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# MODIFICATION OF $^{18}$F-FLUORODESOXY-GLUCOSE ($^{18}$F-FDG) RADIOPHARMACEUTICAL BY OXIME CONJUGATION

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***Abstract.*** *The isotope $^{18}$F is one of the attractive positron emitters with commercial cyclotron production by the following nuclear reaction $^{18}$O (p, n) $^{18}$F. Basically, the radionuclide $^{18}$F is used for the production of $^{18}$F-labeled radiopharmaceuticals applied in positrone-emission tomography (PET). The most widely used among them is $^{18}$F-fluorodeoxy-glucose ($^{18}$F-FDG). $^{18}$F-FDG as glucose analog can be used to assess the metabolism in the brain and heart, and also to study malignancies. It plays an important role in the planning of radiation therapy for pathologies such as lung cancer, head and neck cancer, colon cancer. $^{18}$F-fluorodeoxy-glucose has been used in recent years as a prosthetic group for indirect radiofluorination of biomolecules such as peptides and proteins under relatively mild reaction conditions, which allows the development and synthesis of more specific PET radio tracers. A method has been developed to directly modify $^{18}$F-FDG in the clinic environment and equipment. Simple and reliable procedure was done with formation of an oxime chemical bond with a bifunctional compound. The optimal reaction conditions were carried out by varying the buffer, temperature and catalyst used. The progress of the reaction is monitored by radio TLC - chromatography.*

***Keywords:*** *Radionuclide $^{18}$F, PET-CT, $^{18}$F-FDG, prosthetic group, oxime formation, click chemistry, tetrazine*

## 1. INTRODUCTION

$^{18}$F is one of the attractive radionuclides used in positron emission tomography. Most often, for the purposes of radiopharmaceutical synthesis, $^{18}$F is obtained in a cyclotron by proton bombardment of $^{18}$O enriched water by the reaction $^{18}$O(p,n)$^{18}$F. $^{18}$F is a positron emitter and its half-life is about 110 minutes [1]. Its main application is for the production of $^{18}$F-labeled radiopharmaceuticals. The decay of the radionuclide leads to the production of $^{18}$O by the following reaction:

$$^{18}_{9}F \rightarrow {}^{18}_{8}O + e^{+} + \upsilon$$

Positron emission tomography is used for imaging diagnostics of various diseases. Its principle consists in the detection of radioactive elements - positron emitters, as a result of which two- and three-dimensional images of the spread of radioactivity in the patient's body are reconstructed. PET-CT is considered to be one of the imaging techniques that provide quantitative information about biochemical and physiological processes. The technique can not only give a complete assessment of the patient's condition, but also give guidelines for personalized therapy. It makes it possible to accurately determine the site of biopsy, to assess the effect of treatment already performed [2].

## 2. $^{18}$F-FDG - CHARACTERISTICS AND METHODS OF SYNTHESIS

The most widely used PET radiopharmaceutical in most Nuclear Medicine Centers is $^{18}$F-deoxyglucose ($^{18}$F-FDG). $^{18}$F-FDG is an analog of glucose and enters cells by the same mechanisms. It is also phosphorylated to give $^{18}$F-FDG-6-phosphate. And here is the difference in the behavior of the phosphorylated analogue. The second enzyme of the glycolytic cycle cannot act on $^{18}$F-FDG-6-phosphate because it lacks the OH group at the second C-atom and therefore remains in the cytoplasm of the cell. And this is what is used to obtain images in PET diagnostics. Cancer cells use more glucose than normal cells [3]. The structure of the molecules is shown in Figure 1.

