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Quality Control of Colloid and Particulate ^{99m}Tc-Labeled Radiopharmaceuticals

Elias P. Belkas and Spyridon Archimandritis

Nuclear Research Center "Demokritos", Isotopes Division, Athens, Greece

Abstract. A procedure for the radiochemical purity control of colloid and particulate ^{99m}Tc-labeled radiopharmaceuticals is described. The proposed technique is based on the use of two chromatograms, using in both, 15% H_3PO_4 as solvent and impregnated glass fiber media (Gelman ITLC type SG) as stationary phase. A pretreatment of the radiopharmaceutical with 6 *N* NaOH is involved prior to one chromatographic run. The procedure is fast and the different species (free pertechnetate, ^{99m}Tc-Sn-colloid and labeled ^{99m}Tc) in a ^{99m}Tc-labeled radiopharmaceutical can be determined accurately and with reliability.

Introduction

Increased knowledge of the biological behaviour of the various chemical forms of ^{99m}Tc present in biomedical products and the rapidly growing use of kits or on-site preparations, have increased the need for simple, fast and more accurate and reliable routine methods of quality control. The determination of "hydrolyzed-reduced technetium" is still the main problem.

The time-limiting methods of Colombetti et al. (1976) and Archimandritis and Belkas (1978) seem to solve the problem for water-soluble radiopharmaceuticals, but there is no such method for colloid or particulate preparations. The methods proposed by Eckelman and Richards (1972) and by Billinghurst (1973) can not be considered as simple quality tests suitable for small laboratories because of problems concerning the paper-saline system and gel-filtration (Billinghurst, 1973; Valk et al., 1973). Also, the gel chromatography column scanning (GCS) method of Persson (1975) needs a well organized nuclear medicine department for its practical application (Hladik et al., 1977).

In this study we tried to find a selective and quantitative way to differentiate and alter the mobility of the reduced uncomplexed ^{99m}Tc or the colloid/particulate complex of ^{99m}Tc. For this purpose a number of reagents were tested in combination with various chromatographic systems.

Using the finally proposed procedure, the percentage of the different forms of ^{99m}Tc may be estimated rapidly and with reliability.

Materials and Methods

I. ^{99m}Tc as pertechnetate, processed by the solvent extraction method from ^{99}Mo.

II. ^{99m}Tc-tin-colloid, prepared electrolytically.

III. ^{99m}Tc-S-colloid and ^{99m}Tc-HSA-macroaggregated, obtained from commercial available kits, were used as representatives of colloid and particulate preparations respectively.

The appropriate yield of the above (I, II and III) forms of ^{99m}Tc was checked by paper chromatography with 85% methanol.

IV. The reagents used to differentiate the insoluble forms of ^{99m}Tc (^{99m}Tc-Sn-colloid, ^{99m}Tc-S-colloid or ^{99m}Tc-HSA-MAA) were H_2O_2, $KMnO_4$, HNO_3, H_2SO_4, $NaNO_2$ and NaOH.

V. The employed supporting media for the chromatography were, 2 cm × 14 cm, strips of Whatman No 1 paper and Gelman ITLC type SG.

VI. 85% CH_3OH, 0.9% NaCl, CH_3COCH_3 and 15% H_3PO_4 (Archimandritis and Belkas, 1978) were used as mobil phases. It is known that in these chromatographic systems the ^{99m}Tc-Sn-colloid remains at the origin, the pertechnetate moves to a distance appropriate for its determination while the ^{99m}Tc-S-colloid and the ^{99m}Tc-HSA-MAA either remain at the origin or move in a way not sufficient for their separation form the ^{99m}Tc-Sn-colloid.

The influence of the tested reagents against ^{99m}Tc-Sn-colloid, ^{99m}Tc-S-colloid and ^{99m}Tc-HSA-MAA was found as follows: 0.5 ml of the different ^{99m}Tc forms were separately treated with each one of the reagents under various conditions (concentration, time and heating) and then an amount of 5–10 µl of the sample was applied to the supporting medium 2 cm from the end that

