Rapid Determination of Oxidation State of Unbound ^{99m}Tc and Labeling Yield in ^{99m}Tc-Labeled Radiopharmaceuticals

Lelio G. Colombetti, Stephen Moerlien, Ghanshyam C. Patel, and Steven M. Pinsky

Michael Reese Hospital and Medical Center, Chicago, Illinois

Current techniques for determining the radiochemical purity of ^{99m}Tc-labeled radiopharmaceuticals are limited by the variety of compounds that can be tested or the length of time required to complete the test. A chromatographic method, based on the use of two solvents (0.9% saline and acetone) and a stationary phase made of silica-coated cellulose strips, solves these problems for water-soluble ^{99m}Tc-labeled radiopharmaceuticals. With this method, the oxidation state of unbound ^{99m}Tc and the labeling yield of ^{99m}Tc-labeled radiopharmaceuticals can be quickly determined: the whole procedure takes only a few minutes to run. This system compares favorably with lengthier procedures and with a commercially available kit.

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The prevalent use of radiopharmaceuticals labeled with ^{99m}Tc has created the need for a rapid accurate technique to test the radiochemical purity of these compounds. Because the different chemical states of technetium show different biologic behaviors, knowing the relative amounts of each (i.e., free pertechnetate, reduced uncomplexed ^{99m}Tc, and labeled ^{99m}Tc) is important to the proper evaluation and use of ^{99m}Tc-labeled radiopharmaceuticals.

Unfortunately, present techniques are difficult to use when all three states of technetium must be determined. Thin-layer and paper chromatography are widely accepted as reliable methods, but two liquid separation phases are required, each taking 30–40 min to run. Billinghurst (*1*) has mentioned a method of separating free pertechnetate and reduced technetium using acetone and saline, respectively, as solvents. Alternative schemes are separation of Tc(IV) with saline (*2*) and separation of Tc(VII) from reduced uncomplexed ^{99m}Tc and labeled ^{99m}Tc with 85% methanol (*2*).

Column chromatography (*3*) is unsatisfactory because it does not separate bound technetium from the reduced uncomplexed form. Gel filtration with Sephadex has been found useful in revealing the chemical state of technetium (*4*) and the radiochemical purity of ^{99m}Tc-labeled compounds (*5–6*). However, Valk (*7*) has questioned the biologic applicability of the results of Sephadex filtration because of artifacts produced with some ^{99m}Tc-labeled compounds. Although microfiltration has been used to analyze ^{99m}Tc-sulfur colloid preparations (*8*), this procedure is limited to colloids and particulate matter, and the reduced species is not distinguished from unbound technetium.

This paper presents a rapid reliable microchromatographic method, which we call "Michrom," for investigating the radiochemical purity of ^{99m}Tc-labeled pharmaceuticals and the oxidation state of their unbound technetium. The results of this technique were compared to those obtained with a kit that employs a similar technique (MAC-1, General Radioisotope Products, San Ramon, Calif.). This commercial product consists of two chromatographic strips of unspecified composition, used with two solvents stated to be saline and a mixture of acetic acid and acetone.

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For reprints contact: Lelio G. Colombetti, Div. of Nuclear Medicine, Michael Reese Hospital and Medical Center, 29th St. & Ellis Ave., Chicago, IL 60616.

MATERIALS AND METHODS

Our technique employs a cellulose base coated with silica gel as the stationary phase, and two mobile phases. Saline is used to separate reduced uncomplexed ^{99m}Tc from $^{99m}TcO_4^-$ and labeled technetium, and acetone is used to separate $^{99m}TcO_4^-$ from the reduced uncomplexed and labeled ^{99m}Tc. The stationary phase is prepared from pure cellulose sheets, 1 mm thick, of chromatographic quality (Gelman Instrument Co., Ann Arbor, Mich.), cut into strips 7 mm wide by 57 mm long. These strips are saturated by submersion in a 6% suspension of silica gel, 60–200 mesh grade 62 (Matheson, Coleman, & Bell, Norwood, Ohio), and air-dried. As shown in Fig. 1, the strips to be used with the saline medium are marked transversely with a graphite pencil at a point 20 mm from the bottom. Similarly, the strips to be used in the acetone solution are marked 17 mm from the top of the strip. These marks are used later in cutting the strips into two sections.