The use of PET-CT with [$^{18}$F]-FDG plays an important role in the planning of radiation therapy in pathologies such as lung cancer, head and neck cancer, colon cancer, Hodgkin's lymphoma [4]. $^{18}$F-FDG allows assessment of glycolytic activity, which is more potent in tumor cells than in normal cells. It is also used in the

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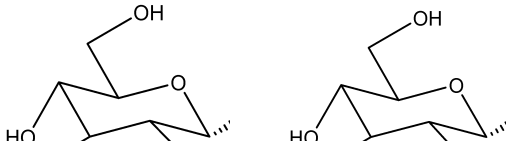
assessment of heart and neurological diseases. Fluorodeoxy-glucose [$^{18}$F]FDG is used as a universal marker in the study of any type of tumor. Nevertheless, it is not a highly tumor-specific radiopharmaceutical. There are other physiological processes in which large amounts of glucose are absorbed, such as infectious or inflammatory processes, which can give false positive results [5]. This is a major reason to seek and develop new, more specific radiopharmaceuticals based on [$^{18}$F]FDG.




glucose $^{18}$F-fluoro-desoxyglucose

Figure 1. Structure of glucose and fluoro-deoxyglucose

The synthesis of [$^{18}$F]FDG can be carried out by both electrophilic and nucleophilic radiofluorination mechanisms. The first synthesis of [$^{18}$F]FDG was based on electrophilic radifluorination with a fluorinating reagent [$^{18}$F] $F_2$ or with the less reactive electrophile [$^{18}$F] FOAc [6]. This method of synthesis was developed by Reivich et al., 1979 [7], who used 3,4,6-tri-O-acetyl-D-glucal as a precursor. The resulting difluoroglucose was isolated and subjected to hydrolysis with hydrochloric acid to give the desired product 2-fluoro-2-deoxyglucose [8]. The synthesis of $^{18}$F – FDG can also be performed by nucleophilic fluorination. One variant was developed by Hamacher et al., 1986 [9], who used the so-called Kryptofix222 (K222) as a catalyst for the reaction. [10]. Mannose triflate is used as a precursor. Nucleophilic fluorination is the more commonly used method for the synthesis of $^{18}$F-FDG due to the higher yield, better selectivity and the possibility of easy automation [8].

## 3. $^{18}$F – FDG - APPLICATION AS A PROSTHETIC GROUP

Apart from being a universal PET radiopharmaceutical, $^{18}$F-FDG can also be used as a prosthetic group for indirect labeling of biomolecules such as peptides, proteins and others under relatively mild reaction conditions. Despite the convenient automated synthesis and good availability of [$^{18}$F] FDG, there are few examples using the molecule as a prosthetic group for indirect radiofluorination. The introduction of [$^{18}$F] FDG as a sugar moiety into peptides may improve the pharmacokinetics of radiolabeled products [11]. Labeling of peptides and proteins with the $^{18}$F positron emitter is important for PET diagnosis. However, direct radiofluorination is difficult due to the lack of functional groups required for nucleophilic substitution. The high temperatures in these reactions may denature proteins tertiary structure. Therefore, their labeling is done indirectly by [$^{18}$F]-containing prosthetic groups [12]. The use of [$^{18}$F]FDG as an $^{18}$F-containing building block is based on the equilibrium observed in aqueous solutions between the cyclic and acyclic forms (Figure 2).

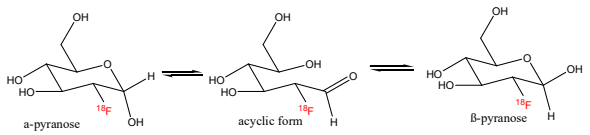



Figure 2. Equilibrium of glucopyranose in aqueous solution

The isomerization process between the two anomers proceeds with the formation of an acyclic aldehyde form. It allows the reaction with aminooxy groups to give the corresponding oximes [11]. [$^{18}$F]FDG, like p-fluorobenzaaldehyde, can form a chemoselective oxime bond. The formation of such a bond is usually carried out in an aqueous medium at pH 2-4 and temperatures between 60 and 100°C. Under these conditions, large biomeolecules are unstable, so this method of fluorination is suitable for simpler peptides and proteins or oligonucleotides [13]. The formation of hydrazone and oxime bonds between α-nucleophiles (eg hydrazines, alkoxyamines) and carbonyl compounds is convenient and widely used in many fields of study. While the reagents are simple, a significant drawback is the relatively slow reaction at neutral pH. A strategy for accelerating these reactions is described using bifunctional buffer compounds that not only control the pH but also catalyze the reaction. Buffers can be used at pH 5–9 (5–50 mM) and accelerate reactions by several orders of magnitude [14]. Such catalysts with higher activity not only increased the reaction rate, but also their low concentrations lead to suppression of potential toxicity and background reactions that may occur at high concentrations [15].

