use as it is highly resistant to corrosion from water and acids, and is stable at high temperatures (Doremus, 1979). Borosilicate glass has therefore been a natural choice also in automated systems for [^{18}F]fluoride chemistry. The container for non-irradiated [^{18}O]water, reagent vials and product collection vials are usually made of borosilicate glass.

It is known that reactor vessels made of borosilicate glass can lead to adsorption of [^{18}F]fluoride to the surface walls (Mudrova and Svoboda, 1972; Nickles et al., 1986). There are practical means of reducing this problem, but little is known of the actual causes of adsorption or how the measures taken to avoid it actually work. It was therefore a keen interest to investigate the use of borosilicate glassware more in depth in order to identify factors that may cause such detrimental effect and predictability of the [^{18}F]fluoride reactivity.

Normal borosilicate glassware (Pyrex-7740) consists of: 81% SiO_2, 13% B_2O_3, 4% Na_2O, 2% Al_2O_3 (Doremus, 1979). This type of glass is also referred to as Type 1 glass in the US and European pharmacopeias (EP, 2010; USP, 2011). Due to its composition, type 1 glass has a high hydrolytic resistance and is therefore suitable for both parenteral and non-parenteral pharmaceutical preparations (EP, 2010). In spite of being highly durable, type 1 glass is still

prone to hydrolytic attack of hydroxide ions at alkaline pH (Borchert et al., 1989). The work described in paper II was based on the hypothesis that all constituents of borosilicate glass could be found as water-soluble leachables or extractables.

The storage of a prefilled eluent vial containing aqueous MeCN, K_{222} and K_2CO_3 was studied in terms of extractables from the borosilicate glass wall. Although the vial of interest was specifically customized for use in a FASTlab, the results are of general importance as different types of borosilicate type 1 glass differ little in composition. Aluminum was the element of greatest interest as it is well known that aluminum binds [^{18}F]fluoride strongly (Clark and Silvester, 1966; Mudrova and Svoboda, 1972). Indeed, it was shown in paper II that significant levels of silicon, boron and aluminum were detected after only a week of storage at room temperature. Thereafter, levels of all three elements increased gradually with storage time. Thus, the results were in agreement with earlier observed alkaline dissolution of glass (Borchert et al., 1989).

The borosilicate v-vial commonly used to collect irradiated [^{18}O]water as it enters the hot cell was investigated as a source of water-soluble constituents of borosilicate glass. The tested v-vial included in this work is shown in Figure 8. The results showed that transfer of irradiated [^{18}O]water into the collection v-vial released ppm levels of silicon and boron with normal use. Furthermore, it was shown that the glass itself was the cause of these elements, as replacing the v-vial with a plastic vial resulted in no detectable levels (paper II). The mechanism for the leaching was not likely caused by alkaline dissolution, as measured pH from irradiated [^{18}O]water samples varied between pH 3.6-6.9 (paper IV). Instead, it is possible that the observed leachables could arrive from washing the v-vial between different syntheses. E.g. a glassware-washer could leave residuals of impurities on the glass-wall after cleaning.

The Sep-Pak® QMA light Accell Plus cartridge is a strong anion-exchange cartridge; containing 130 mg of silica-based, hydrophilic resins with quaternary methyl ammonium (QMA) functional groups. The cartridge is originally designed for extracting anionic compounds from aqueous or organic solutions, but in 1997, the group of Dr. Zhang described how the cartridge also was applicable for separating [^{18}F]fluoride from bulk [^{18}O]water (Zhang et al., 1997). Having excellent adsorption and elution capabilities, and being commercially available made it rapidly the preferred choice in [^{18}F]fluoride chemistry. Today, its use remain popular and can be exemplified in a wide variety of [^{18}F]fluoride PET tracer syntheses (Hockley and Scott, 2010; Li et al., 2010; Tang et al., 2010; Yao et al., 2010;

Bourdier et al., 2012). In spite of the widespread current use, this cartridge itself has received little scientific attention. The recommended pH for the Sep-Pak QMA ranges from pH 2-8, while in [^{18}F]fluoride chemistry the normal procedure is to condition the Sep-Pak QMA with aqueous potassium carbonate. We discovered that regardless of how well the cartridge was dried; there was always residual water present at the end of the drying (paper IV). This would result in an internal pH 11-12 or above, depending on the amount of residual water. The equilibrium between CO_3^{2-} and OH^- in the residual water may then lead to dissolution of the underlying silica due to attack from OH^-. Dissolution of silica could be observed already in freshly conditioned and dried cartridges. A gradual increase of dissolved silica then developed with storage time.

