General precautions related to preparation and use of ^{99}Mo/^{99m}Tc generators based on chromatographic alumina columns

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ABSTRACT

As ^{99}Mo/^{99m}Tc alumina column generator is the main source of ^{99m}Tc, it is essential to take into consideration the parameters affecting the ^{99}Mo loading efficiency in addition to the ^{99m}Tc elution yield, radionuclidic purity, radiochemical purity and specific activity. Those parameters include (i) generator assembling quality, (ii) pH of the ^{99}Mo loading solution, (iii) oxidants in the ^{99}Mo loading solution, (iv) pH of ^{99m}Tc eluent, (v) radiolysis of water (on the column) and organic impurities and (vi) elution time. Thus, general precautions, related to such parameters, should be taken into account to obtain the optimum preparation and use of ^{99}Mo/^{99m}Tc generators, from which the eluted ^{99m}Tc is widely used for preparation of many radiopharmaceuticals utilized in a variety of diagnostic imaging processes in the field nuclear medicine.

1. Introduction

Technetium-99m is a decay product of ^{99}Mo (87.9 %), along with ^{99}Tc (12.1 %), which is then decays to ^{99}Tc (> 99 %) and ^{99}Ru (Figure 1). The wide use of ^{99m}Tc in diagnostic applications of nuclear medicine is attributed to its unique nuclear properties, since it is a short-lived radionuclide and, in the same time, a single-photon gamma-ray emitter with ideal energy for imaging applications ($t_{1/2}$ = 6.01 h, γ-ray energy = 140.5 keV) [1]. In addition, many successful ^{99m}Tc-based radiopharmaceuticals are widely used in diagnosis of renal, hepatic, hepatobiliary, bone, cardiac and oncological diseases [2]. In nuclear medicine centers, the main source of ^{99m}Tc is ^{99}Mo/^{99m}Tc chromatographic column generators based on alumina and loaded with a high-specific activity fission ^{99}Mo. However, chromatographic column gel generators, e.g., those based on zirconium molybdate, loaded with low-specific activity (n,γ)^{99}Mo are also known as an alternative to meet the shortage in fission ^{99}Mo. Surface-modified alumina and nano alumina can be loaded with low-specific activity ^{99}Mo achieving high loading capacities [3, 4]. Other types of generators have been reported including solvent extraction, sublimation, thermochromatographic and electrochemical generators [3]. In addition to the generators using (n, f)^{99}Mo and ^{99}Mo(n,γ) produced in nuclear reactors, ^{99m}Tc can also be produced in accelerators either as a direct product of nuclear reactions or form the nuclear decay of ^{99}Mo, e.g., ^{100}Mo(p,2n)^{99m}Tc, ^{100}Mo(d,3n)^{99m}Tc, ^{98}Mo(d,n)^{99m}Tc and ^{98}Mo(d,p)^{99}Mo, ^{97}Mo(α,2p)^{99}Mo, etc. Then, separation of ^{99m}Tc from the irradiated targets can be achieved via solvent extraction, column chromatography, thermochromatography and chemical precipitation [5]. Turning back to the ^{99}Mo/^{99m}Tc chromatographic alumina column generators loaded with fission ^{99}Mo as the main commercial source of ^{99m}Tc used in nuclear medicine, this paper discusses some precautions that should be taken into account in order to obtain the optimum preparation and use of ^{99}Mo/^{99m}Tc generators, in other words to obtain a high ^{99}Mo loading efficiency and high ^{99m}Tc elution yield with specifications meeting nuclear medicine requirements. Finally, to avoid any confusion during routine production of many ^{99}Mo/^{99m}Tc generators in the same patch, a valid spreadsheet calculation model can be used to determine ^{99m}Tc activity to be eluted at the maximum ^{99m}Tc activity growth time (t_{max}). In addition, such calculation model can also be utilized during generator use to determine the expected ^{99m}Tc activity and specific activity against elution time.

