Original Article

Significance of Cartridges and Resins used in a Purification Column during ^{18}F-fluorodeoxyglucose Synthesis

Abstract

Aims and Objective: The aims and objective of this study to share the 15 years of working experience regarding importance of cartridges and resins used in a Purification Column during 18F-FDG synthesis. **Materials and Methods:** 18F- flourodeoxyglucose (18F-FDG) is common radiotracer used in positron emission tomography (PET). 18F-FDG is synthesized in a PET radiochemistry synthesis module and purification column is an integral part of 18F-FDG synthesis. The purification column has four integral parts, namely of ion exchange retardation resin, cation exchange resin, C18 bonded silica, Neutral Alumina. **Conclusion:** The purification column plays a very important role during the synthesis of 18F-FDG. If all parts of the purification column are intact, then maximum transfer of produced 18F-FDG from reaction vessels to the final product vial takes place. The total yield of 18F-FDG is also dependent upon the purification column. If all components of purification column placed tightly and properly charged then there is a high possibility of maximum yield of final product without impurities.

Keywords: *Alumina, C-18, Ag-50, Ag-11, 18F-FDG, PET, purification column, radiochemistry module*

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Introduction

The most common radiotracer in use today is ^{18}F-fluorodeoxyglucose (^{18}F-FDG) which is a radiolabeled sugar molecule. It is glucose sugar labeled with radioactive fluorine-18.[1] Although it behaves as glucose in many situations, some important differences should be understood. Like glucose, ^{18}F-FDG is taken up into living cells by facilitated transport and then phosphorylated by hexokinase. Unlike glucose, ^{18}F-FDG cannot undergo further metabolism because the hydroxyl group at the 2-carbon is a requirement for the process. Intracellular accumulation of ^{18}F-FDG is a good indicator of glucose uptake and cell viability.[2,3]

^{18}F-FDG is the most commercially successful and widely employed radiotracer in the field of positron emission tomography (PET) imaging. ^{18}F-FDG whole-body PET-computed tomography is commonly performed for cancer staging and follow-up, evaluation of myocardial viability or sarcoidosis, and assessment of neurological conditions including epilepsy and dementia.[4]

The purification column is an integral part of ^{18}F-FDG synthesis in any PET radiochemistry module [Figures 1 and b, 2]. It is placed inside the radiochemistry module to remove all impurities by absorbing them during the final stage of ^{18}F-FDG of synthesis. It is consisting four-part cation-exchange resin, ion retardation resin, neutral alumina, and C18-bonded silica. All four parts of the purification column are having different properties, and they are removing different types of impurities. In some radiochemistry, module cartridge is placed in the purification column, and in some modules, it is placed individually with a different brand name, but the purpose is the same to remove all impurities.

^{18}F-fluorodeoxyglucose Synthesis

^{18}F-FDG can either be produced by electrophilic fluorination reaction or by nucleophilic substitution reaction. Nowadays, nucleophilic substitution reaction for ^{18}F-FDG synthesis has become the method of choice over electrophilic synthesis due to its higher yield.[5] During the synthesis of ^{18}F-FDG, ^{18}F produced in a medical cyclotron is trapped on an anion exchanger [quaternary ammonium anion exchange in Figure 3] which is released by a stock solution/tetrabutylammonium

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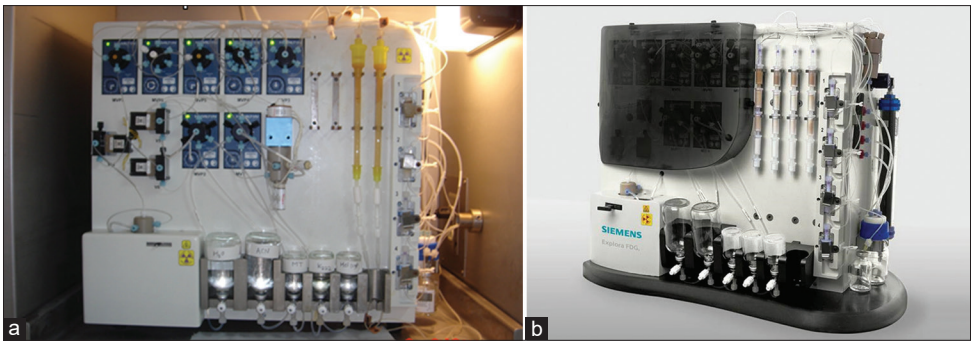

Figure 1: Pictures of Explora fluorodeoxyglucose-4 for synthesis of ^{18}F-fluorodeoxyglucose with help of purification column: (a) Ready-made purification column, (b) Manual purification column