In order to evaluate the importance of the leachables and extractables from borosilicate glassware and the Sep-Pak QMA; an experimental design study was conducted to investigate correlations between the impurities and the reactivity of the [^{18}F]fluoride ion. Based on knowledge on how borosilicate glass dissolves, a full 2-level factorial design study was performed in which specific species of silicon, boron and aluminum were added at relevant ranges to the eluent solution and tested in a S_N2 substitution reaction with [^{18}F]fluoride. Careful considerations were made regarding the type of salts to include. The salts $AlCl_3$, KBO_2 and Na_2SiO_3 were chosen, as they are natural starting points in the most plausible speciation of dissolved glass in aqueous solutions. $AlCl_3$ forms free Al^{3+} ions, KBO_2 is an early intermediate when B_2O_3 is dissolved (Cotton and Wilkinson, 1988), and Na_2SiO_3 will form the monomers $SiO(OH)_3^-$ and $SiO_2(OH)_2^{2-}$ which are the main species when silica (SiO_2) dissolves in dilute alkaline solutions (Alexander et al., 1954; Tanakaa and Takahashib, 2001; Yang et al., 2008).

Of the main impurities originating from borosilicate glass, we have found that only aluminum negatively influenced the RCY in the tested S_N2 reaction (paper II). The effect from aluminum was however very strong. It was originally hypothesized that all three salts included in the study would be able to bind [^{18}F]fluoride. The reason being that from non-radioactive chemistry it has been described a wide diversity of conceivable species of fluorosilicates (Busey et al., 1980; Urbansky, 2002), fluoroborates (Mesmer et al., 1973; Mesmer and Rutenberg, 1973), and aluminum-fluoride complexes (Martin, 1988; Martin, 1996; Martinez et al., 1996; Scancar and Milacic, 2006). However, these complexes only exist at neutral or acidic pH. At pH > 5-8, these complexes will in general hydrolyze and release free fluoride (Wamser, 1948; Martinez et al., 1996; Urbansky, 2002). Therefore, in light of

the alkaline pH introduced by the $K_2CO_3/KHCO_3$ based eluent solutions used in this project; it was somewhat surprising to see how strongly aluminum would bind [^{18}F]fluoride, causing substantial reductions in RCY.

In order to assess the observed effects from aluminum one must consider aluminum´s behavior in aqueous solutions. For aluminum, the speciation occurs in a stepwise manner as pH shifts from acidic to alkaline. At pH <5, cationic species like Al^{3+}, $Al(OH)^{2+}$ and $Al(OH)_2^+$ exist, at neutral pH, aluminum merely exist as insoluble $Al(OH)_3$, and at pH >8, aluminum is completely converted into the water soluble $Al(OH)_4^-$ (Scancar and Milacic, 2006). When fluoride is present, it will compete with hydroxide ions for filling one or more of the four ligand positions in the ternary aluminum complex ($Al(OH)_nF_m$); e.g. species like $Al(OH)_3F^-$ or $Al(OH)_2F_2^-$ can be found at pH 7.5 (Martin, 1988; Martin, 1996). At around pH 8 and above, the hydroxide ions will displace the fluoride and free fluoride is reported to occur when pure water is used as a solvent (Martinez et al., 1996; Srinivasan et al., 1999). In our situation, the removal of water during evaporation of the eluent solution will increase the basicity of the [^{18}F]fluoride ion markedly since the strong solvation effect from water is gradually removed (Bessiere and Bazine, 1989). We believe that at some point during evaporation, the basicity of the [^{18}F]fluoride ion is strong enough to create Al-binding at pH 8 and above, causing unreactive [^{18}F]fluoride. From the experimental design study, we saw interaction effects in that the presence of KBO_2 counteracted the negative effect from aluminum. We can only speculate that BO_2^- would compete for Al-binding in a similar fashion as fluoride/hydroxide and thereby free [^{18}F]fluoride, which would then be available for labeling.