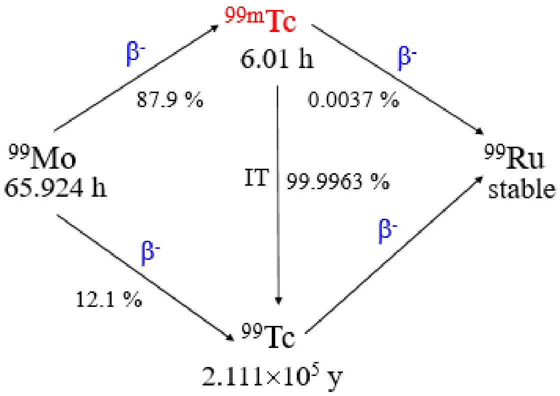

Figure 1. ^{99}Mo decay.

2. Radioactive decay-growth kinetics

Calculation Equations related to ^{99}Mo decay, growth of technetium isotopes (^{99m}Tc and ^{99}Tc) and specific activity of ^{99m}Tc are given below [6, 7].

2.1. Technetium-99m activity at any time *t*

$$A_{Tc1} = D_{f1}\frac{\lambda_{Tc1}}{\lambda_{Tc1}-\lambda_{Mo}}A^0_{Mo}\left(e^{-\lambda_{Mo}t} - e^{-\lambda_{Tc1}t}\right) + A^0_{Tc1}e^{-\lambda_{Tc1}t} \qquad \text{Bq} \qquad (1)$$

Where:

D_{f1} : decay ratio of ^{99}Mo to ^{99m}Tc (0.879).
A_{Tc1} : ^{99m}Tc activity at time t.
A^0_{Mo} : ^{99}Mo activity at $t = 0$.
A^0_{Tc1}: ^{99m}Tc activity at $t = 0$.
λ_{Mo} : decay constant of ^{99}Mo ($\lambda_{Mo} = 0.693/t_{1/2(Mo)}$; $t_{1/2(Mo)} = 65.924$ h).
λ_{Tc1} : decay constant of ^{99m}Tc ($\lambda_{Tc1} = 0.693/t_{1/2(\mathrm{Tc})}$; $t_{1/2(Tc1)} = 6.01$ h).

Assuming that $A^0_{Tc1} = 0$ at $t = 0$, Eq. 1 becomes:

$$A_{Tc1} = D_{f1}\frac{\lambda_{Tc1}}{\lambda_{Tc1}-\lambda_{Mo}}A^0_{Mo}\left(e^{-\lambda_{Mo}t} - e^{-\lambda_{Tc1}t}\right) \qquad \text{Bq} \qquad (2)$$

2.2. Time of maximum ^{99m}Tc activity growth (t_{max})

$$t_{max} = \frac{\ln\left(\frac{\lambda_{Tc1}}{\lambda_{Mo}}\right)}{\lambda_{Tc1}-\lambda_{Mo}} \qquad \text{h} \qquad (3)$$

2.3. Simplified approximation of Eq. 2 (at transient equilibrium; at $t \geq t_{max}$)

$$A_{Tc1} = D_f\frac{\lambda_{Tc1}}{\lambda_{Tc1}-\lambda_{Mo}}A_{Mo} \qquad \text{Bq} \qquad (4)$$

2.4. Technetium-99 activity and technetium-99m specific activity at any time *t*

$$N_{Tc1} = D_{f1}\frac{\lambda_{Mo}}{\lambda_{Tc1}-\lambda_{Mo}}N^{0}_{Mo}\left(e^{-\lambda_{Mo}t} - e^{-\lambda_{Tc1}t}\right) \qquad (5)$$

$$N_{Tc2} = D_{f1}N^{0}_{Mo}\left(1 + \frac{\lambda_{Mo}}{\lambda_{Tc1}-\lambda_{Mo}}e^{-\lambda_{Tc1}t} - \frac{\lambda_{Tc1}}{\lambda_{Tc1}-\lambda_{Mo}}e^{-\lambda_{Mo}t}\right) + D_{f2}N^{0}_{Mo}\left(1 - e^{-\lambda_{Mo}t}\right) \qquad (6)$$

$$m_{Tc} = m_{Tc1} + m_{Tc2} = \frac{(N_{Tc1}+N_{Tc2})\times 99}{A_v} \qquad \text{g} \qquad (7)$$

$$A_{Tc1} = \lambda_{Tc1}N_{Tc1} \qquad \text{Bq} \qquad (8)$$

$$A_{Tc2} = \lambda_{Tc2}N_{Tc2} \qquad \text{Bq} \qquad (9)$$

$$A_{sTc1} = \frac{A_{Tc1}}{m_{Tc}} \qquad \text{Bq/g} \qquad (10)$$

Where:

D_{f2}: decay ratio of ^{99}Mo to ^{99}Tc (0.121).
N^{0}_{Mo}: number of ^{99}Mo nuclei at $t = 0$.
N_{Tc1} and N_{Tc2}: number of ^{99m}Tc and ^{99}Tc nuclei, respectively, at time *t*.
λ_{Tc2}: decay constant of ^{99}Tc ($\lambda_{Tc2} = 0.693/t_{1/2(Tc2)}$; $t_{1/2(Tc2)} = 1.85\times10^{9}$ h).
m_{Tc}: total mass of ^{99m}Tc and ^{99}Tc.
m_{Tc1} and m_{Tc2}: ^{99m}Tc and ^{99}Tc individual masses, respectively, at time *t*.
A_v: Avogadro's number (6.023×10^{23}).
A_{sTc1}: specific activity of ^{99m}Tc at time *t*.

3. Parameters affecting generator performance

3.1. Generator assembling quality

The column should be carefully packed with alumina to achieve homogeneity and avoid formation of channels. After assembling (Figure 2), the assembled generator should be tested on cold (via passing 10 ml of 0.9 % saline solution) to ensure good sealing, absence of leakage or blockage, clarity of the collected solution and sufficient evacuation level in 'eluate collection vials' (to collect the whole eluate volume).

3.2. pH of the ^{99}Mo loading solution

pH-value of the ^{99}Mo loading solution is a key parameter for optimum loading and performance of ^{99}Mo/^{99m}Tc generators. pH-range of 2-3 is the optimum for loading, to avoid unaccepted levels of ^{99}Mo breakthrough in ^{99m}Tc eluates. In such pH range (pH 2-3), in the case of high-specific activity ^{99}Mo, the predominant species is MoO4^{2-} [8, 9]. The adsorption mechanism of ^{99}Mo on alumina is the electrostatic attraction between MoO_4^{2-} anions and positively-charged alumina surface, at pH-values < PZC (point of zero charge) [8]. Figure 3 shows the alumina surface charge at different pH ranges. At pH-values > PZC, alumina surface becomes negatively-charged. At highly-acidic solutions, the predominant ^{99}Mo species in solution is $(MoO_3.H_3O)^+$ cations [8]. It is worth mentioning that PZC varies according to alumina type; it lies in the range of pH ~ 7-10 [10].

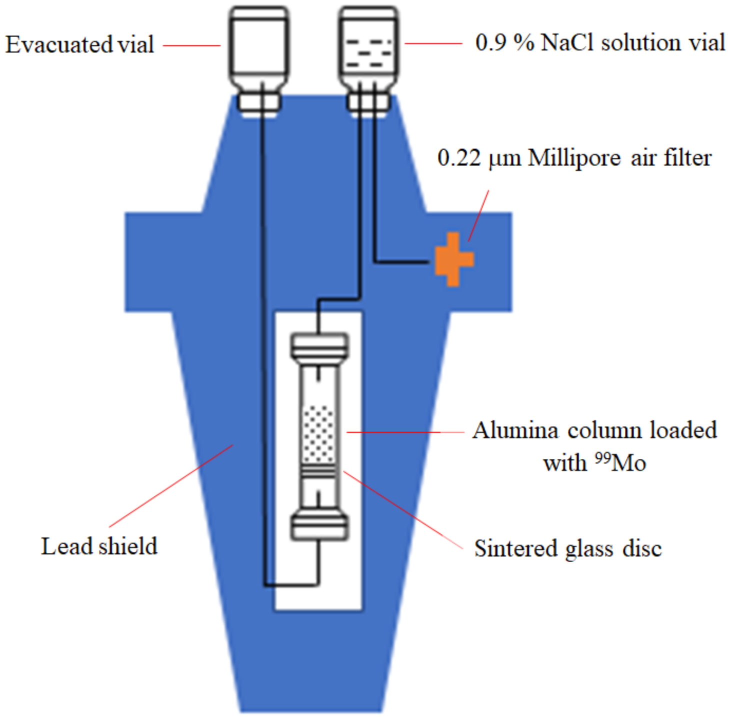

Figure 2. ^{99}Mo/^{99m}Tc chromatographic alumina column generator.