For convenience, each strip is spotted 10 mm from the bottom (Fig. 1) with 2 λ of a soluble dye solution. These colored spots will be used as points of application. Because of their solubility, the dyes will follow the liquid front closely. Methyl orange was chosen as the indicator for the saline strips (which will be called strips A), and gentian violet was used for the acetone strips (strips B). After the dye has dried, the strips are stored in air-tight vials until used.

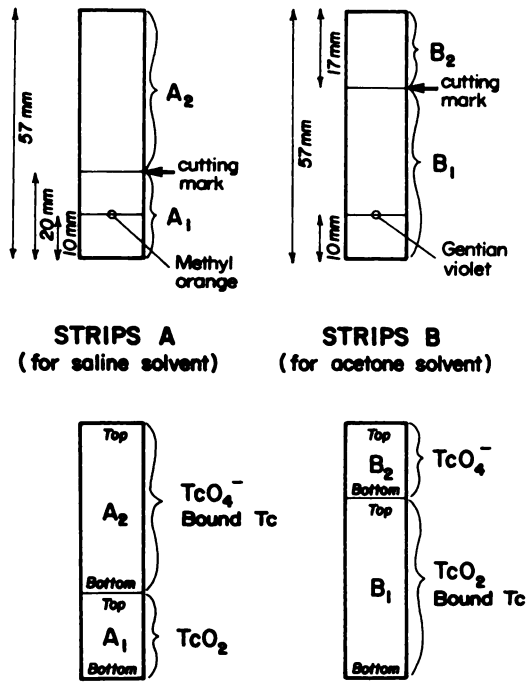

FIG. 1. Stationary phases used in Michrom system. Both strips are made of pure cellulose sheets coated with silica gel. Application points are shown as dye spots in upper two drawings. Cutting marks and distributions of ^{99m}Tc species are also shown. Reduced uncomplexed technetium is shown as TcO_2.

The species of technetium found in radiopharmaceuticals are separated as follows. A 5–10-λ drop of the sample is placed on the dye spot of strip A and another drop on strip B. Without drying, the strips are inserted vertically into flat-bottomed vials containing 2 mm of the corresponding mobile phase. The vials need not be stoppered because, being small, they remain saturated with vapors from the mobile phases. Just before the solvent front reaches the top of each chromatographic strip (in 40–45 sec), as indicated by the dyes, the strips are removed from the vial. They are then cut at the pencil marks and the two components are placed, bottom end down, in counting tubes for measurement in a well counter or a dose calibrator. These sections (Fig. 1) are designated as A_1 and A_2 for strip A and B_1 and B_2 for strip B.

The percentages of reduced uncomplexed ^{99m}Tc, free pertechnetate, and labeled ^{99m}Tc are calculated from the counts obtained, using the following formulas:

$$\text{Reduced uncomplexed } ^{99m}Tc = \frac{A_1}{(A_1 + A_2)} \times 100\% = R$$

$$\text{Free pertechnetate} = \frac{B_2}{(B_1 + B_2)} \times 100\% = F$$

$$\text{Labeled } ^{99m}Tc = 100\% - (R + F).$$

This system was tested with eluates obtained from ^{99}Mo–^{99m}Tc generators and with pertechnetate reduced by stannous chloride. Over 98% of the radioactivity due to free pertechnetate progressed with the liquid phase using strips A, while more than 99% of the activity in tests of reduced samples remained at the bottom of strip B. These results support the formulas presented above.

Using the same samples, the new procedure was compared with conventional instant thin-layer chromatography (ITLC) (2) and with the commercially available kit MAC-1. Silica gel paper for ITLC was used with three solvents: saline, acetone, and 85% methanol in water. Saline transports the $^{99m}TcO_4^-$ and bound ^{99m}Tc, but leaves reduced ^{99m}Tc at the application point because the $SnCl_2$ by hydrolysis forms a colloid that binds the reduced ^{99m}Tc (9). The acetone transports free $^{99m}TcO_4^-$ rapidly, leaving the reduced (bound and unbound) ^{99m}Tc close to the application point. Methanol was found to produce many artifacts, and its use was abandoned. From the percentages of reduced ^{99m}Tc and free $^{99m}TcO_4^-$, the percentage of bound ^{99m}Tc can be calculated. The commercially available MAC kit was

TABLE 1. COMPARISON OF RESULTS WITH MICHROM SYSTEM AND INSTANT THIN-LAYER CHROMATOGRAPHY (ITLC)