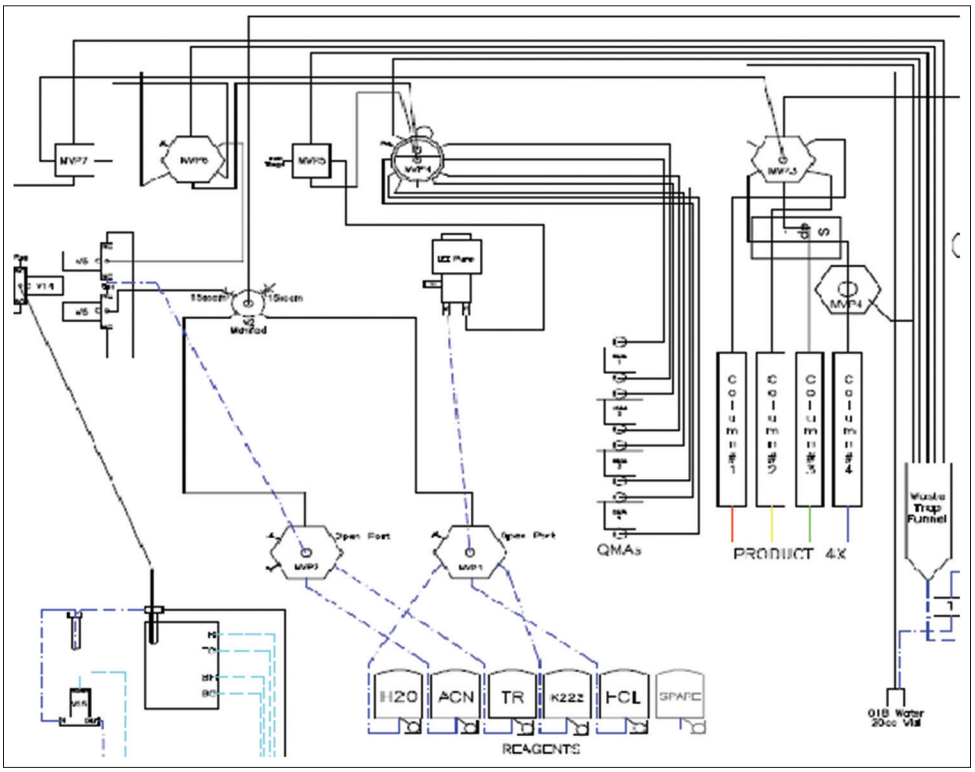

Figure 2: Schematic diagram of Explora fluorodeoxyglucose-4 for synthesis of ^{18}F-fluorodeoxyglucose with the help of four purification columns

bicarbonate ($TBAHCO_3$) into the reaction vessels. The use of Kryptofix or TBA depends upon the manufacturer. The stock solution is a mixture of Kryptofix, potassium carbonate, acetonitrile, and water. In this solution, ^{18}F is in hydrous condition; in hydrous condition, ^{18}F is unreactive, so it must be converted into an anhydrous form. Hence, the solution is mixed with acetonitrile and azeotropically dried. The vapors generated during the reaction are stored in the waste gas system**.** Now, ^{18}F is ready for a reaction, so the addition of acetylated sugar derivative (mannose triflate dissolved in acetonitrile) takes place, and the mixture is heated in a closed system. Nucleophilic substitution reaction happens and acetylated ^{18}F-FDG is formed. The final stage of synthesis is hydrolysis. In acidic hydrolysis, hydrolysis takes place by refluxing it with hydrochloric acid, which removes all the acetyl protecting group from fluorinated glucose in the reaction vessels and is separated using cartridge and resin in the purification column. ^{18}F-FDG obtained is in crude form. To get a purified form, ^{18}F-FDG must be passed through the purification column, which absorbs all the impurities present in the solution and collected them into a product vial through a 0.22-µm filter. The collected ^{18}F-FDG is ready to inject after the quality control test. The average yield of the product was approximately 60% or above.

Significance of Purification Column

Purification of the final ^{18}F-fluorodeoxyglucose product

Purification of the final ^{18}F-FDG can be performed by purification column which is having a series of cation- and anion-exchange column, C18 reversed-phase column, and

alumina column. Most automatic synthesizers can produce ^{18}F-FDG of radiochemical purity over 95% routinely.

In the purification column, a series of resin and cartridges were used. From top to bottom, cation-exchange resin (Ag50), ion retardation resin (Ag11), neutral alumina, and C18-bonded silica were used [Figure 4].