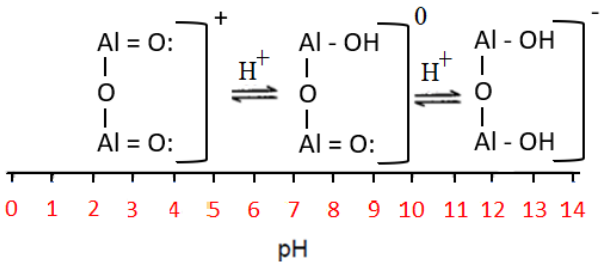

Figure 3. Alumina surface charge at different pH-ranges.

3.3. Oxidants in the ^{99}Mo loading solution

Nitrate can be added to ^{99}Mo solution to consume the hydrogen generated as a result of water radiolysis; it acts as hydrogen free-radical scavenger. In the same time, nitrate addition prevents formation and precipitation of the reduced species of ^{99}Mo, e.g., hydrated MoO_2 [11]. Also, addition of NaOCl in alkaline ^{99}Mo solution (before adjusting pH-value to be in acidic range) is recommended to lower the reduction effect of radiolysis, via re-oxidation of the reduced ^{99}Mo species [12].

3.4. pH of ^{99m}Tc eluent

The usual eluent of ^{99m}Tc from alumina generators is 0.9 % NaCl solution (pH 5.5), which elutes ^{99m}Tc predominantly as pertechnetate ($^{99m}TcO_4^-$). It is important to pre-condition the alumina column (after ^{99}Mo loading from acidic solution and before the 1st elution) by passing 10 ml of 0.9 % NaCl, since at lower pH-values, reduced technetium species, of Tc(IV), can be formed [13, 14] which adversely affects the elution yield and the radiochemical purity of ^{99m}Tc (Table 1).

Table 1. ^{99m}Tc species at acidic pH-values.

pH	**Species (other than $^{99m}TcO_4^-$)**
pH < 1.5	$^{99m}TcO^{2+}$
1.5 < pH < 2.2	$^{99m}TcO(OH)^+$ and $^{99m}TcO(OH)_2$
pH > 2.2	$^{99m}TcO(OH)_2$ and $[^{99m}TcO(OH)_2]_2$
pH 3-4	$^{99m}TcO_2.2H_2O$

3.5. Radiolysis of water (on the column) and organic impurities

Radiolysis of water with ionizing radiation occurs in three stages (physical, physicochemical and chemical stages) and produces hydrous electrons, hydrogen peroxide, hydrogen gas and free radicals (Figure 4) [15].

The destruction rate of H_2 and H_2O_2 is equal to that of their production in water:

$$HO^\bullet + H_2 \rightarrow H_2O + H^\bullet$$
$$H^\bullet + H_2O_2 \rightarrow H_2O + HO^\bullet$$

The reduced technetium species can be formed according to [16, 17]:

$$TcO_4\text{-} + e_{aq}^- \rightarrow TcO_4^{2-}$$
$$TcO_4^- + H^\bullet \rightarrow TcO_4^{2-}$$
$$2TcO_4^{2-} \rightarrow TcO_4^- + Tc(V)$$
$$2Tc(V) \rightarrow TcO_4^- + Tc(IV)$$

Thus, keeping the column dry is advantageous for maintaining high ^{99m}Tc elution yields (dry column mode). Otherwise, for wet column mode, Cu(II) can be used as a radical scavenger by adsorption on alumina (0.3-0.4 mg Cu/g Al_2O_3). The Cu(II)-modified alumina can be used as the upper column layer (the lower layer is pure alumina) [18].