The use of sodium as the counter-cation to added silica was expected to have little influence on RCY of [^{18}F]FACBC. Although sodium is a harder cation than potassium, it has been shown that use of Na_2CO_3 rather than $CsCO_3$ give comparable RCY as with use of K_2CO_3 in the synthesis of [^{18}F]*p*-fluoronitrobenzene (Schlyer et al., 1993). The results in paper II confirmed that addition of 2.4–12 μmol of sodium as Na_2SiO_3 in the eluent solution did not have an impact on ^{18}F-labeling yield in the [^{18}F]FACBC reaction.

5.2 Degradation impurities

The storage of prefilled eluent vials revealed an issue that was not related to the container material. Eluent solutions of K_{222}, K_2CO_3 in aqueous acetonitrile degraded upon storage and influenced the RCY (paper III). Acetonitrile hydrolyzes at alkaline pH, forming acetamide and ammonium acetate in a two-step mechanism as shown in figure 7. Although alkaline hydrolysis of acetonitrile is well known in conventional chemistry literature (Chin, 1991), it had to our knowledge not been evaluated as an issue in relation to [^{18}F]fluoride chemistry. This could be attributed to eluent solutions traditionally being mixed manually at the day of a synthesis with minimal storage or that the degradation has not been realized as an issue for RCY. Modern PET centers and new automated systems utilize the benefit of making prefilled vials or bulk solutions that simplifies routine productions. The use of prefilled vials allows more well-defined, reliable and reproducible synthesis processes. In addition, prefilled vials can be made with a low bioburden and a documented shelf life, which serves as a better starting point for GMP compliance compared to manually mixing solutions on the day of synthesis.

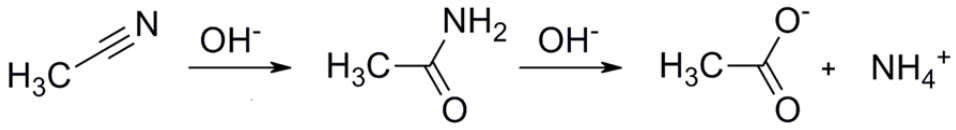


Figure 7*. Base catalysed hydrolysis of acetonitrile to acetamide and ammonium acetate.*

The investigation of the two prefilled eluent solutions revealed that hydrolysis was relatively fast and that storage temperature was of a major importance. For instance, storage at 5 ^{o}C resulted in negligible degree of hydrolysis, while storage at room temperature or above resulted in mg/ml levels of acetamide and ammonium acetate after few weeks of storage. The hydrolysis of acetonitrile creates two implications. First, the formation of acetate could affect the ^{18}F-labeling yield. It has been shown elsewhere that acetate works as an inhibitor when replaced with carbonate as the phase transfer catalyst (PTC) counter-ion (Liotta et al., 1974; Gatley and Shaughnessy, 1982). Secondly, acetamide is a well-known carcinogen and it is thus important to understand its formation and control it to acceptable low levels in the final

product. It was not believed that acetamide would negatively impact RCY as acetamide is a known [^{18}F]fluoride labeling solvent (Knust et al., 1982; Knust et al., 1986).

The findings in paper III confirmed that formation of acetate did cause significant reductions in RCY if present at sufficient milligram levels in the eluent solution. Although the formation of ammonium gradually decreased the pH of the eluent, it was shown through a spiking-study that acetate was the main reason for reductions in RCY (paper III). Most likely, the acetate anion would act as a competing nucleophile to [^{18}F]fluoride. However, several hundred ppm of acetate was needed in the labeling mixture to get significant RCY reductions in the two reactions investigated.