The component used in Purification Column

AG50 resin

This is an example of strong acid cation-exchange resin. It consists of sulfonic acid functional groups attached to the styrene-divinylbenzene lattice. The amount of resin cross-linking determines the bead pore size and hence the permeability to high-molecular-weight substances. Higher cross-linking (e.g., AG50W-X8 8% resin [Bio-Rad]) has a lesser wet diameter, so it swells less.[6] They have been used for cation removal from the monosaccharide (AG50W-X8 resin for Kryptofix). It is also autoclavable and stable in acid, base, and organic medium. It should be kept away from ultraviolet (UV) light and strongly oxidizing solutions.

AG11 resin

This is an example of ion-exchange retardation resin. A mixed bed of ion-exchange resins can be used for achieving the desalination of high-molecular-weight molecules. Usually, the bead size and surface of these resins are modified to achieve the removal of small cations and anions (<1000 Da), while allowing passage of large organic molecules. These can result in acidification of the final solution.[7] However, ion retardation resins uptake ions by absorption, thus bringing about desalination without pH change. AG11 A8 resin (Bio-Rad) is made by polymerizing acrylic acid inside AG 1-X8 resin to produce spherical beads containing paired anion (quaternary ammonium)- and cation (carboxyl)-exchange sites.[8] It has been used for the removal of HCl from the labeled product. It is stable for 5 years at room temperature. It is also autoclavable and stable in acid, base, or organic medium. It should be kept away from UV light and strongly oxidizing solutions.

C18 column

These are Sep-Pak cartridges. It consists of molecules with 18 carbon atoms as stationary phase, bound to silica. The other atoms can vary, leading to different characteristics among different preparations within this group. It is employed for reversed-phase chromatography using hydrophobic stationary phase to adsorb nonpolar molecules. Elimination of K222 requires adsorption of ^{18}F mannose tetra-acetate intermediate on C18 cartridge. Thus, it also provides solid support to the intermediate for hydrolysis.[9,10]

Alumina N

Alumina (Al_2O_3) provides an extremely polar surface for retention, similar to silica, but it is more stable in high pH. These cartridges contain a highly active grade of alumina

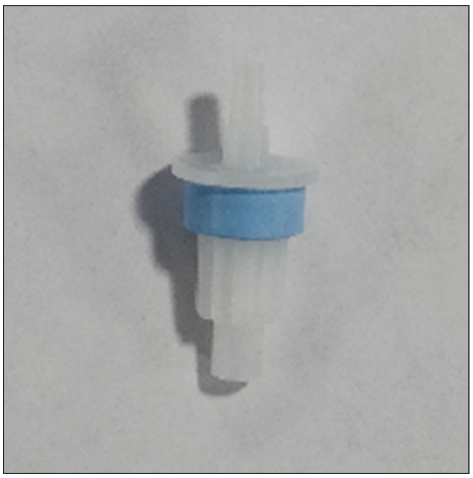

Figure 3: Picture of quaternary ammonium anion

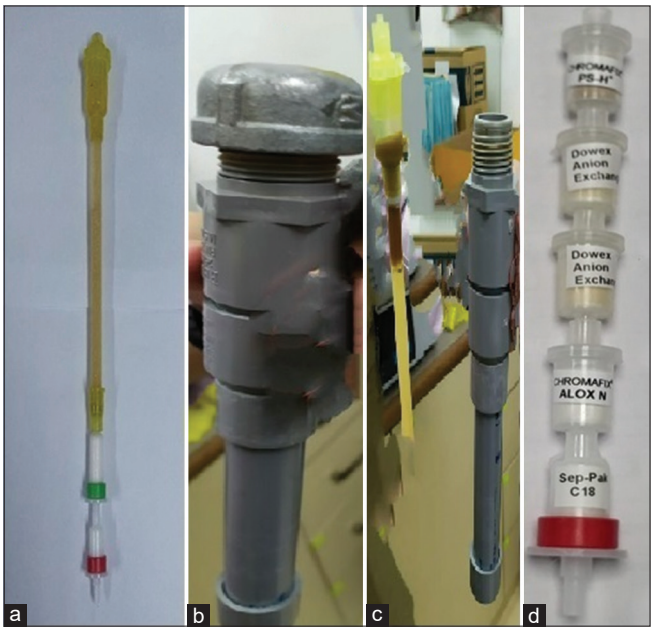

Figure 4: (a) Picture of purification column, (b) Purification column holder, (c) Purification column with its holder, (d) Ready-made column

