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Improvement of Technetium-Labeled Phytate Radiochemical Quality Control

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1. Introduction

Technetium-99m holds a special place in nuclear medicine with chemical and nuclear characteristics ideals to application in Single Photon Emission Tomography imaging. Furthermore, non-radioactive labeling kits have the advantage of chemical versatility and ability to bind to many molecular substrates, leading to a broad application, both in research and clinical purposes.[1] Quality control of non-radioactive lyophilized reagents (LR) is often performed before commercial release. In nuclear medicine clinics, the labeling with Tc-99m is performed and some quality control assays must be performed to ensure safety and efficiency of the preparation, including pH, percentage of radiochemical purity (RP) to quantify $^{99m}TcO_2$ and free pertechnetate ion, ($^{99m}TcO_4^-$) usually through quick planar chromatography (PC) systems. ^{99m}Tc-phytate forms an insoluble species with Ca^{+2} in vivo and is trapped in the reticuloendothelial system, it is a traditional radiopharmaceutical used in lymphatic ways and sentinel lymph node imaging. However, up to now, PC is only used to quantify $^{99m}TcO_4^-$ and there is not a system to quantify $^{99m}TcO_2$ as radiochemical impurity in ^{99m}Tc-phytate [2,3]. The objective of this work was to evaluate $^{99m}TcO_2$ in ^{99m}Tc-phytate using new chromatographic systems based on surfactant mixture solution as mobile phase.

2. Methodology

Phytate LR as FITA-TEC and $^{99m}TcO_4^-$ eluate of IPEN-TEC Generator were from Nuclear and Energy Research Institute (IPEN-CNEN) Radiopharmacy Center. Whatman 1 (W1) and Whatman 3MM (W3MM) chromatography papers were from General Electric and TLC-SG was from Merck, sodium dodecyl sulphate (SDS) and glycerol reagents were from Merck. Purified water was obtained from Gehaka purifier. FITA-TEC formulation is described in Table I.

Table I: Composition of FITA-TEC LR.[3]

Reagent	Quantity (mg)/vial
Phytic Acid	20.0 mg
Stannous Chloride Dihydrate	1.0 mg

Retention Factor (Rf) establishes a relation between the distance traveled by a chemical species (Ds) and the

total distance traveled by the elution front (De) in a chromatographic system and is expressed by Equation 1.

$$Rf = \frac{Ds}{De} \quad (1)$$

Rf of $^{99m}TcO_2$, $^{99m}TcO_4^-$ and ^{99m}Tc-phytate were determined in each chromatographic system. In experiments of Rf determination, $^{99m}TcO_2$ solution was prepared mixing stannous chloride in HCl, $^{99m}TcO_4^-$ and adjusting the pH to 8 with NaOH.

10% glycerol, 0.02 M SDS, 0.04 M SDS, 5% glycerol in 0.02 M SDS, 5% glycerol in 0.04 M SDS, and W1, W3 and TLC-SG strips of 12.5 cm long were prepared. The percentage per segment (Ai) was calculated by Equation 2 and the results were expressed as media of triplicate. Ai is the counts of an individual segment and At is the total activity of the chromatographic strip.[4]

$$\%Ai = \frac{Ai}{At} \times 100 \quad (2)$$

Labeling of FITA-TEC with Tc-99m was performed according to the leaflet, using 10 mCi (370 MBq) total activity in 3 mL total volume, resulting 3.33 mCi/mL specific activity.[3] Percentage of free pertechnetate ($^{99m}TcO_4^-$) in FITA-TEC was determined using Whatman 3MM chromatography paper and acetone as mobile phase.[3]

After the chromatographic run, the strips were dried, cut into 1 cm segments and the activity (as counting per minute, cpm) during 12 seconds was measured in an automatic gamma counter Hidex in a 70 to 210 keV energy window.

3. Results and Discussion

Up to now, there is not a chromatographic system reported in literature to separate $^{99m}TcO_2$ from ^{99m}Tc-phytate. Traditional chromatographic systems used in quality control of radiopharmaceutical preparations are not applicable to evaluate $^{99m}TcO_2$ in ^{99m}Tc-phytate. That is the reason that in radiochemical purity determination of ^{99m}Tc-phytate, only the percentage of $^{99m}TcO_4^-$ is determined as radiochemical impurity of the preparation.[1,2]

Table II shows that when W1 and W3MM and micellar solutions of SDS above critical micelle concentration (CMC=0.008 M) [5,6] were employed, both free pertechnetate and ^{99m}Tc-phytate migrated with the front of solvent and $^{99m}TcO_2$ remained at the origin. 10% glycerol solution as mobile phase showed a carrier effect on $^{99m}TcO_2$ and ^{99m}Tc-phytate. TLC-SG was not a good stationary phase to maintain $^{99m}TcO_2$ and to carry ^{99m}Tc-phytate with the solvent studied. When a mixture of glycerol in SDS and W1 was used, $^{99m}TcO_2$ remained at the origin and ^{99m}Tc-phytate migrated with the front of the solvent, resulting in high percentage of activity (%Ai) at the Rf range of $^{99m}TcO_2$ and ^{99m}Tc-phytate, respectively. Free Pertechnetate presented Rf of 0.5-0.8 in this chromatographic system.