Although the two eluent solutions studied in paper III had relatively similar degradation profile, the impact on RCY differed substantially between the two S_N2 reactions tested. While storage of eluent led to a gradual decrease in RCY in the [^{18}F]FACBC reaction, the RCY in the [^{18}F]FDG reaction did not change to any extent before the eluent was stored at temperatures as high as 50 ^{o}C. It was discovered that coincidental factors like differences in volumes of eluent and labeling solvent made a substantial impact on the acetate concentration during labeling. Thus, smaller volume of eluent and larger volume of labeling solvent made the [^{18}F]FDG less sensitive to eluent degradation. This suggests that [^{18}F]FDG synthesis performed on different process rigs may be more prone to eluent degradation than observed in this study. For instance, a higher volume of eluent, would introduce higher amount of acetate into the reaction vessel.

It was a keen interest to develop an acetonitrile-free eluent, as acetonitrile was the mere reason for degradation. It was decided to modify the eluent solution for the [^{18}F]FACBC reaction by replacing acetonitrile with an alternative organic solvent. Methanol was considered the best candidate for several reasons. Methanol is much more resistant towards alkaline pH and therefore more suited for storage. Methanol-based eluents have demonstrated excellent eluting properties, together with a potential of shorter evaporation time as eluents can be made with 100% methanol. (Jewett et al., 1990; Lee et al., 2011; Seo et al., 2011). Ethanol was also tested, but it was not possible to achieve the same labeling yields as with fresh eluents based on methanol or acetonitrile. Hence, only methanol was investigated further as a candidate in developing an eluent that was more suitable for storage. The results did indeed show that methanol was a suitable organic phase for both storage and in use. The

methanol-based eluent showed no degradation and no difference in RCY even after 6 months of storage at 50 ^{o}C.

5.3 Cationic impurities

It has been suggested that cationic impurities such as Al^{3+} and Ca^{2+} may bind to [^{18}F]fluoride and form unreactive complexes (Nickles et al., 1986; Tewson et al., 1988; Tewson, 1989). In paper II, we investigated the presence of aluminum in the irradiated [^{18}O]water from one PET center. Although aluminum was not detected in these samples, we were yet determined to investigate other PET centers, as aluminum has been found as a contaminant at relatively high levels (up to 1.25 ppm) elsewhere (Avila-Rodriguez et al., 2008). Furthermore, the fact that aluminum binds fluoride stronger than 60 other metals (Martin, 1996), suggests that aluminum might serve be an important detrimental impurity in [^{18}F]fluoride chemistry. The strong bonding between [^{18}F]fluoride and aluminum has in recent years even been exploited as a promising labeling technique (McBride et al., 2009; McBride et al., 2010). In spite of this knowledge, it has not been investigated in detail what role aluminum and other cationic impurities such as Ca^{2+} and Mg^{2+} play when present in irradiated [^{18}O]water.

Analysis of irradiated [^{18}O]water from seven different PET sites revealed that only Ca^{2+}, Mg^{2+}, Zn^{2+} and Al^{3+} were detected as significant cationic impurities (0.02–5.34 ppm). There was a great variability between the sites; which in itself was interesting as it is a common belief that much of the variation in RCY observed between sites is due to different impurity-profiles of the irradiated [^{18}O]water. Somewhat surprisingly, transition metals such as Co, Cr, Fe, Ni, Mn, and Cu were not detected in any samples (unpublished results). Considering that all sites investigated used Havar foils which consists of: Co 42%, Cr 19.5%, Fe 19.3%, Ni 12.5%, W 2.6%, Mo 2.2%, Mn 1.7% and C 0.2%, it was expected to see some of these elements as detectable impurities. It has been shown in a recent paper that sputtering Havar foils with Nb or Ta increased the RCY of [^{18}F]FDG of around 5% (Wilson et al., 2008; Gagnon et al., 2011). Most likely, less etching of transition metals from the sputtered Havar foil was the reason for higher RCY.

