Comparison of FASTlab ^{18}F-FDG Production Using Phosphate and Citrate Buffer Cassettes

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The objective of this research is to determine whether there are significant differences in the ^{18}F-FDG produced by either the phosphate or the citrate buffer cassettes in the FASTlab synthesizer. **Methods:** Forty batches of ^{18}F-FDG were produced with each cassette and analyzed retrospectively. The analysis consisted of determining the mean radiochemical yield (RCY)—uncorrected and corrected for decay—radiochemical purity (RCP), pH, and residual solvent content (ethanol and acetonitrile). An independent *t* test (alpha error [α], 0.05) was performed to determine whether the differences were statistically significant. **Results:** The mean decay-corrected RCYs for ^{18}F-FDG produced by phosphate and citrate cassettes were 82.9% ± 17.4% and 79.2% ± 5.0%, respectively. The uncorrected RCY was 57.5% ± 16.7% for phosphate- and 58.8% ± 6.0% for citrate-buffered ^{18}F-FDG, leading to a difference of 4.4% and *P* value of 0.11 for corrected RCY and a difference of 2.2% and *P* value of 0.32 for uncorrected RCY. Thus, the RCY differences are neither statistically nor clinically significant. The mean RCPs were 99.4% ± 0.2% for the phosphate-buffered ^{18}F-FDG and 99.0% ± 1.1% for the citrate-buffered ^{18}F-FDG. There was a 0.5% difference and a *P* value of 0.021, meaning that the difference was statistically significant. The average pHs for ^{18}F-FDG produced by phosphate and citrate buffer cassettes were 5.9 ± 0.1 and 5.3 ± 0.2, respectively, resulting in a 9.6% difference and a *P* value close to zero (2.6×10^{-19})—a statistically significant difference. The difference between ethanol content was also dramatic. Phosphate-buffered ^{18}F-FDG contained 0.08% ± 0.02% ethanol, whereas the citrate-buffered ^{18}F-FDG contained 0.20% ± 0.07%. No difference was found in the acetonitrile content of the 2 cassettes. **Conclusion:** The differences in yield between cassettes are due to statistical variability. The results confirm our hypothesis that there is no significant difference in RCY. The differences seen in the statistically significant data (those with a *P* value > 0.05) turn out to be insignificant in a real-world setting because all values fell within the limits set by the United States Pharmacopeia and Food and Drug Administration. Therefore, determining which cassette to use is a matter of the preference of the institution.

Key Words: PET radiochemistry; ^{18}F-FDG; radiopharmacy; quality control

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For determining metabolism in the brain and detecting various tumors, ^{18}F-FDG is a commonly used radiopharmaceutical in PET imaging. ^{18}F-fluoride is produced in a cyclotron by the following reaction: ^{18}O(p,n)^{18}F. The ^{18}F-fluoride is then substituted onto a deoxyglucose analog by a multistep reaction. The reaction, from introduction of ^{18}F-fluoride to end of synthesis (EOS), can vary depending on the type of PET synthesizer used (*1*). However, GE Healthcare's FASTlab synthesizer using an ^{18}F-FDG cassette decreases the reaction time dramatically to around 22–25 min, depending on the cassette used (*2,3*).

The FASTlab synthesizer allows for single-use cassettes to be inserted into the unit. The cassettes contain all the necessary chemicals for synthesis of bulk ^{18}F-FDG. GE Healthcare manufactures 2 cassette types: one in which ^{18}F-FDG is diluted in 15 mL of phosphate buffer and another in which ^{18}F-FDG is diluted in 22–38 mL, depending on the volume desired, of sodium citrate buffer (*2,3*). The Mayo Clinic PET Radiochemistry Facility in Rochester, Minnesota, uses 29 mL for the final volume of ^{18}F-FDG produced with the citrate cassette. Each cassette is barcoded, allowing the radiopharmaceutical being produced to be traced. The synthesis is controlled and monitored with vendor-supplied custom software, which automates the process, reducing exposure to the user while also minimizing or eliminating any variability caused by the operator. It is the objective of this study to determine whether any significant differences exist between ^{18}F-FDG produced by phosphate and by citrate buffer cassettes and to make users of the FASTlab system aware of these differences.