Like other types of imine formation, oxime formation is a reversible reaction. However, due to the nitrogen effect in the aminoxy group, the reaction equilibrium favors the formation of an oxime bond and in many cases the reactions can reach completion. The efficiency of the oxime bond reaction can be dramatically increased with a number of catalysts (e.g. aniline). Aniline forms a Schiff base with an aldehyde or ketone and undergoes rapid transification to the oxime through the formation of an intermediate [16].

## 4. MATERIALS AND METHODS

### *4.1. Production of [$^{18}$F]FDG*

In the production of $^{18}$F-FDG in the Clinic of Nuclear Medicine at the University Hospital "St. Marina" is used cyclotron model ABT BG-75, complete with automated radiochemical synthesis module and automated QC-system. In the first stage of the production cycle, the production of the radionuclide $^{18}$F is carried out by bombarding $^{18}$O-enriched water with protons with an energy of 7.5 eV. $^{18}$F is obtained in the form of an anion in aqueous solution, which in the next step enters the synthesis module. The radiochemical process is performed, after which the quality control is

carried out. The synthesis is based on the nucleophilic method for radiofluorination. The reaction is shown in Figure 3.

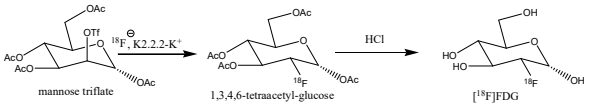



Figure 3. Synthesis of [$^{18}$F] FDG

For the chemical process, a reagent kit is used, which is loaded once when the device is started, and disposable synthesis cards. After $^{18}$F enters the reactor, Kryptofix2.2.2 and acetonitrile are added successively. This is followed by azeotropic drying at 110°C. The role of Kryptofix2.2.2 is to convert the fluorine anion from the aqueous to the organic phase and to shorten the time of azeotropic drying. In the next step, the precursor mannose triflate is added, in which the competing OH groups are protected with acetate. The radiofluorination is performed at a temperature of about 80°C. Acid hydrolysis was performed with 2M HCl to remove acetate protection. The product is diluted with water for injection and passed through a chromatographic column for purification. It is then passed through a sterile 0.2 μm filter and fed into a syringe as a ready-to-inject dose.

### *4.2. Modification of [$^{18}$F]FDG by oxime conjugation.*

Development of a methodology for modification of the [$^{18}$F] FDG using a bifunctional derivative of tetrazine will provide clickable fluorination agent for bioactive trans cyclooctene moieties. Part of the [$^{18}$F] FDG synthesized in the clinic, which was not administered to patients, was used for our experiment. The idea is to carry out the process under clinical conditions, the labeling of tetrazine should be simple and take place at a not very high temperature and for a relatively short time, given the half-life of $^{18}$F. A scheme of the reaction can be seen in Figure 4b.

The used aminooxy functionalized tetrazine (O- {4-(6-phenyl- [1,2,4,5] tetrazin-3-yl) -phenoxy] -decyl} -hydroxylamine used) was synthesized on order from the organic synthesis group at Sofia University St. Kliment Ohridski, part of the current project group. The purified and NMR-H characterized compound was used for further synthesis. The structure of the tetrazine used is shown in Figure 4a.

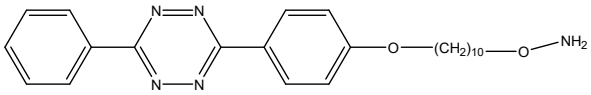


Figure 4a. Structure of tetrazine used

From the point of view of radiation safety, the experiments were performed with low starting radioactivity of [$^{18}$F] FDG (in the range of 50-200 uCi). So far, we are conducting experiments in a tenfold excess of glucose relative to the amount of tetrazine. To compensate for the low concentration of [$^{18}$F] FDG, an amount of non-radioactive glucose was added as carrier. The syntheses were carried out by varying the reaction conditions - temperature, buffer and the type of catalyst used. The buffers used are sodium acetate, phosphate (PBS) and HEPES, and aniline and p-methoxyaniline are used as catalysts. The reactions are performed in a clinical setting, not in a specialized radiochemical laboratory therefor we used only thin layer chromatography (TLC) and radio TLC for monitoring progress of reaction. The reaction temperature is between 40 and 80°C, and the heating is performed in a thermostated water bath. Working under this manner we define optimal condition for the modification of [$^{18}$F] FDG and finally the reaction was run without carrier and monitored by radio TLC.