			Michrom			ITLC		
Compound	Batch No.	No. of replicates	TcO_2 (%)	TcO_4 (%)	Labeled (%)	TcO_2 (%)	TcO_4 (%)	Labeled (%)
Pyrophosphate	523	3	26.0	0.6	73.4	19.0	7.5	73.5
	572	2	10.5	0.2	89.3	7.1	5.8	87.1
	573	3	9.5	0.1	90.4	2.6	8.5	88.9
	580	2	9.5	2.5	88.0	5.8	11.4	82.8
	582	2	19.4	0.1	80.5	12.5	8.9	78.6
	585	3	14.7	0.9	84.4	6.7	6.3	87.0
	601	2	25.2	0.4	74.4	11.7	10.7	77.6
	605	3	16.2	1.6	82.2	14.7	10.8	74.5
DTPA	203	2	2.6	4.0	93.4	1.8	6.2	92.0
	211	3	3.9	3.4	92.7	1.7	4.2	94.1
	214	3	1.5	1.5	97.0	1.7	7.2	91.1
Citrate	4	1	0.7	1.4	97.9	1.6	—	98.4
	5	1	2.5	4.1	93.4	1.0	0.8	99.2
	6	2	1.1	3.9	95.0	—	—	100.0
	7	1	1.6	6.9	91.5	—	—	100.0
Osteoscan	112	2	6.0	0.1	93.9	1.8	1.2	97.0
	113	3	5.1	0.0	94.9	7.6	1.4	91.0

TABLE 2. COMPARISON OF RESULTS WITH MICHROM AND MAC SYSTEMS*

			Michrom			MAC		
Compound	Batch No.	No. of replicates	TcO_2 (%)	TcO_4 (%)	Labeled (%)	TcO_2 (%)	TcO_4 (%)	Labeled (%)
Pyrophosphate	612	2	9.5	2.7	87.8	35.2	3.7	61.1
	622	3	9.7	0.1	90.2	28.5	0.2	71.3
	630	2	11.0	0.2	88.8	41.2	0.3	58.5
Osteoscan	114	2	6.7	0.3	93.0	2.1	0.2	97.7
DTPA	230	3	0.9	0.5	98.6	0.7	0.9	98.4
Citrate	4	2	0.7	1.4	97.9	—	—	100.0

* Note that results diverge for reduced technetium for a few batches of the ^{99m}Tc-pyrophosphate.

also used, according to the manufacturer's instructions, and these results were compared with those obtained using the similar Michrom procedure.

RESULTS AND DISCUSSION

The conventional ITLC tests tend to show relatively higher levels of free pertechnetate, which could be significant. As shown in Table 1, ITLC gives percentages of free pertechnetate up to ten times those given by Michrom. This result may be ascribed to oxidation of the reduced species of ^{99m}Tc as the sample is slowly separated by the saline in ITLC media. This oxidation effect is less noticeable in the Michrom procedure because the separation takes only 45 sec, compared to the 30 min required by ITLC. For this reason, we believe that the Michrom technique gives a more accurate indication of the species of ^{99m}Tc in radiopharmaceuticals than the lengthier ITLC procedure. Because the body distribution of reduced ^{99m}Tc differs from that of free $^{99m}TcO_4^-$, these differences in test results are important for the clinician.

The Fisher t-test showed no difference, at the 95% confidence limit, between the labeled ^{99m}Tc values of Michrom tests and those of ITLC. The resulting 5% uncertainty level is generally acceptable for analytic test results (*10*).

Table 2 shows a divergence in results for ^{99m}Tc-pyrophosphates using the Michrom technique and the commercial MAC kit. This kit gave unusually high values for uncomplexed reduced technetium, with correspondingly low values for labeled ^{99m}Tc. Clinical findings with the same samples failed to support the MAC results, but an excellent correlation was found with the Michrom data.