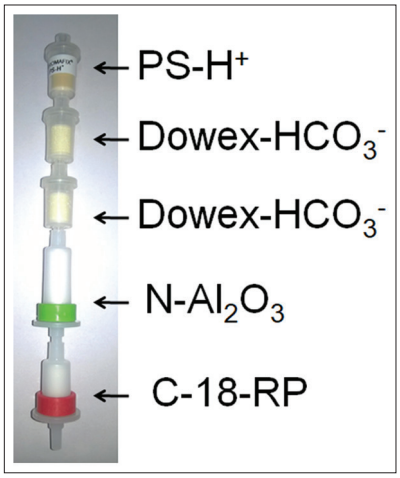

Figure 5: Component of purification column

with neutral surface (N) chemistry. It is also used for the purification of unlabeled 18-fluorine [Figure 5].

Preparation of Purification Column for ^{18}F-Fluorodeoxyglucose Purification

There are two types of purification columns available, the glass purification column and the ready-made purification column. In the glass purification column, both resins are dissolved in sterile water in the different beakers and filled in the glass column. The lower part of the glass column is filled with ion retardation resin (3 gm/column), and above it, cation-exchange resin (1.5 g/column) is placed. The approximate length of AG11 is 10 cm and AG50 is 4–5 cm in the glass purification column. Alumina is flushed with 10-ml sterile water. C18 is flushed with 5-ml ethanol followed by dry air after that 10-ml sterile water followed by dry air. Now, it is ready for assembly and arrangement according to Figure 2. The total time taken in the preparation of four purification column sets is about 30 min.

In the ready-made purification column, it is used readily available SPE cartridges such as PSH + cation exchanger, C18 reversed-phase, and neutral alumina. Both cation exchanger and C18 cartridges were used after conditioning with 10 mL of absolute ethanol followed by 20 mL of sterile water for injection (SWFI). Alumina cartridge is conditioned by passing 60 mL of SWFI in the reverse direction before its use so that it should not block its path.

Anion exchanger were prepared by filling 850-900 mg of Dowex (1×8, in Chloride form) in empty barrel and were conditioned by passing 20 mL of saturated sodium bicarbonate solution. The barrel is then rinsed with 20 mL SWFI to remove excess of sodium bicarbonate. After that 10 mL absolute ethanol was passed in the same barrel followed by 40 mL of SWFI to remove ethanol. The combination of different cartridges from top to bottom, used in sequence were cation-anion-alumina-C18.

Cation removal

Macherey-Nagel Chromafix PS-H + SPE Cartridge is a strong PS/DVB cation exchanger polymer-based SPE phase. It functions over the whole pH range from 0 to 14

Anion removal

For removal of anion, anion exchanger was used. DOWEX (1X8 chloride form, having mesh size 100-200) has strong anion exchange capacity of 1.2 milliequivalent/mL and act as strong anion exchanger. Anion exchanger Dowex 1 × 8 chloride form was converted to bicarbonate form before use. During the solid-phase extraction process, the bicarbonate anion exchanger plays dual roles. Primarily, it neutralizes hydrochloric acid used for precursor hydrolysis ($HCl + NaHCO_3 \rightarrow NaCl + H_2O + CO_2$), and secondarily, it traps unreacted fluoride ions. Incomplete neutralization of hydrochloric acid leads to the dissolution of alumina from the cartridge as aluminum chloride and comes into a final product. Hence, the quantity (number of milliequivalents) and quality of bicarbonates in an anion exchanger are very important. Degradation in the quality of bicarbonate anion exchanger, due to long time storage, may lead to inferior radiochemical purity as well as leaking of soluble alumina into the final ^{18}F-FDG solution. For anion exchanger, commercially available Dowex resin (chloride form) can be used because it has a higher milliequivalent capacity and can be used in acidic pH. Dowex resin (chloride form) was freshly converted into bicarbonate form before its use. The quantity of Dowex was optimized to neutralize hydrochloric acid. Since the anion exchanger is freshly conditioned before its use, it does not lose its potential in neutralizing acid and efficiently trapping unreacted fluoride ions. Hence, it maintains consistency in product quality.

Usually, there is no leakage from the purification column, if it is properly tightened. It was experienced that if the purification column was not tightening properly, it might be possible that it leaks. The leak may be from any part of the purification column, it may be from the upper part of the column, the glass part of the column, or the cartridge of the purification column. To prevent leakage, it should change the glass column on a regular basis. The time duration of the change of the purification column purely depends upon the usage and experience of the PET radiochemist. To avoid leakage, some centers use purification holder [Figure 1b], to hold the purification column. By using this purification holder, possibility of leakage of synthesized 18F-FDG from the purification column becomes very less.