MATERIALS AND METHODS

^{18}F-FDG Production

Phosphate Buffer Cassette. The cyclotron was used to irradiate ^{18}O-enriched water with 16.5-MeV protons, producing ^{18}F-fluoride. The ^{18}F-fluoride was then transferred into the FASTlab synthesis unit. There, the water containing ^{18}F-fluoride was passed through an anion exchange column, to which it became bound. A solution composed of aminopolyether (Kryptofix, K222; GE Healthcare) and potassium carbonate in acetonitrile and water was then passed through the exchange column and eluted the ^{18}F-fluoride, which was collected in a reaction vessel. Approximately 1.7 mL of acetonitrile was added to the reaction chamber, and the solvents were evaporated by heating to 125°C. The reaction vessel contents were dried using nitrogen and the application of a vacuum for approx-

imately 9 min. Mannose triflate, dissolved in acetonitrile, was then added to the reaction chamber while being heated for approximately 2 min, allowing the nucleophilic substitution of the triflate group for ^{18}F-fluoride, forming ^{18}F-fluoro-tetra-acetyl-glucose (^{18}F-FTAG). The contents of the reaction chamber were then mixed with water and passed through a tC_{18} cartridge (Sep-Pak; Waters), which retained the ^{18}F-FTAG. The unbound materials were washed through the cartridge into a waste container. Sodium hydroxide was then passed through the cartridge to elute and hydrolyze the ^{18}F-FTAG. The elution was mixed with water and the pH adjusted using phosphate buffer. The buffered solution was then passed through tC_{18} and alumina cartridges (Sep-Pak; Waters) to remove any remaining impurities. Finally, the synthesized ^{18}F-FDG was passed through a 0.22-μm filter and collected in a sterile vial. The synthesis time was around 22 min (*3*). The quantity of ^{18}F-FDG was determined by measuring the bulk quantity with a dose calibrator (CRC15-PET; Capintec). This measurement was deemed to be EOS. The radiochemical yield (RCY) was determined by the fraction of activity present at EOS divided by the activity entering the synthesis unit after the end of bombardment (EOB). In essence, (activity at EOS/activity at EOB) × 100 = uncorrected RCY percentage. RCY can be corrected for the decay during synthesis with the application of the decay equation.

Citrate Buffer Cassette. For the citrate buffer cassette, the same procedure was followed as for the phosphate buffer cassette; however, ^{18}F-FDG was diluted in sodium citrate and pushed through reversed-phase and alumina cartridges (Sep-Pak; Waters). The synthesis time was generally 25 min (*2*).

Quality Control of ^{18}F-FDG

In compliance with the guidelines of the Food and Drug Administration (FDA) for PET drug current good manufacturing practice and the United States Pharmacopeia (USP) monograph, quality control (QC) procedures were performed on newly synthesized ^{18}F-FDG to ensure that it not only meets safety and purity requirements but also has the correct identity, strength, and quality, (*4,5*).

Radiochemical Purity (RCP). RCP was determined using radio–thin-layer chromatography (TLC) in compliance with the USP monograph for ^{18}F-FDG (*4*). QC samples were analyzed using aluminum-backed silica gel plates (Whatman) in acetonitrile and water 90:10. The plates were then analyzed with a radio-TLC chromatograph (AR-2000; Bioscan). The acetonitrile causes ^{18}F-FDG to travel along the TLC plate, whereas free ^{18}F-fluoride remains at the origin. The radiochromatogram typically contains 2 peaks: one at origin (^{18}F-fluoride) and one at an R_f of approximately 0.6 (^{18}F-FDG). By comparing the areas under the radiopeaks, the RCP of ^{18}F-FDG can be calculated. The current USP monograph allows up to 10% ^{18}F-fluoride (*4,5*).

pH. The pH was measured using indicator strips with a range of 4.0 to 7.0 ± 0.2. The acceptable range for pH, as stated in the USP monograph, is between 4.5 and 7.5 (*4*).

