

**Mesoporous alumina (MA) based double column approach for development of clinical scale  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator using (n, $\gamma$ ) $^{99}\text{Mo}$ : An enticing application of nanomaterial**

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**Supplementary Information S1: The synthesis and characterization of mesoporous alumina (MA)**

The synthesis of MA was carried out by hydrolysis of aluminium isopropoxide in aqueous medium in the presence of glucose template adopting procedure similar to that reported by Xu et al.<sup>30</sup> In brief, 32 g of aluminium isopropoxide was gradually added to 1 L aqueous solution containing 30 g of glucose. The resultant solution was stirred at room temperature for 5 h. The pH of the resulting suspension was adjusted to ~5 by adding dilute nitric acid. The mixture was heated at 100 °C in open air to remove water and all other volatiles. The resultant black residue was then calcined at 600 °C for 4 h to remove the template, completely. The material obtained was crushed mechanically and sieved to obtain particles of 50-100 mesh size (149-297  $\mu\text{m}$ ).

X-ray diffraction measurements (10–70°) were carried on the powder for the crystallite size estimation, using monochromatized Cu-K $\alpha$  radiation on a Philips X-ray diffractometer, Model PW 1927. Silicon was used as an external standard for correction due to instrumental

broadening. FT-IR spectra of the synthesized sorbent were recorded in a JASCO FT-IR-420 spectrometer. Thermogravimetry and differential thermal analysis studies were carried out using Setaram Simultaneous TG-DTA (Setsys Evolution) instrument. The analysis was conducted in argon atmosphere with the flow rate  $20 \text{ ml min}^{-1}$  at a heating rate of  $10 \text{ }^{\circ}\text{C min}^{-1}$ , using  $\alpha$ -alumina as the reference in a platinum crucible. Atomic force microscopic (AFM) measurements were performed using NT-MDT solver model instrument with  $50 \text{ }\mu\text{m}$  scanner head and silicon nitride tip in contact mode. AFM grade mica sheet was used as the substrate. Transmission Electron Microscopy (TEM) data were recorded using TEM, using a JEOL FX microscope (Jeol Ltd., Tokyo, Japan), on the powder sample. The preparation of samples for TEM analysis involved sonication in ethanol for 5 min and deposition on a carbon-coated copper grid. The accelerating voltage of the electron beam was 200 kV. Small-angle x-ray scattering (SAXS) measurements were performed using a laboratory based SAXS instrument with  $\text{Cu K}_{\alpha}$  x-ray radiation. The scattered intensities  $I(q)$  were recorded as a function of scattering vector  $q (= 4\pi \sin \theta / \lambda$ , where  $2\theta$  is the scattering angle, and  $\lambda$  is the X-ray wavelength). The surface area analysis were carried out by the standard BET technique with  $\text{N}_2$  sorption using Quantachrome, Autosorb-1 analyser. Gamma activity of  $^{99\text{m}}\text{Tc}$  was assayed using a NaI (Tl) scintillation counter (100–160 keV). The chemical analysis for the determination of trace level of metal contaminations was done using inductively coupled plasma-atomic emission spectroscopy (ICP-ES JY-238, Emission Horiba Group, France).

### **Supplementary Information S2: The production and radiochemical processing of $^{99}\text{Mo}$**

The target material, natural  $\text{MoO}_3$  (2.5 g) in the form of powder was packed in an Al capsule, sealed by cold-welding method, checked for leakage by quality control procedures and neutron irradiated at a position having neutron flux of  $\sim 9.6 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$  in the Dhruva reactor for 1 week. After irradiation, the target was dissolved in 50 mL of 3 M NaOH solution at 80 °C. The activity of the  $^{99}\text{Mo}$  solution was assayed in an ion-chamber.