The high values for reduced unbound technetium

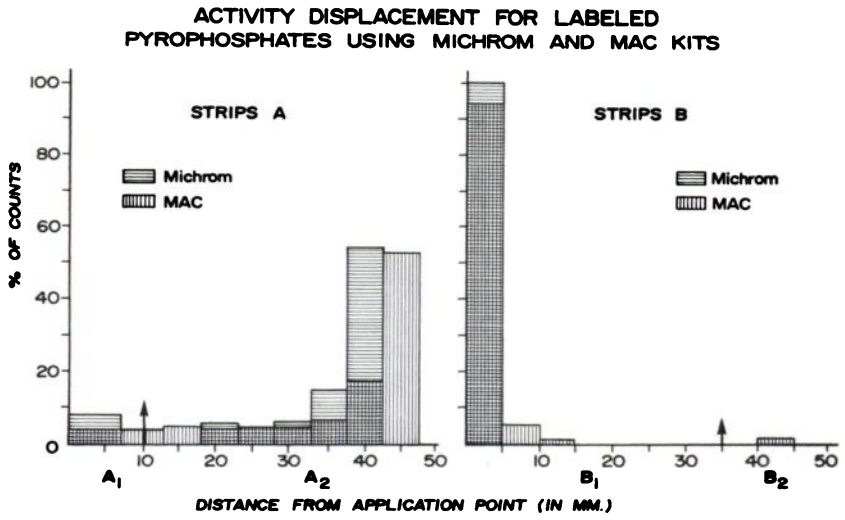

FIG. 2. Separation efficiency of Michrom and MAC for ^{99m}Tc-pyrophosphate. Cutting marks are shown by arrows. Strips A measure reduced uncomplexed ^{99m}Tc, while strips B measure free pertechnetate.

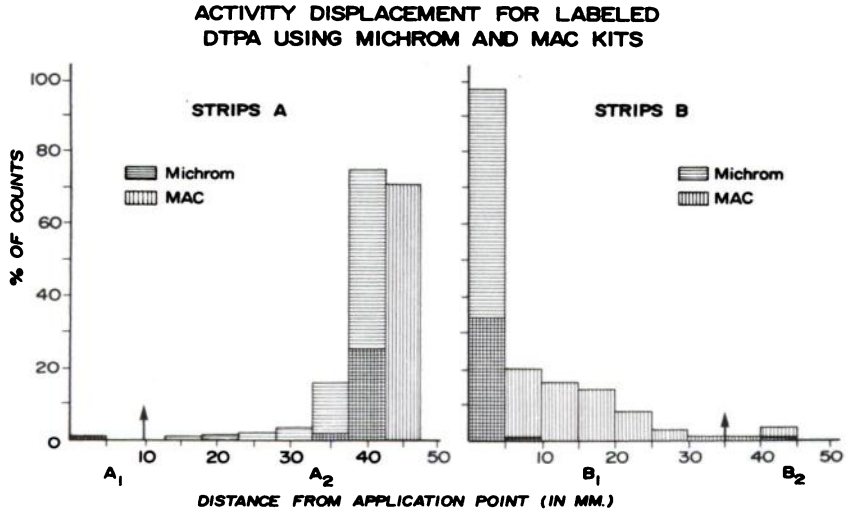

FIG. 3. Separation efficiency of Michrom and MAC for ^{99m}Tc-DTPA. Cutting marks are shown by arrows. Strips A measure reduced uncomplexed ^{99m}Tc, while strips B measure free pertechnetate.

obtained with the MAC kit are difficult to explain. The acetic acid contained in the acetone solvent may have dislodged a fraction of the bound technetium, releasing it as the reduced species and hindering its movement with the saline front. Another possible problem with the MAC kit is that the reduced technetium may form technetium acetate in the presence of acetic acid. The acetate thus formed is soluble in acetone and will travel with this solvent, giving erroneous results.

Figures 2 and 3 show that the separation of reduced uncomplexed technetium (strips A) from pertechnetate and labeled ^{99m}Tc was sharper in the Michrom procedure, whereas the separation of pertechnetate from uncomplexed reduced and labeled ^{99m}Tc was similar for both kits. These bar graphs present an example in which the Michrom and MAC values for the different species of technetium are approximately equal, so that the separation efficiencies may be more easily compared. However, in many samples tested, the MAC values for reduced uncomplexed technetium were significantly higher than those using our Michrom technique (Table 2). The chromatographic development times for both methods differed in these experiments because the MAC strips are about 5 mm longer.

The technique described in this paper has limitations. The Michrom method is not useful in testing compounds that are insoluble in water, such as particulate radiopharmaceuticals (colloids, macroaggregates, microspheres), because the labeled fraction of these compounds does not move in the liquid front when saline is used as a solvent, as it does when chelates, inorganic salts, etc., are tested.

CONCLUSION

The method presented is a rapid, inexpensive, and chemically reliable process that may be used in any nuclear medicine department where it is desired to test water-soluble ^{99m}Tc-labeled radiopharmaceuti-

cals for labeling efficiency before administration to patients. The preparation of the materials necessary to perform this test is simple and rapid. The extra cost, time, and effort are minimal in any nuclear medicine setting.

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