Fig. 1 and 2 show the profile of $^{99m}TcO_2$ and ^{99m}Tc-phytate in W1 using 5% Glycerol in 0.04 mol/L SDS, respectively.

Rf of $^{99m}TcO_2$ and ^{99m}Tc-phytate in glycerol and SDS solutions as mobile phase are presented in Table II.

Table II: Rf of $^{99m}TcO_2$ and ^{99m}Tc-phytate in chromatographic systems using glycerol in micellar solutions as mobile phase.

Mobile phase	Radiochemical species	Stationary phase – strips Rf and % activity in Rf range		
		W1M[1]	W3MM[1]	TLC–SG[1]
0.02 M SDS	$^{99m}TcO_2$	0.0-0.1 (99%)	0.0-0.1 (97%)	0.0-0.1 (88%)
	^{99m}Tc-phytate	0.1-1.0 (94%)	0.1-1.0 (93%)	0.1-1.0 (85%)
0.04 M SDS	$^{99m}TcO_2$	0.0-0.1 (99%)	0.0-0.1 (99%)	0.0-0.1 (97%)
	^{99m}Tc-phytate	0.1-1.0 (95%)	0.1-1.0 (93%)	0.1-1.0 (83%)
10% Glycerol	$^{99m}TcO_2$	0.0-0.1 (92%)	0.0-0.1 (98%)	0.0-0.1 (71%)
	^{99m}Tc-phytate	0.1-10 (96%)	0.1-10 (64%)	0.1-10 (95%)
5% glycerol in 0.02 M SDS	$^{99m}TcO_2$	0.0-0.1 (87%)	0.0-0.1 (98%)	0.0-0.1 (74%)
	^{99m}Tc-phytate	0.1-1.0 (98%)	0.1-1.0 (96%)	0.1-1.0 (89%)
5% glycerol in 0.04 M SDS	$^{99m}TcO_2$	0.0-0.1 (98%)		
	^{99m}Tc-phytate	0.1-1.0 (99%)		

1-Total percentage of activity in the range of Rf

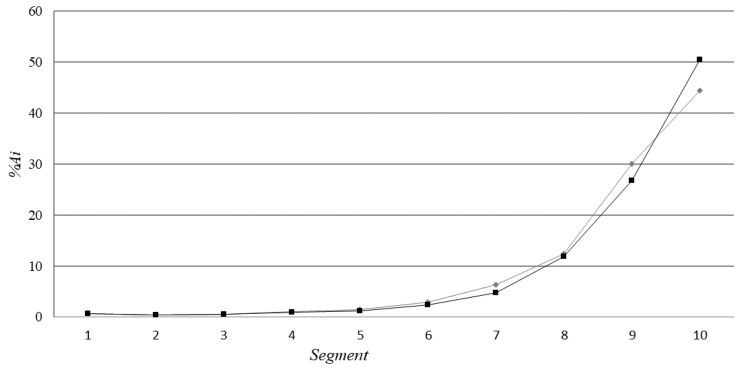

Figure 1: ^{99m}Tc-phytate profile in 5% Glycerol in 0.04 mol/L SDS and W1 chromatographic system.

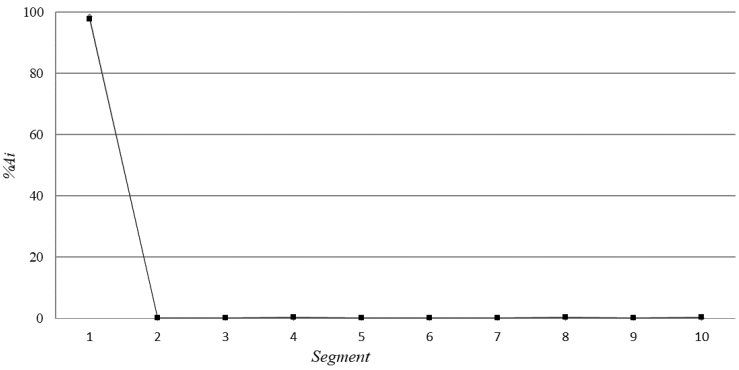

Figure 2: $^{99m}TcO_2$ profile in 5% Glycerol in 0.04 mol/L SDS and W1 chromatographic system.

Use of this system to calculate the radiochemical purity of FITA-TEC radiopharmaceutical resulted in 99.21%, with $\%^{99m}TcO_2$ of 0.66 and $\%^{99m}TcO_4^-$ of 0.13.

4. Conclusions

In this work, a new chromatographic system for separation of $^{99m}TcO_2$ from ^{99m}Tc-phytate using glycerol as modifier of micellar solutions of SDS was successfully developed, improving the radiochemical purity quality control assay of FITA-TEC radiopharmaceutical.

Acknowledgements

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