### **Supplementary Information S3: Study of the sorption characteristics of MA**

The distribution coefficients ( $K_d$ ) of  $\text{MoO}_4^{2-}$  and  $\text{TcO}_4^-$  ions were determined by batch process at different pH, using  $^{99}\text{Mo}$  and  $^{99\text{m}}\text{Tc}$  radiotracers. In each experiment, 100 mg of sorbent was suspended in 11 mL solution containing the radioactive metal ions, in a 20 mL sealed vial. The vials were shaken in a wrist arm mechanical shaker for 1 h at 25 °C and then filtered. The activities of the solution before and after equilibration were measured in a HPGe counter using an appropriate  $\gamma$ -ray peak (140 keV for  $^{99\text{m}}\text{Tc}$  and 181 keV for  $^{99}\text{Mo}$ ). The distribution ratios were calculated using the following expression:

$$K_d = \frac{(A_i - A_{\text{eq}})V}{A_{\text{eq}} m} \text{ L g}^{-1}$$

where,  $A_i$  is the initial total radioactivity of 1 mL the solution,  $A_{\text{eq}}$  is the unadsorbed activity in 1 mL of the solution at equilibrium,  $V$  is the solution volume ( $\text{cm}^3$ ) and  $m$  is the mass (g) of the sorbent. In order to determine the time required to attain sorption equilibrium, the  $K_d$  values of  $\text{MoO}_4^{2-}$  ions were studied as a function of time at pH 3.

The static sorption capacity of MA was determined by batch equilibration method by taking 0.5 g aliquots of accurately weighed sorbent in a stoppered conical flask containing 50 mL of sodium molybdate solution (10 mg of Mo mL<sup>-1</sup>) spiked with ~3.7 MBq (100 µCi) of <sup>99</sup>Mo. The sorbent was allowed to equilibrate in contact with the radioactive solution by shaking the conical flask using a mechanical shaker for 30 min at room temperature. At the end, the contents were filtered through a Whatman filter paper (No. 542). The activities of <sup>99</sup>Mo in the solution before and after absorption were estimated by using HPGe detector coupled to a multichannel analyzer, by measuring the counts at 181 keV peak corresponding to <sup>99</sup>Mo in 1 mL aliquots. All measurements were carried out at 25° C in triplicate. The sorption capacity was calculated using the following expression:

$$\text{Capacity} = \frac{(A_o - A_e)V.C_o}{A_o m},$$

where, A<sub>o</sub> and A<sub>e</sub> represented the radioactivity of <sup>99</sup>Mo in 1 mL of supernatant solution before and after sorption, respectively, C<sub>o</sub> was the total Mo content (10 mg) in 1 mL of solution before sorption, V was the volume of solution and m was the mass (g) of the sorbent.

The dynamic sorption capacity and breakthrough pattern were studied by passing sodium molybdate solution (25 mg Mo mL<sup>-1</sup>) at pH 3, spiked with <sup>99</sup>Mo radiotracer through borosilicate glass columns of dimension 6 cm × 1 cm (i.d.) with sintered disc (G<sub>0</sub>) at the bottom containing 5 g of nano-alumina at a flow rate of 2 mL min<sup>-1</sup>. From the feed <sup>99</sup>Mo solution, 2 mL was kept as reference and the eluted solution was collected in 2 mL aliquots. The radioactivity associated with the reference solution (A<sub>o</sub>), as well as each fraction (A<sub>i</sub>) was measured in a HPGe detector by counting the 181 keV peak of <sup>99</sup>Mo. The sorption capacity of MA for Mo under dynamic conditions was estimated by the study of the breakthrough profile determined using these data. In

order to determine the practical sorption capacity of MA, 100 mL of sodium molybdate solution (25 mg Mo mL<sup>-1</sup>) at pH 3, spiked with <sup>99</sup>Mo radiotracer was passed through the column containing 5 g of MA at a flow rate of 2 mL min<sup>-1</sup>. Subsequently, the column was washed with 400 mL of 0.9% solution and the activities of the effluent and the washing were measured.