The procedure of the performed syntheses is as follows: Prepare 0.2 M catalyst solutions in appropriate buffers. 0.5 ml of the resulting solutions are taken and transferred to the reaction vessel. To this was added the required amount of inactive glucose together with [$^{18}$F]FDG in some of the experiments or only [$^{18}$F]FDG in a larger amount. Heat for 15-20 minutes at the given temperature, then add tetrazine at a concentration of about 700 ppm, previously dissolved in $CH_3CN$. Heat the reaction system again for another 15 minutes, then read the result by TLC.

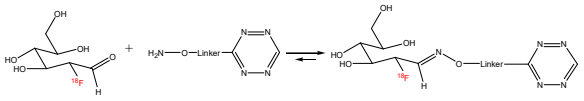


Figure 4b. Modification of [$^{18}$F]FDG by oxime conjugation

## 5. Results and discussion

To date, only the course of the oxime formation reaction between [$^{18}$F]FDG and the aminooxy derivative of tetrazine has been qualitatively established by TLC. In the eluent using DCM, the catalysts and tetrazine are moved to the front, and the glucose and the resulting product (different color spot) are registered at the start. Radioactivity was reported in the newly obtained color spot, which confirmed the successful course of the reaction.

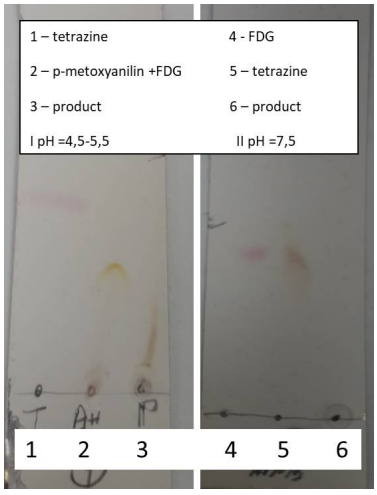
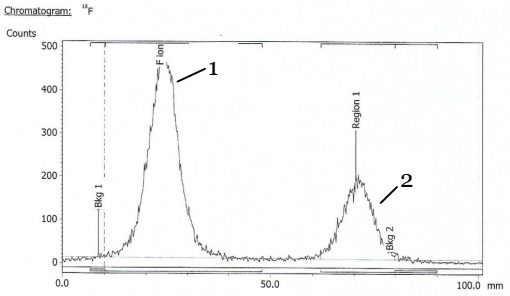



Figure 5. Chromatograms of performed syntheses with inactive glucose carrier

Chromatograms show that at pH 4.5-5.5 there is binding, while at pH 7.5, the reaction does not proceed.

Once the conditions were established, the syntheses were performed in larger quantities and with higher radioactivity of [$^{18}$F]FDG without the use of inactive glucose. We changed the polarity of the elution phase to achieve better separation of the spots on the TLC plate. DCM/methanol (1:1) was used as eluent in these experiments. Under these conditions, only FDG remains at the start, and the remaining substances move to the front. The results are presented in Figure 6. Approximate radiochemical yield of the product (between 25 and 35%) was calculated based on radio-TLC.




Legend: Peak 1 – [$^{18}$F]FDG
Peak 2 – [$^{18}$F]FDG + tetrazine

Figure 6. Radiochromatogram of performed syntheses without carrier

Reaction conditions will be optimized to improve process efficiency and radiochemical yield.

## 6. Conclusions

- Based on the obtained results, the following conclusions can be made:
- $^{18}$F-FDG is a convenient prosthetic group for indirect fluorination;
- Oxyme formation is a practical method of modification;
- Of the catalysts used, p-methoxy-aniline is more effective;
- Better binding is observed at pH 4.5-5.5.
- The modification of FDG makes it possible to develop new, more specific PET radiopharmaceuticals.