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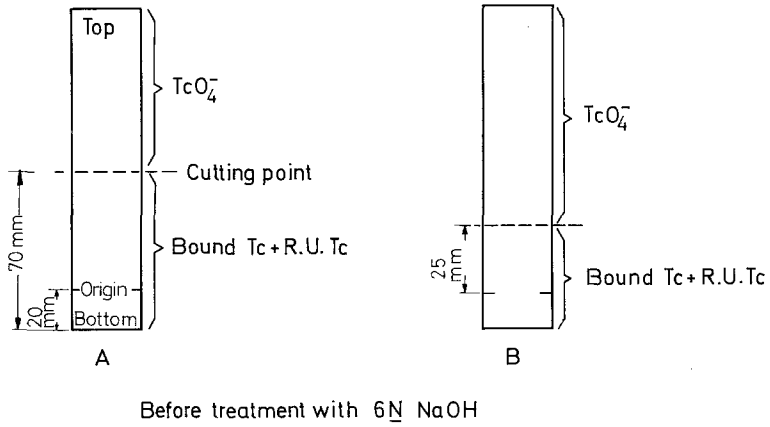
Table 1. Percent remaining at the origin. Supporting medium Gelman ITLC-SG

N NaOH	Time after treatment with NaOH (min)	Developing solvent											
		15% H_3PO_4			CH_3COCH_3			85% CH_3OH			0.9% NaCl		
		Tc(IV)	Tc-SC	Tc-HSA	Tc(IV)	Tc-SC	Tc-HSA	Tc(IV)	Tc-SC	Tc-HSA	Tc(IV)	Tc-SC	Tc-HSA
1	0.5	15	98	99	61	99	98	30	99	99	27	99	99
	5	10	98	99	33	99	98	20	99	99	19	99	99
	10	7	98	99	26	99	98	20	99	99	15	99	99
2	0.5	15	98	99	58	99	96	30	98	98	21	98	98
	5	8	98	99	30	99	96	20	98	98	16	98	98
	10	6	98	99	26	99	96	20	98	98	11	97	98
4	0.5	14	96	98	50	99	95	27	98	97	21	98	96
	5	5	96	98	25	99	95	17	98	97	14	98	96
	10	3	96	97	23	99	95	17	98	97	11	97	96
6	0.5	5	96	97	35	98	94	20	97	96	11	98	95
	5	3	96	96	20	98	94	13	97	96	7	97	92
	10	2	96	96	16	98	94	12	97	96	5	97	92
8	0.5	4	94	97	10	98	94	13	97	96	7	97	95
	5	2	94	95	6	98	93	13	97	96	5	96	92
	10	2	94	95	6	98	92	11	97	96	4	96	92
10	0.5	2	94	96	9	97	93	13	96	96	4	97	95
	5	2	94	95	5	97	93	12	96	96	3	96	92
	10	2	94	95	3	97	90	10	96	96	3	96	92



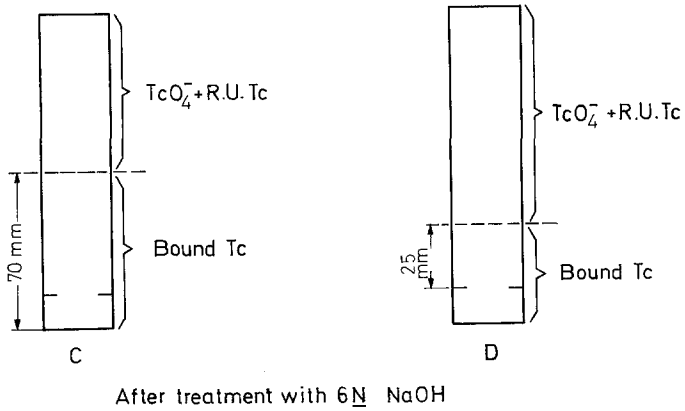


Fig. 1A–D. Chromatography strips used. **A** Gelman ITLC-SG with all solvents used. Also, Whatman No 1 with CH_3COCH_3; **B** Whatman No 1 with 15% H_3PO_4 or 85% CH_3OH or 0.9% NaCl; **C** Gelman ITLC-SG with 15% H_3PO_4; **D** Whatman No 1 with 15% H_3PO_4. *R.U.Tc:* Reduced Uncomplexed Technetium

was to be dipped into the different developing liquids. The chromatograms were allowed to develop until the solvent front just reached the top of the strips. After air drying the chromatograms were radioscanned and autoradiographed. The segment of a particular spot was cut and counted in a well-type NaI(Tl) counter. The activity of each segment was then expressed as a percentage of the toal activity on the strip.

The results show that in all but the case of NaOH, no quantitative and selective separation between ^{99m}Tc-Sn-colloid and ^{99m}Tc-S-colloid or ^{99m}Tc-HSA-MAA could be achieved and so we extensively studied the use of NaOH. In a test tube 0.5 ml of the ^{99m}Tc preparation form to be tested was mixed with 50 µl of NaOH at concentrations from 1–10 *N*. The mixture was vortexed for 30 s. A volume of 5–10 µl was taken immediately after shaking and at 5 and 10 min intervals for chromatographic analysis.