From a total of 39 samples irradiated [^{18}O]water, only one sample contained detectable levels of aluminum. Despite this low occurrence, it was our opinion that Al should still be

considered as a potential contaminant for two reasons. First, we were unable to achieve good detection limits for Al when analyzing the samples with ICP-OES. While the typical detection limits for other elements were in the range of 0.02-0.05 ppm, the average detection limit for Al was as high as 0.15 ppm. Secondly, the pH of the irradiated [^{18}O]water samples were measured in the range of 3.6-6.9. In the neutral pH range, Al will form as the insoluble aluminum hydroxide ($Al(OH)_3$) (Scancar and Milacic, 2006). Around 50% of the measured samples had a pH in the range of which Al can form insoluble $Al(OH)_3$ and thus be unavailable for detection. Clearly, pH plays a major role for the presence of Al in irradiated [^{18}O]water. Presumably, Al could deposit on contact surfaces that later dissolve if pH is <5. Interestingly, it was found in a single experiment that adding aliquots of hydrochloric acid into an empty, but used [^{18}O]water borosilicate receiving v-vial, washed off 2 ppm of aluminum.

After identifying Al^{3+}, Ca^{2+}, Mg^{2+} and Zn^{2+} as potential impurities in irradiated [^{18}O]water, the next question was whether or not these cations would follow the [^{18}F]fluoride into the reaction vessel after passing the AEC. The use of an AEC has been suggested as a mean for removing cationic impurities in addition to separating the [^{18}F]fluoride from the [^{18}O]water (Nishijima et al., 2002). The rationale being that positively charged species would pass the cartridge while the [^{18}F]fluoride is trapped. The results in paper IV showed that this assumption was too simplistic. The carbonate and bicarbonate salts used for conditioning the cartridge will influence the solubility of the cationic impurities. For instance, while $CaCO_3$ and $MgCO_3$ have sufficient solubility to avoid precipitation, $ZnCO_3$ did most likely precipitate; explaining why Zn was retained on the AEC. In addition, the shift in pH can substantially affect the speciation of the cation in solution. It was shown that aluminum was fully retained on an AEC since any cationic form of aluminum will transpose to either insoluble $Al(OH)_3$ or anionic $Al(OH)_4^-$ (paper IV). In the subsequent elution step, it was shown that aluminum and zinc could be co-eluted with [^{18}F]fluoride to the reaction vessel. Amount of eluted aluminum was however much higher in the polymer-based anion exchange cartridge. The factors involved in creating this difference was not investigated, but such a difference points out how sensitive a synthesis can be towards differences in the process setup.

Some groups routinely wash the AEC after [^{18}F]fluoride adsorption by flushing the cartridge with aliquots of either water or acetonitrile before elution of the [^{18}F]fluoride (Toorongian et al., 1990; Kim et al., 2004). Our experience was that such flushing did not change the degree

of eluted aluminum. Flushing with acetonitrile did however shorten the subsequent drying time with a few minutes (unpublished results).

In order to evaluate the importance of the detected cationic impurities found in paper IV; two full factorial 2-level designs were used to investigate how Al^{3+}, Ca^{2+}, Mg^{2+} and Zn^{2+} could influence an aromatic or an aliphatic substitution reaction with [^{18}F]fluoride. The setup was similar to the design study in paper II, except that the salts were added to the aqueous [^{18}F]fluoride rather than in the eluent vial. This entails that the salts must first be adsorbed and then released from the AEC in order to make an impact on the RCY. In addition, a silica-based AEC was used instead of the polymer-based AEC used in paper II. Again, it was shown that aluminum had a strong negative effect on the RCY. The use of the silica-based rather than the polymer-based AEC greatly influenced the results. The polymer-based AEC released a much larger fraction of retained aluminum (38–86%) compared to the silica-based AEC (1–10%). It was therefore logical that the RCY in the [^{18}F]FACBC reaction was reduced more when the polymer-based AEC was used (paper II). In the [^{18}F]Flutemetamol reaction, using a silica-based AEC conditioned with K_2CO_3 was enough to cause a strong detrimental effect on RCY even though only ~6% of the added aluminum in the aqueous [^{18}F]fluoride reached the reactor vessel. Such a result demonstrates how potent impurity aluminum could be and careful avoidance is therefore an important precaution that should be taken in [^{18}F]fluoride drug manufacturing.