Residual Solvent Content. Residual solvent content was determined using gas chromatography (model 8610C; SRI Instruments) in compliance with the USP monograph (*4*). A 0.5-μL sample was pushed through the gas chromatography column (inside diameter, 30 m × 0.53; MXT Crossbond Carbowax [Restek]) at 10 mL/min using helium gas. The temperature was initially 40°C for 2 min and then increased 20°C per minute up to 130°C. The gas chromatograph allowed for determination of the amount of ethanol and acetonitrile in the final ^{18}F-FDG product. The maximum allowable content of ethanol and acetonitrile as stated by guidelines of the USP and FDA are 0.5% and 0.04%, respectively (*4,5*).

Data Analysis

Data for 40 batches of ^{18}F-FDG for both the phosphate and citrate buffer cassettes were analyzed retrospectively. The analysis consisted of determining the mean RCY (uncorrected and corrected for decay), RCP, appearance, RSC, and pH. An independent *t* test (alpha error [α], 0.05) was performed to determine whether any differences were statistically significant.

RESULTS

The results for RCY, RCP, pH, and RSC are shown in Table 1.

RCY

Forty batch productions for each FASTlab cassette type were analyzed retrospectively. The mean RCY was calculated to determine the radiochemical efficiency for each cassette. Of the 40 batches of phosphate-buffered ^{18}F-FDG, the mean activity at EOS was 133 GBq (3.6 Ci), ranging from 59 to 263 GBq (1.6–7.1 Ci). The mean activity of the citrate-buffered ^{18}F-FDG at EOS was 133 GBq (3.6 Ci), with a range of 41–244 GBq (1.1–6.6 Ci). There was a 4.4% difference and a *P* value of 0.11 for the decay-corrected yield and a 2.2% difference and a *P* value of 0.32 for the uncorrected yield. Because α was set at 0.05, these differences were determined to be statistically insignificant and caused by normal statistical variance.

RCP

Because the *P* value fell below the α-value (0.05), it can be said that the difference in RCP is statistically significant. However, the difference was clinically insignificant because both cassette means fell well within the limit of at least 90% set by the USP monograph for ^{18}F-FDG (*4*).

pH

A 9.6% difference in pH existed between the cassettes, with a *P* value of nearly 2.6×10^{-19}. Thus, the pH of the final

TABLE 1
Relationship Between RCY, RCP, RSC, and pH of Each Cassette

Cassette	Average corrected RCY (%)	Average uncorrected RCY (%)	Average RCP (%)	Average pH	Average EtOH (%)	Average MeCN (%)
Phosphate cassette	82.9 ± 17.4	57.5 ± 16.7	99.4 ± 0.19	5.9 ± 0.1	0.08 ± 0.02	<0.01
Citrate cassette	79.2 ± 5.0	58.8 ± 6.0	99.0 ± 1.1	5.3 ± 0.2	0.20 ± 0.07	<0.01
Percentage difference	4.36	2.16	0.53	9.62	35	Not applicable
P	0.11	0.32	0.021	2.6×10^{-19}	2.96×10^{-15}	Not applicable

solution was dependent on which cassette was used in the synthesis of ^{18}F-FDG, and the difference was statistically significant. Again, however, statistical significance did not lead to clinical significance. The pH values of all 80 batches fell within the 4.5–7.5 range stated in the USP monograph for ^{18}F-FDG (*4*).

RSC

A 35% difference existed in ethanol content between the cassettes, with a statistically significant *P* value. There was no recorded difference in acetonitrile content between the cassettes. The difference in ethanol content is insignificant because the value falls within the 0.5% allowance stated in the USP monograph (*4*).

DISCUSSION

The Mayo Clinic PET Radiochemistry Facility switched from producing ^{18}F-FDG with the phosphate buffer cassette to the citrate cassette in December 2011. The change was because of the revised abbreviated new drug application (ANDA) requirements for ^{18}F-FDG set forth by the FDA. The guidance states that the reference listed drug of ^{18}F-FDG is diluted to a concentration of 4.5 mg/mL of sodium chloride and 7.2 mg/mL of citrate in water for injection (*6*). Thus, the citrate buffer cassette is used to allow PET radiochemistry facilities to submit ANDAs with the same composition as the reference listed drug in the FDA guidance. Hence, one can have peace of mind in switching from phosphate buffer cassettes to citrate cassettes in the FASTlab synthesis unit.