Results and Discussion

The results show that after treatment with NaOH the reduced uncomplexed ^{99m}Tc moves on the chromatographic paper with an Rf similar to that of ^{99m}Tc(VII) in contrast to the labeled forms of ^{99m}Tc (^{99m}Tc-S-colloid and ^{99m}Tc-HSA-MAA) which remain at the application point. We identified the chemical form of the reduced uncomplexed ^{99m}Tc by repeating the process against a ^{99m}Tc(VII) reference in 85% CH_3OH. Both forms had the same Rfs and so we concluded that, under the influence of the NaOH, the reduced uncomplexed ^{99m}Tc changes to ^{99m}Tc(VII). It was also observed, that the quantitative movement of the ^{99m}Tc-Sn-colloid depends on the mobil phase used. No significant differences were found with the nature of the supporting medium (Whatman No 1 or Gelman SG).

The results obtained using supporting medium Gelman SG are given in Table 1. From these data, it is quite evident that the best results are registered for the system using solvent 15% H_3PO_4 for developing and concentrations of NaOH of more than 6 *N*. In order to test the applicability of the method to a non acceptable kit preparation (impurities more than 5%) we repeated experiments with mixtures of different amounts of Tc-Sn-colloid, Tc(VII) and Tc-S-colloid or Tc-HSA-MAA. The results agree with those shown in Table 1.

The technique entails the use of two chromatograms. One is used for the determination of the percentage of free TcO_4^- by using as a solvent 15% H_3PO_4, acetone, 85% CH_3OH or normal saline, and a second for determining the percent of the labeled ^{99m}Tc remaining at the origin. For the later 15% H_3PO_4 is used as solvent and it is run 5 min after 30 s vortexing with a mixture of 0.5 ml of the sample and 50 µl of 6 *N* NaOH. The fraction of the reduced uncomplexed ^{99m}Tc is obtained by subtraction of the two above figures from 100%.

The agreement of the percentages of bound Tc in all the systems is excellent. The ratio TcO_4^-: reduced uncomplexed-Tc is not significant altered by changing the stationary phase and the solvent in the first chromatogram.

The protocol we employed for cutting the strips and for the distributions of ^{99m}Tc species are shown in Fig. 1.

Conclusion

The described procedure has the great advantage of allowing the determination of reduced uncomplexed technetium in colloid or particulate preparations by performing paper or thin-layer chromatography. All known techniques employ filtration (for particulates greater than 0.2 µm) or centrifugation with successive washings.

From the chromatographic systems studied we propose the use of Gelman SG strips, as stationary phase, since it is considerably faster and can be cut in two halves. For developing solvent we prefer 15% H_3PO_4 since both chromatograms (before and after treatment with NaOH) can be run in the same jar simultaneously. Finally, for determination of the total impurities (sum of TcO_4^- and reduced uncomplexed Tc) the first chromatogram can be deleted.

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References

Colombetti, L.G., Moerlien, S., Patel, S.M., Pinsky, S.M.: Rapid determination of oxidation state of unbound ^{99m}Tc and labeling yield in ^{99m}Tc-labeled radiopharmaceuticals. J. Nucl. Med. **17**, 805–809 (1976)

Archimandritis, S., Belkas, E.P.: Analytical procedures for radiochemical control of water-soluble ^{99m}Tc-labeled radiopharmaceuticals. J. Radioanal. Chem. **43**, 287–293 (1978)

Eckelman, W.C., Richards, P.: Analytical pitfalls with ^{99m}Tc-labeled compounds. J. Nucl. Med. **13**, 202–204 (1972)

Billinghurst, M.W.: Chromatographic quality control of ^{99m}Tc-labeled compounds. J. Nucl. Med. **14**, 793–797 (1973)

Valk, P.E., Dilts, C.A., McRae, J.: A possible artifact in gel chromatography of some ^{99m}Tc-chelates. J. Nucl. Med. **14**, 235–237 (1973)

Persson, B.R.R.: Gel chromatography column scanning (GCS). A method for identification and quality control of ^{99m}Tc radiopharmaceuticals. In: Radiopharmaceuticals, Subramanian, G., Rhodes, B.A., Cooper, J.F., Sodd, V.J. (eds.), pp. 228–235. New York: Society of Nuclear Medicine 1975

Hladik III, W.B., Study, K.T., Gallagher, J.H., Rhodes, B.A.: Quality control of radiopharmaceutical purity by a rapid feedback system. J. Nucl. Med. Technol. **5**, 94–100 (1977)

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