#### **Supplementary Information S4: Details regarding the preparation of <sup>99</sup>Mo/<sup>99m</sup>Tc generators**

A borosilicate glass column of 1 cm internal diameter (12 cm × 1 cm) with a sintered disc (G<sub>o</sub>) at the bottom was packed with 9 g of MA and kept in a lead shielded container. The column matrix was conditioned at pH 3 by passing 0.01 N HNO<sub>3</sub> solution. A small piece of glass wool was placed on top of the sorbent bed to prevent disturbance of the bed during passage of eluents. Input/output connections were made with standard teflon tubings of 1 mm inner diameter and connectors. All the operations were carried out in the closed cycle system using connecting tubes. The generator column, connectors and connection tubings were integrated within a small portable lead shielded unit throughout experimental use for radioprotection purpose. Only the feed and output vials were accessible externally. A disposable 0.22 µm membrane filter was attached to the generator column output by teflon tubing. The column was then loaded with 26 GBq (700 mCi) of <sup>99</sup>Mo solution maintained at pH 3. The loaded column was washed with 500 mL of 0.9% NaCl solution.

A double column <sup>99</sup>Mo/<sup>99m</sup>Tc generator having two columns (**Fig. 1**) connected in tandem was prepared adopting the procedure similar to that for single column generator. The internal diameter of the generator columns were kept fixed at 1 cm, as in the case of single column

generator. Two columns each of dimension 6 cm  $\times$  1 cm were packed with 4.5 g of the sorbent and connected in tandem using suitable connectors. The elution of the generators was carried out using evacuated vials. The elution performance of both these generators was compared over a period of 1 week. After allowing adequate time (24 h) for  $^{99m}\text{Tc}$  build up, the generators were eluted with 0.9% NaCl solution at a flow rate of 2 mL min $^{-1}$ . In order to examine the elution profile, the eluate was collected as 2 mL aliquots and each fraction was counted for gamma activity.

#### **Supplementary Information S5: Quality control of $^{99m}\text{Tc}$ and its complexation with different ligands**

The evaluation of the radionuclidic purity, radiochemical purity, labeling efficacy of  $^{99m}\text{Tc}$  were carried out using standard procedures as described below. Radionuclidic purity measurements were made using a calibrated HPGe detector. The  $^{99}\text{Mo}$  contamination level in  $^{99m}\text{Tc}$  was quantified by allowing the  $^{99m}\text{Tc}$  samples to decay for 2 days and then measuring the 181 keV  $\gamma$ -ray peak, corresponding to emission from  $^{99}\text{Mo}$  contaminant.

To evaluate the radiochemical purity of  $^{99m}\text{TcO}_4^-$ , 5  $\mu\text{L}$  of activity was applied on paper chromatographic strips (Whatman 12 cm  $\times$  1 cm) at 1.5 cm from the lower end. The strips were developed in ethanol and 0.9% saline solutions. After chromatography, the paper strips were dried, cut into 1 cm segments, placed in test tubes and counted in a NaI(Tl) scintillation counter.

In order to determine the presence of metal contamination (chemical impurities), the  $^{99m}\text{Tc}$  samples were allowed to decay for 10 days. The presence of trace level of Al ion contamination in the decayed samples was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

To evaluate the labeling efficacy,  $^{99m}\text{Tc}$  eluted from the generator was used to prepare complexes of dimercapto succinic acid, methylene diphosphonate (MDP) and ethyl cysteinate dimer (ECD) using standard commercial kits, obtained from the Board of Radiation and Isotope Technology, India.

The  $\gamma$ -ray spectra of freshly eluted  $^{99m}\text{Tc}$  and decayed  $^{99m}\text{Tc}$  samples are shown in **Fig. S1**. The  $\gamma$ -spectrum of freshly eluted  $^{99m}\text{Tc}$  (**Fig. S1 a**) did not show the presence of such peaks, thereby confirming the absence of  $^{99}\text{Mo}$  in the eluted  $^{99m}\text{Tc}$ . The analysis of the decayed samples of  $^{99m}\text{Tc}$  by  $\gamma$ -spectrometric technique (**Fig. S1 b**) revealed that the level of  $^{99}\text{Mo}$  present in the eluate was below  $10^{-3}$  %. The radiochemical purity of  $^{99m}\text{Tc}$  in pertechnetate form as determined by paper chromatography in ethanol and saline medium was  $>99\%$  which was within the prescribed limits as per the pharmacopoeias.<sup>36</sup> The results are shown in **Fig. S2**.