In total, these results show that cationic impurities in the irradiated [^{18}O]water cannot be neglected even though an AEC is in place. Impurities arriving from the cyclotron are especially important threats as they are by large not dependent on the type of synthesizer used.

6. CONCLUSIONS

In this thesis we have investigated a typical synthesis setup in search for common pharmaceutical-chemical impurities that may influence labeling yields in nucleophilic substitution reactions with [^{18}F]fluoride. The overall findings illustrate the diversity of issues that may influence the [^{18}F]fluoride chemistry.

Aluminum was identified as a potent impurity with a strong detrimental effect on RCY in nucleophilic substitution reactions with [^{18}F]fluoride. Aluminum, if present as a contaminant during labeling, might be an important contributor to inconsistent ^{18}F-labeling yields. Possibly the best strategy to handle aluminum contamination is by removal rather than taking extensive actions to prevent its introduction. For instance, a cation-exchange cartridge positioned upstream to the AEC may be sufficient to remove aluminum and other cationic impurities present in the irradiated [^{18}O]water.

The use of borosilicate glassware as a mean of storing alkaline eluent solutions with K_2CO_3 caused extractables to be released from the glass wall; releasing ppm levels of silicon, boron and aluminum. It was shown that aluminum present in the eluent solution could pass a polymer-based AEC and cause detrimental effect on RCY. These results suggest that alkaline eluent solutions should be stored in either borosilicate glass that is treated to withstand the alkaline pH or in alternative container materials.

Eluent solutions with K_{222}, K_2CO_3 in aqueous acetonitrile were found unsuited for storage at room temperature or above as the alkaline pH caused hydrolysis of acetonitrile to acetamide and ammonium acetate. Acetate at sufficient levels (several hundred ppm) acted as a negative inhibitor in nucleophilic substitution reactions with [^{18}F]fluoride. Methanol can replace acetonitrile without negative effect on radiolabeling and without detrimental degradation.

Calcium, magnesium, zinc and aluminum were identified as potential impurities in irradiated [^{18}O]water samples. The use of an AEC hindered Ca and Mg from subsequently entering the reaction vessel. Al and Zn could to a variable degree be co-eluted with [^{18}F]fluoride depending on type of AEC used. Eluted Al had a strong detrimental effect on RCY.

7. FUTURE PERSPECTIVES

One of the most notable findings in this study was the strong negative effect aluminum made on RCY in nucleophilic substitution with [^{18}F]fluoride. There is a need to further enquire the extensiveness of aluminum contamination and do a wider study of the contamination level at different PET centers — including parameters such as cyclotron type, targetry type and age, transfer line materials, washing routines etc. From literature, we were only able to find one paper of which aluminum was explored as a contaminant in irradiated [^{18}O]water (Avila-Rodriguez et al., 2008). In our work, the achieved detection limit for aluminum was relatively high and as a consequence we were not able to detect aluminum in the range of 0–0.14 ppm. It was shown that aluminum could significantly decrease the RCY even at this low range.

It was demonstrated that the commonly used Sep-Pak® QMA light Accell Plus cartridge would free relatively large amounts of silicon-based leachables if pre-conditioned with K_2CO_3. Although our results suggest that dissolved silica would not influence the tested [^{18}F]fluoride chemistry, it is still not optimal to allow for such hydrolysis. Either, a less alkaline counter-ion should be used for pre-conditioning or consider an alternative anion-exchange material that withstand the alkaline pH from K_2CO_3 conditioning. Furthermore, it would be beneficial to considerably reduce the amount of AEC resin. Perhaps a valuable strategy is to develop a smaller AEC in conjunction to a cation-exchange purification cartridge. It has been reported that inclusion of a cationic-exchange cartridge upstream of the AEC can greatly enhance the trapping efficiency of [^{18}F]fluoride (Tewson et al., 2004). A small amount AEC resin (<10 mg) should be optimal for microfluidic devices. As already mentioned earlier, there is a current need to improve the pre-concentration of [^{18}F]fluoride in microfluidic devices in order to create complete integrated stand-alone microfluidic systems.

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Paper I

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Paper IV

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