Because USP and FDA requirements for QC procedures for ^{18}F-FDG did not change with the new ANDA requirements, it was the objective of the researchers to ensure that the new citrate cassette produced high-quality batches of ^{18}F-FDG.

The differences seen between the batches produced with the 2 cassettes, as stated previously, are clinically insignificant. The large difference seen in ethanol content can be attributed to GE Healthcare's striving to produce a more stable end product, because ethanol is a known radiolysis inhibitor at high amounts of radioactivity (*7,8*). This inhibition may contribute to the large SD seen in the yield of the phosphate-buffered ^{18}F-FDG, because the phosphate buffer cassette has a lower ethanol content and higher radioactive concentration (Table 2). Thus, the citrate cassette has a higher likelihood of better stability at high radioactive concentrations, which are present in PET radiochemistry facilities.

Not all QC parameters required by the USP and FDA were analyzed in this research. These QC parameters were selected for evaluation because they are the most likely to be affected by the type of cassette used in the production of ^{18}F-FDG.

CONCLUSION

The RCY, RCP, pH, and RSC of 80 batches of ^{18}F-FDG, produced in GE Healthcare's FASTlab synthesis unit using the phosphate buffer cassette or citrate buffer cassette, were analyzed and compared to determine whether any significant differences existed between the 2 cassettes. Some statistically significant differences did exist in RCP, pH, and ethanol content. The increased ethanol content of the citrate cassette supports a more stable end product at high concentrations of radioactivity, because of the ability of ethanol to inhibit radiolysis. However, all values for both cassettes fell within acceptable limits set forth by the USP monograph and FDA. Therefore, the biggest differences found were that the citrate buffer is stated explicitly in the reference listed drug composition of ^{18}F-FDG in the FDA guidance on new drug application and ANDA submissions and has a higher likelihood of stability at high radioactive concentrations. Thus, using the citrate buffer cassette to produce ^{18}F-FDG ensures peace of mind for the PET radiochemistry facility.

TABLE 2
Radioactive Concentration of Each Cassette

Cassette	Average radioactive concentration		Range of radioactive concentration	
	mCi/mL	GBq/mL	mCi/mL	GBq/mL
Phosphate cassette*	339	12.5	67–462	2.48–17
Citrate cassette†	134	5.0	61–238	2.26–8.8

*15 mL, final volume.
†29 mL, final volume.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Saha B. *Fundamentals of Nuclear Pharmacy.* 5th ed. New York, NY: Springer-Verlag; 2004.
2. GE Healthcare *FASTlab™ Regulatory Support Information: White Paper.* 2011. Waukesha, WI: GE Healthcare.
3. GE Healthcare White Paper. *FASTlab™ Regulatory Support Information: FDG Citrate Cassette.* 2011. Waukesha, WI: GE Healthcare.
4. United States Pharmacopeial Convention. *Fludeoxyglucose F18 Injection. The United States Pharmacopeia 34: The National Formulary 29. 2011.* Rockville, MD: United States Pharmacopeial Convention; 2011.
5. United States Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2011. *Guidance: PET Drugs—Current Good Manufacturing Practice (CGMP): Draft Guidance.* Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM266640.pdf.
6. United States Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2011. *Guidance: PET Drugs–Content and Format for NDAs and ANDAs.* Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078738.pdf.
7. Walters L, Martin K, Jacobson M, Hung J, Mosman E. Stability evaluation of ^{18}F-FDG at high radioactive concentrations. *J Nucl Med Technol.* 2012;40:52–56.
8. Jacobson M, Dankwart H, Mahoney D. Radiolysis of 2-[^{18}F]fluoro-2-deoxy-d-glucose ([^{18}F]FDG) and the role of ethanol and radioactive concentration. *Appl Radiat Isot.* 2009;67:990–995.

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