In order to examine the suitability of  $^{99m}\text{TcO}_4^-$  for radiolabeling studies, it was used for the preparation of  $^{99m}\text{Tc}$ -MDP,  $^{99m}\text{Tc}$ -DMSA and  $^{99m}\text{Tc}$ -ECD using lyophilized kits. The paper chromatography patterns for  $^{99m}\text{Tc}$ -MDP,  $^{99m}\text{Tc}$ -DMSA and  $^{99m}\text{Tc}$ -ECD are shown in **Fig. S3**. In case of  $^{99m}\text{Tc}$ -MDP and  $^{99m}\text{Tc}$ -DMSA, in the TLC using acetone as solvent, the complex and  $^{99m}\text{TcO}_4^-$  migrated to the solvent front and hydrolyzed technetium ( $^{99m}\text{TcO}_2$ ) remained at the origin. When acetone was used as the solvent, hydrolyzed technetium and  $^{99m}\text{Tc}$ -MDP and  $^{99m}\text{Tc}$ -DMSA remained at the point of spotting and  $^{99m}\text{TcO}_4^-$  migrated to the solvent front. By combining the results of both the paper chromatography patterns, the complexation yields were estimated to be  $>90\%$ . In the paper chromatography patterns, using methanol solvent for  $^{99m}\text{Tc}$ -ECD, only the complex migrated towards the solvent front, whereas hydrolyzed technetium and  $^{99m}\text{TcO}_4^-$  remained at the origin. The complexation yields were estimated to be  $\sim 98\%$ . These

results justify the suitability of  $^{99m}\text{Tc}$  obtained from the generator for radiopharmaceutical applications.

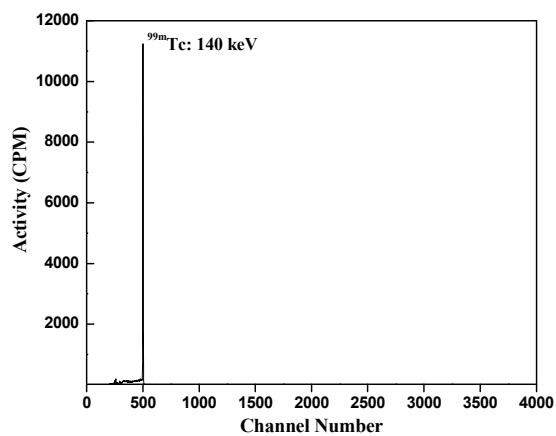
**Figure captions:**

Fig. S1:        Gamma spectrum of  $^{99m}\text{Tc}$  (a) freshly eluted (b) decayed samples

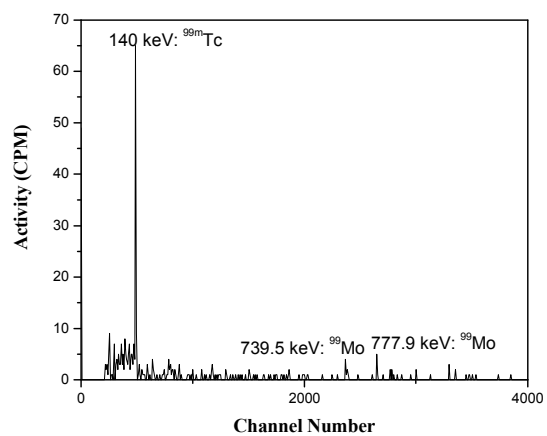
Fig S2:        Paper chromatographic pattern to determine the radiochemical purity of the  $^{99m}\text{TcO}_4^-$  eluted

Fig. S3:        Paper chromatographic patterns of (a)  $^{99m}\text{Tc}$ -MDP, (b)  $^{99m}\text{Tc}$ -ECD and (c)  $^{99m}\text{Tc}$ -DMSA