Trace organic impurities (e.g., those leached from rubber or plastic closures), undergo radiolysis and produce reducing gases leading to the formation Tc(V) and Tc(VI) species which, in turn, reduce the elution yield [19].

Despite several oxidants are tested for oxidizing Tc reduced species to pertechnetate (such as H_2O_2, $S_2O_8^-$, MnO_4^-, OCl^-, BiO_3^-, FeO_4^{2-}, O_3, Ce^{4+}) [20, 21], there is a strong restriction before use one of them during elution to avoid any possible adverse effect during further use for medical purposes. However, H_2O_2 can carefully be added to the eluent, taking into consideration the further required reduction before preparation of the different ^{99m}Tc-radiopharmaceuticals used in various imaging processes [22].

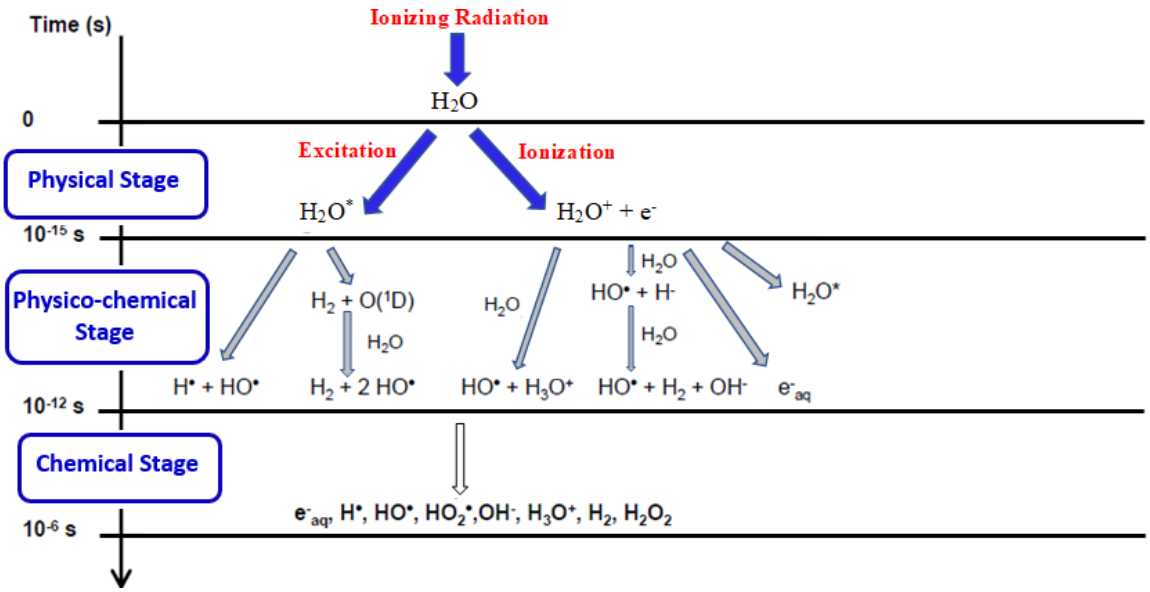

Figure 4. Water radiolysis stages.

3.6. Elution time

Technetium-99m activity is a function of elution time. It is important to use a calculation model based on valid equations, in order to determine the elution yield during QC testing (at times $< t_{max}$), to elute the required ^{99m}Tc activity during use and to estimate the specific activity of the eluted ^{99m}Tc.

Starting from the last elution time (assuming a complete ^{99m}Tc elution), the ^{99m}Tc percent growth reaches 11, 29.7, 81.8 and 100 % after 1, 3, 10 and 22.86 h, respectively (Figure 5). Thus, during testing the elution of the prepared generators, by dividing the ^{99m}Tc eluted activity (at any time $t < t_{max}$) on such growth factors, ^{99m}Tc to be eluted at t_{max} can be estimated.

It is noticed that, the percent ratio of ^{99m}Tc/^{99}Mo increases with time till reaching 87.9 % at t_{max} (22.86 h) and slightly continues increase till reaching 96.7 % (Figure 6) as a result of continuous ^{99m}Tc build-up beyond t_{max} (along with its decay and ^{99}Mo decay), so Eq. 2 (not Eq. 4) is favorable for such more accurate calculations (which may be useful for further studies). On the other hand, the long-lived ^{99}Tc contribution continuously increases with increasing time elapsed between elutions (Figure 6).