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## References

1. M. R. Kilbourn, *Fluorine-18 labeling of radiopharmaceuticals*, Washington DC, USA: The National Academies Press, 1990.
Retrieved from:
https://www.nap.edu/catalog/20467/fluorine-18-labeling-of-radiopharmaceuticals
Retrieved on: Jan. 30, 2021
2. A. O. Valdivia, J. L. López, Y. M. Vargas-Rodríguez, O. C. González, "Producción de radiofármacos para tomografía por emisión de positrones (PET) y su aplicación en el diagnóstico de diversas enfermedades," *Educación Química*, vol. 27. núm. 4, páginas 292 – 299, Oct. 2016.
(A. O. Valdivia, J. L. López, Y. M. Vargas-Rodríguez, O. C. González, "Production of radiopharmaceuticals for positron emission tomography (PET) and their application in the diagnosis of various diseases," *Chem. Education*, vol. 27, no. 4, pp. 292 – 299, Oct. 2016.)
Retrieved from:
https://www.elsevier.es/es-revista-educacion-quimica-78-articulo-produccion-radiofarmacos-tomografia-por-emision-S0187893X16300076
Retrieved on: Jan. 30, 2021
3. Y. Chain, L. Illanes, *Radiofármacos en medicina nuclear: fundamentos y aplicación clínica*, La Plata, Argentina: EDULP, 2015.
(Y. Chain, L. Illanes, *Radiopharmaceuticals in nuclear medicine: fundamentals and clinical application*, La Plata, Argentina: EDULP, 2015.)
Retrieved from:
http://sedici.unlp.edu.ar/bitstream/handle/10915/46740/Documento_completo.pdf?sequence=1
Retrieved on: Aug. 13, 2021
4. D. L. De Guevara, "Utilidad clínica oncológica y no oncológica del PET/CT," *Rev. Med. Clin. Condes*, vol. 24, núm. 1, páginas 78 – 87, Enero 2013.
(D. L. De Guevara, "Clinical utility of oncological and no oncological PET/CT," *Rev. Med. Clin. Condes*, vol. 24, no. 1, pp. 78 – 87, Jan. 2013.)
Retrieved from:
https://www.elsevier.es/es-revista-revista-medica-clinica-las-condes-202-articulo-utilidad-clinica-oncologica-no-oncologica-S0716864013701329?referer=coleccion
Retrieved on: Aug. 13, 2021
5. M. A. Áliva-Rodríguez, H. Alva-Sánchez, "Radiofármacos para PET, una nueva perspectiva de la medicina nuclear molecular en México," *El Residente*, vol. 5, núm. 3, páginas 103 – 110, Sep.-Dic. 2010.
(M. A. Áliva-Rodríguez, H. Alva-Sánchez, "Radiopharmaceuticals for PET, a new perspective on molecular nuclear medicine in Mexico," *The Resident*, vol. 5, no. 3, pp. 103 – 110, Sep.-Dec. 2010.)
Retrieved from:
https://www.medigraphic.com/pdfs/residente/rr-2010/rr103c.pdf
Retrieved on: Aug. 13, 2021
6. M. Wagner, F. Wuest, "The Radiopharmaceutical Chemistry of Fluorine-18: Electrophilic Fluorinations," in *Radiopharmaceutical Chemistry*, J. S. Lewis, A. D. Windhorst, B. M. Zeglis, Eds., 1st ed., Cham, Switzerland: Springer, 2019, part II, pp. 285 – 295.
Retrieved from:
http://library.lol/main/EDAC45121994C3798BB6B701AFD5F3F5
Retrieved on: Aug. 13, 2021
7. M. Reivich et al., "The [18F]fluorodeoxyglucose method for the measurement of local cerebral glucose utilization in man," *Circ. Res.*, vol. 44, no. 1, pp. 127 – 137, Jan. 1979.
Retrieved from:
https://www.ahajournals.org/doi/pdf/10.1161/01.RES.44.1.127
Retrieved on: Aug. 13, 2021
8. A. M. Rodríguez-Sánchez, "Establecimiento de controles de calidad para $^{18}$F-FDG sintetizada por hidrolisis básica y evaluación de dosis de cristalino de POE de radiofarmacia PET con EPD, OSL y TLD,"