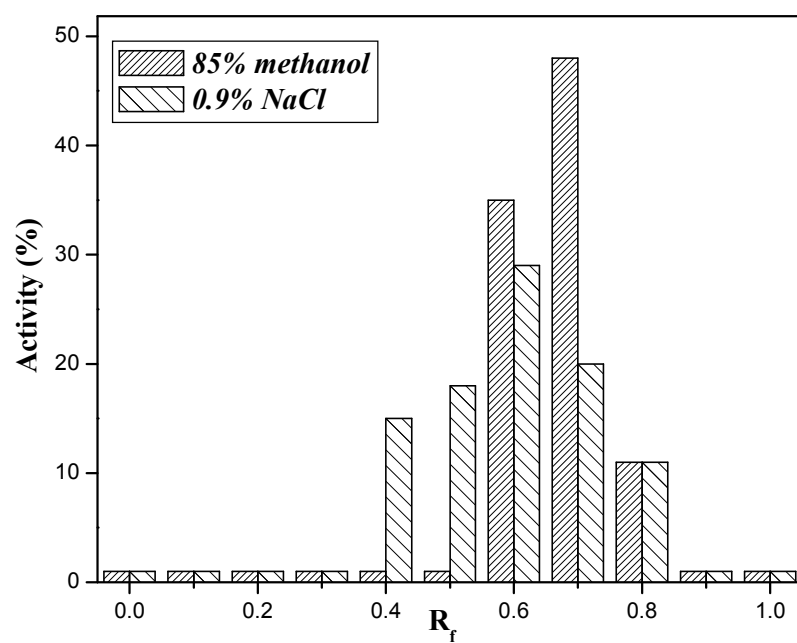


(a)

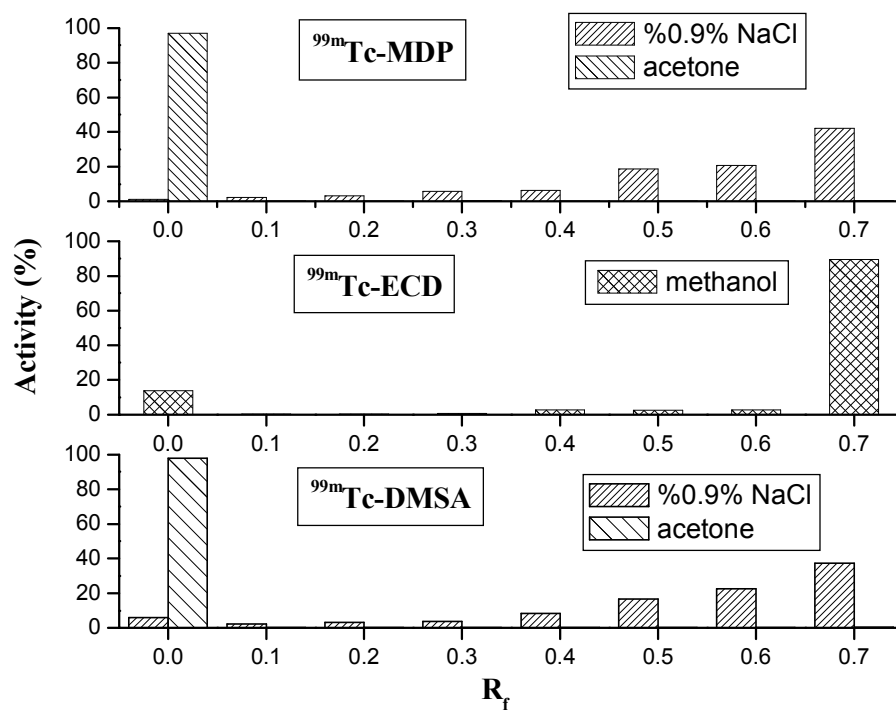


(b)

**Fig. S1: Gamma spectrum of  $^{99m}\text{Tc}$  (a) freshly eluted (b) decayed samples**



**Fig S2: Paper chromatographic pattern to determine the radiochemical purity of the  $^{99m}\text{TcO}_4^-$  eluted.**



**Fig. S3: Paper chromatographic patterns of (a)  $^{99m}\text{Tc-MDP}$ , (b)  $^{99m}\text{Tc-ECD}$  and (c)  $^{99m}\text{Tc-DMSA}$**