Long time periods between successive elutions (several days) are not favorable, because specific activity of the eluted ^{99m}Tc decreases with increasing such time periods (Figure 7), which may decrease the labeling yield of some kits [23].

The prementioned parameters and their related precautions are compiled in Table 2. Finally, ^{99m}Tc eluates should have specifications meeting the requirements of human injection and medical use. Table 3 compiles specifications of ^{99m}Tc eluted from ^{99}Mo/^{99m}Tc generators produced in RPF, ETRR-2 Complex, Egyptian Atomic Energy Authority.

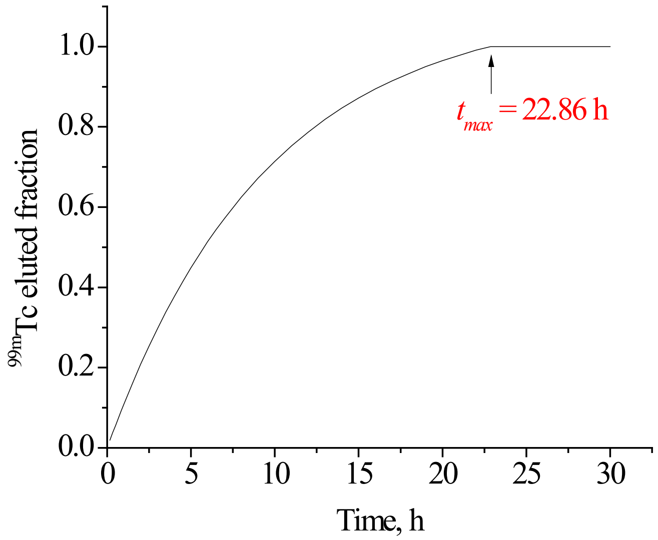

Figure 5. ^{99m}Tc elution fraction against time.

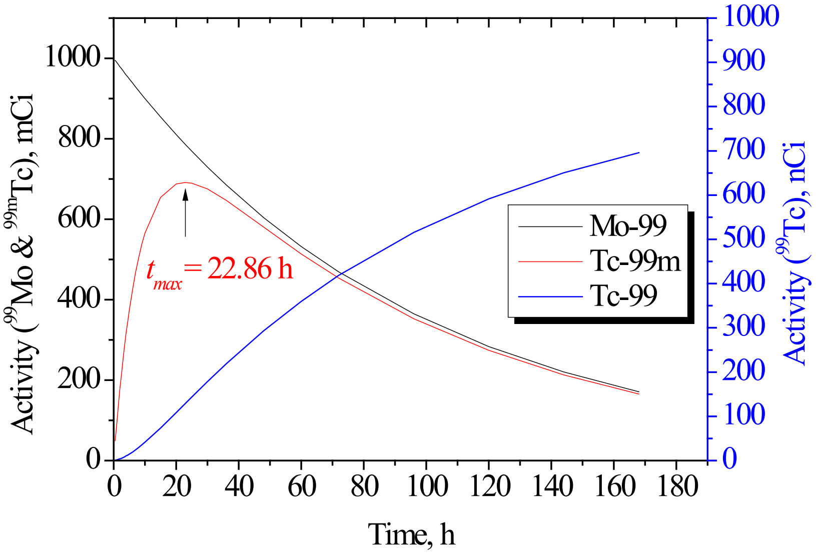

Figure 6. ^{99}Mo, ^{99m}Tc and ^{99}Tc activities against time.