Tesis de maestría en ciencias, Instituto Balseiro, Física médica, San Carlos de Bariloche, Argentina, 2016.
(A. M. Rodríguez-Sánchez, "Quality controls establishment for $^{18}$F-FDG synthesidez by basic hydrolysis and evaluation of lens of the eyes doses of POE of radiopharmacy PET with EPD, OSL and TLD," M.Sc. thesis, Balseiro Institute, Medical Physics, San Carlos de Bariloche, Argentina, 2016.)
Retrieved from:
http://ricabib.cab.cnea.gov.ar/579/1/1Rodriguez_S%C3%A1nchez.pdf
Retrieved on: Jan. 30, 2021

9. K. Hamacher, H. H. Coenen, G. Stöcklin, "Efficient stereospecific synthesis of no-carrier-added 2-[18F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution," *J. Nucl. Med.*, vol. 27, no. 2, pp. 235 – 238, Feb. 1986.
Retrieved from:
https://jnm.snmjournals.org/content/jnumed/27/2/235.full.pdf
Retrieved on: Jan. 30, 2021
10. M. C. López, "Procedimiento de fluoración para la síntesis de 2-[$^{18}$F]-fluoro-2-desoxi-D-glucosa," Patente ES 2347165 T3, España, Agosto 15, 2007.
(M. C. López, "Fluorination procedure for the synthesis of 2- [18F] -fluoro-2-deoxy-D-glucose," Patent ES 2347165 T3, Spain, Aug. 15, 2007.)
Retrieved from:
https://patentimages.storage.googleapis.com/8a/59/46/493718a8dd6706/ES2347165T3.pdf
Retrieved on: Jan. 30, 2021
11. F. Wuest, C. Hultsch, M. Berndt, R. Bergmann, "Direct labelling of peptides with 2-[18F]fluoro-2-deoxy-d-glucose ([18F]FDG)," *Bioorg. Med. Chem. Lett.*, vol. 19, no. 18, pp. 5426 – 5428, Sep. 2009.
Retrieved from:
https://sci-hub.se/10.1016/j.bmcl.2009.07.108
Retrieved on: Mar. 20, 2021
12. Z. Li, P. S. Conti, "Radiopharmaceutical chemistry for positron emission tomography," *Adv. Drug Deliv. Rev.*, vol. 62, no. 11, pp. 1031 – 1051, Aug. 2010.
Retrieved from:
https://sci-hub.se/10.1016/j.addr.2010.09.007
Retrieved on: Mar. 20, 2021
13. O. Jacobson, X. Chen, "PET designated fluoride-18 production and chemistry," *Curr. Top. Med. Chem.*, vol. 10, no. 11, pp. 1048 – 1059, 2010.
Retrieved from:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3617500/pdf/nihms-454834.pdf
Retrieved on: Mar. 20, 2021
14. D. Larsen et al., "Exceptionally rapid oxime and hydrazone formation promoted by catalytic amine buffers with low toxicity," *Chem. Sci.*, vol. 9, no. 23, pp. 5252 – 5259, May 2018.
DOI: 10.1039/c8sc01082j
PMid: 29997880
PMCid: PMC6001384
15. D. K. Kölmel, E. T. Kool, "Oximes and Hydrazones in Bioconjugation: Mechanism and Catalysis," *Chem. Rev.*, vol. 117, no. 15, pp. 10358 – 10376, Aug. 2017.
Retrieved from:
https://sci-hub.se/10.1021/acs.chemrev.7b00090
Retrieved on: Mar. 20, 2021
16. X. G. Li, M. Haaparanta, O. Solin, "Oxime formation for fluorine-18 labeling of peptides and proteins for positron emission tomography (PET) imaging: A review," *J. Fluor. Chem.*, vol. 143, pp. 49 – 56, Nov. 2012.
DOI: 10.1016/j.jfluchem.2012.07.005