Quality Control of ^{18}F-Fluorodeoxyglucose

Since it is going to inject ^{18}F-FDG into the human being, so quality control is mandatory. As purification column is consisting AG resin (AG50 and AG11), C18, and alumina, which removes all impurities present in the final produced 18F-FDG [Table 1]. The patency of the purification column is checked by the quality control process. It tells us about the functional capability of the purification column. If purification column is made and works perfectly, it will absorb all impurities present in the produced ^{18}F-FDG. The requirements of quality control of ^{18}F-FDG are varied from country to country. The various pharmacopeia are United

Table 1: Parts of purification column with its capacity of impurity absorption during synthesis of ^{18}F-fluorodeoxyglucose in an acidic hydrolysis radiochemistry module

Removing part of the purification column	Impurities
Cation-exchange resin (Ag50)	$[K/K222]^+$ complex/TBA complex
Ion retardation resin (Ag11)	Excess acid
Neutral alumina	Unreacted 18-fluoride
C18-bonded silica	Nonpolar species (traces of the tetra-acetyl [^{18}F] FDG intermediate)

FDG: Fluorodeoxyglucose, TBA: Tetrabutylammonium

States Pharmacopeia, British Pharmacopeia, European Pharmacopeia, Indian Pharmacopeia, etc.

The tests of Kryptofix involve spotting the test solution and the reference standard on a thin-layer chromatography (TLC)-silica gel plate and then developing the plate in a mixture of methanol and ammonia (9:1 v/v). The developed plate is then exposed to an iodine vapor chamber. The test solution spot should have a color lighter than the reference solution spot. However, this TLC method is unreliable. The spots can be indistinct. Alternatively, Kryptofix can be determined by placing the TLC plate in an iodine chamber directly or by gas chromatography.

Radiochemical purity is to prove the absence of unreacted 18-fluorine, partially acetylated ^{18}F-fluorodeoxyglucose derivative, or other labeled sugar. TLC is used to detect residue of Kryptofix and unreacted 18-fluorine by 9:1 methanol: ammonium hydroxide and 95:5 acetonitrile: water, respectively. The pH is checked by either a pH meter or pH strip.

Crude ^{18}F-FDG-Purification-^{18}F-FDG

After passing from the purification column, the crude ^{18}F-FDG was converted into pure ^{18}F-FDG. During the process of purification, the purification column absorbs all the impurities such as (K/K222) + complex/TBA complex, excess acid, unreacted 18 flourine, and nonpolar species (traces of the tetra-acetyl ^{18}F-FDG intermediate). The ^{18}F-FDG is sterilized during the heating process of the reaction. Hence, heating plays two roles, completion of the reaction as well as sterilization of the product. Finally, it is passed through a membrane filter into a vial, containing sufficient sodium chloride and buffer solution to render the final solution isotonic and ready for injection.[11]

Determination of TBA + Ion in the Final Product

Test for TBA + ion concentration in the final ^{18}F-FDG solution was performed by color spot test following the reported procedure. The color developed with the standard solution was visually compared with the samples. According to the reported procedure, 2 µL of sample is spotted in TLC plate and dried in hot air. 10 µL of ammonium hydroxide: methanol (1:9) was added to the dried spot. The plate was placed inside iodine chamber immediately for 1 min. The intensity of color developed was directly proportional to the TBA + ion concentration. TBA + concentration was found below the allowed limit (<2.6 mg/volume injected).[12]

Conclusion

As impurities present in the produced ^{18}F-FDG are unnecessary and may be harmful for biological use. All four components of the purification column play a very important role in removing all impurities present in the finally produced ^{18}F-FDG. It absorbs all the impurities present with ^{18}F-FDG and purifies it. Cation-exchange resin (Ag50), ion retardation resin (Ag11), neutral alumina, and C18-bonded silica help remove (K/K222) + complex/ TBA Complex, Excess acid, unreacted 18 flouride and Non-polar species (traces of the tetra-acetyl [18F] FDG intermediate) respectively. The leak may happen from any part of the purification column, especially from its top. During the final delivery, it was observed that final product i.e. 18F-FDG may leak from its cap, if it is not tighten properly. If the purification column is functioning properly, all the synthesized activity is collected in the collection vial, otherwise, there are chances of leak of the maximum amount of produced ^{18}F-FDG. The distinct advantage of using the SPE method using readily available solid phage cartridges and resins for routine ^{18}F-FDG synthesis in automated radiochemistry module is reliability and consistency.

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Conflicts of interest

There are no conflicts of interest.

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