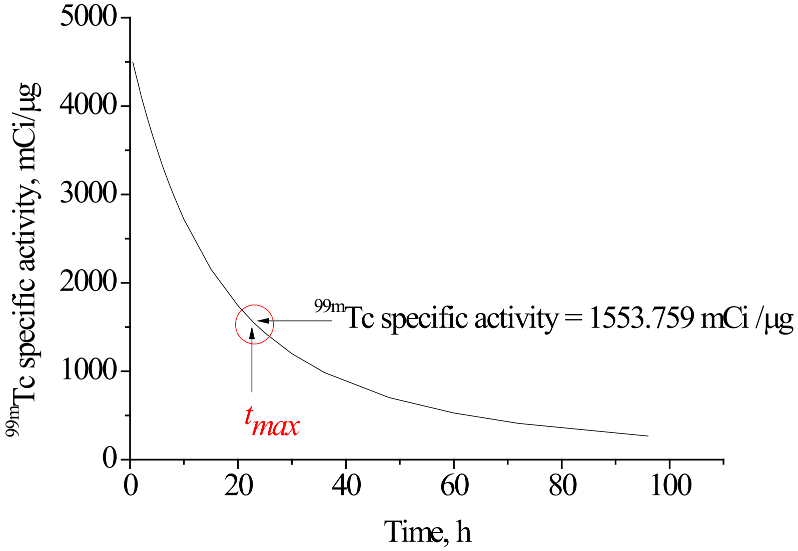

Figure 7. ^{99m}Tc specific activity against time.

Table 2. General precautions during preparation and use of $^{99}Mo/^{99m}Tc$ chromatographic alumina column generators.

Parameter	**Precautions**
Generator assembling quality	Be sure of: - The homogeneous packing of alumina in the chromatographic column. - Good sealing. - Absence of blockage. - Sufficient evacuation level in the 'eluate collection vials'; a quick collection of the whole saline solution volume in the evacuated vials. - Clarity of the collected solution.
pH of the ^{99}Mo loading solution	- pH should be in the range of 2-3 to avoid lower loading efficiency and higher unaccepted levels of ^{99}Mo breakthrough in ^{99m}Tc eluates.
Oxidants in the ^{99}Mo loading solution	Avoid the formation of reduced ^{99}Mo species (with oxidation states lower than VI) via addition of (e.g.): - NO_3^- (free-radical scavenger). Or - NaOCl.
pH of ^{99m}Tc eluent	pH should be nearly neutral, 0.9 % of NaCl solution (pH 5.5) is usually used, to avoid: - Lower elution yields (due to formation of reduced ^{99m}Tc species). - Lower unaccepted radiochemical purity of ^{99m}Tc eluate. Alumina column should be pre-conditioned after ^{99}Mo loading and before the 1st elution (by passing 10 ml of 0.9 % NaCl solution).
Radiolysis of water (on the column) and organic impurities	To reduce the effect of water radiolysis: - Keep the column dry between elutions (dry mode). - Modify the alumina column by adsorbing Cu(II) to act as a radical scavenger (wet mode). In case of lower elution yields due to radiolysis of water or organic impurities, H_2O_2 can be carefully added to the eluent solution.
Elution time	It should be taken into account to: - Determine elution yield (during testing) or estimate ^{99m}Tc activity in the eluate (during use). - Estimate ^{99m}Tc specific activity in the eluate to avoid the possible adverse effects during labeling of some kits.

Table 3. Specifications of ^{99m}Tc eluted from ^{99}Mo/^{99m}Tc generators produced in RPF, ETRR-2 Complex, Egyptian Atomic Energy Authority.

Test	Acceptance criteria
^{99}Mo breakthrough	^{99}Mo:^{99m}Tc < 0.1 %
Radiochemical purity	> 95 % (as TcO_4^-)
pH	4.5-7.5
Sterility	Sterile (no growth)
Pyrogenicity	Bacterial endotoxins <175 EU/V (ml)

4. Conclusion

The optimum performance of the ^{99}Mo/^{99m}Tc chromatographic alumina column generators can be obtained by taking into consideration some precautions related to the parameters of (i) generator assembling quality, (ii) pH of the ^{99}Mo loading solution, (iii) oxidants in the ^{99}Mo loading solution, (iv) pH of ^{99m}Tc eluent, (v) radiolysis of water (on the column) and organic impurities and (vi) elution time.

Acknowledgements

The author would like to thank all the staff members of Radioisotope Production Facility (RPF), ETRR-2 Complex, Egyptian Atomic Energy Authority for their expended effort till the success of the continuous production of ^{99}Mo/^{99m}Tc generators.

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