CHROMATOGRAPHIC QUALITY CONTROL OF ^{99m}Tc-LABELED COMPOUNDS

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The increasingly general use of a number of ^{99m}Tc-labeled compounds makes the need for an understanding of the capabilities of simple quality-control procedures urgent. A number of ^{99m}Tc radiopharmaceuticals which are already in general use have been studied in various chromatographic procedures which might potentially provide adequate quality control. The comparative effectiveness of each of these chromatographic procedures for the accurate quality control of each radiopharmaceutical may be readily observed and an adequate procedure decided upon. The practical ease of handling and the technical skills necessary for the accurate handling of each chromatographic system is discussed. It is recommended that quality control of ^{99m}Tc-albumin be done on a Sephadex G50 column and that of ^{99m}Tc-DTPA be done with thin-layer silica gel chromatography in both saline and acetone or butyl acetate. The quality-control procedure for ^{99m}Tc-polyphosphate is shown to be dependent on the commercial kit which is used. Two such commercial kits are discussed.

In a recent letter to the editor (*1*) attention was drawn to the need for good routine quality-control systems for radiopharmaceuticals, particularly those which are prepared in situ. Some laboratories are using 85% methanol as a developing solvent in either paper or silica gel thin-layer chromatography for the quality control of ^{99m}Tc-serum albumin and ^{99m}Tc-DTPA. However, Eckelman and Richards (*2*) have shown that a paper chromatogram developed in 85% methanol does not distinguish between these products and some other form of ^{99m}Tc which they refer to as "hydrolyzed-reduced technetium IV." In some preparations they show that this contaminant was not only significant but of greater magnitude than the free pertechnetate which is the principle contaminant evaluated by paper chromatography in methanol. It is therefore of limited value to carry out quality-control procedures which fail to separate the product from this hydrolyzed-reduced technetium. Eckelman and Richards (*2–4*) use both paper chromatography with saline as the developing solvent and Sephadex gel chromatography. While both of these systems do provide a separation between the product, with both pertechnetate and hydrolyzed-reduced technetium the paper chromatography must be performed with careful attention to technique. Although this may be readily carried out accurately in the larger specialized laboratories, many of the smaller nuclear medicine departments do not have the necessary expertise in paper chromatography to use this procedure routinely and obtain accurate analyses. On the other hand, the Sephadex gel chromatography is technically easier to operate but requires increased technician involvement unless a fraction collector is available.

I believe that at this stage it is desirable to study the relative merits of various chromatographic procedures so that the users of in situ prepared radiopharmaceuticals may know what systems may be expected to give an adequate analysis of the radioactive preparation before in vivo use. This paper describes such a study.

METHODS AND MATERIALS

In this study, the primary quality-control procedures were thin-layer or paper chromatography because in our experience these techniques are the

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most suitable for the quality control of short half-life radiopharmaceuticals. The paper used was Whatman No. 1 chromatography paper and the thin-layer chromatography media used were Gelman silica gel ITLC, Baker-flex polyamide 6, and Baker-flex aluminum oxide 1B thin-layer chromatography strip. These last two use a flexible, inert polyethylene backing material while the Gelman strip uses a woven glass fiber backing which makes it much easier to handle. The solvents used were 0.9% w/v sodium chloride solution, 85% v/v methanol solvent, n-butyl acetate, and acetone.

Two other types of analysis systems were also investigated. These two were paper electrophoresis in three different buffers and at two different constant voltages and Sephadex gel chromatography. The electrophoresis was carried out using acetate buffer containing 6.8 gm of sodium acetate and 3.0 ml of acetic acid per liter, a phosphate buffer containing 3.6 gm of sodium monohydrogen phosphate and 3.6 gm of sodium dihydrogen phosphate monohydrate per liter, and a barbiturate buffer containing 20.6 gm of sodium barbitone and 3.68 gm of barbitone per liter. The paper used was Whatman No. 1 chromatography paper. The gel column chromatography was carried out using a 0.9 × 60-cm column packed with Sephadex G50 medium and a 0.9 × 30 column packed with Sephadex G25 medium. The eluant was physiological saline. Under such conditions, chelated species are eluted according to their molecular size between the void volume and the total column volume. Pertechnetate is eluated at the total column volume while the hydrolozed-reduced technetium is retained by the gel as discussed by Eckelman and Richards (*2*).

The ^{99m}Tc-human serum albumin was prepared by a modification of the method of Stern, et al (*5*) and the ^{99m}Tc-DTPA by a modification of the procedure of Hauser, et al (*6*). Two commercially available polyphosphate kits were used to prepare the ^{99m}Tc-polyphosphate. The ^{99m}Tc-iron ascorbate was prepared as follows.

One hundred milligrams of ascorbic acid was dissolved in 0.7 ml of 2 *N* hydrochloric acid. Five

TABLE 1. PAPER AND THIN-LAYER CHROMATOGRAPHY DATA AS PERCENTAGE OF TOTAL COUNTS

Medium	Solvent	R_f	$^{99m}TcO_4^-$	^{99m}Tc-HSA	^{99m}Tc-DTPA	^{99m}Tc-poly (X)	^{99m}Tc-poly (Y)	^{99m}Tc-FeAs
Paper	Saline	0.0	0	Incomplete resolution	6	77	81	4
		0.75	100		0	8	0	13
		1.0	0		94	15	19	83
	85% methanol	0.0	0	80	91	96	66*	7
		0.5	100	20	9	4	34*	93
	Acetone	0.0	0	93	100	97	68*	89
		1.0	100	7	0	3	31*	11
	Butyl acetate	0.0	100	99	100	100	99	100
	Saline	0.0	0	75	5	14	5	5
		1.0	100	25	95	86	95	95
	85% methanol	0.0	0	79	Incomplete resolution	74	60*	6
		1.0	100	21		26	39*	94
Silica gel	Acetone	0.0	0	93	100	98	65*	88
		1.0	100	7	0	2	35*	12
	Butyl acetate	0.0	0	95	100	98	67*	90
		0.8	100	5	0	2	33*	10
	Saline	0.0	100	79	17	90	95	19
		1.0	0	21	83	10	5	81
	85% methanol	0.0	100	94	100	98	96	67
		0.8	0	6		2	4	33
Polyamide 6	Acetone	0.0	100	99	99	99	99	99
	Butyl acetate	0.0	100	99	100	99	99	99
	Saline	0.0	0	84	90	90	95	86
		0.7	100	16	10	10	5	14
	85% methanol	0.0	0	89	92	94	96	87
		0.5	100	11	8	6	4	13
Alumina	Acetone	0.0	0	92	92	97	67*	88
		0.5	100	8	8	3	33*	12
	Butyl acetate	0.0	100	100	99	99	99	100

* Standard deviations > ±20. All other standard deviations less than ±3.

milliliters of pertechnetate solution followed by 0.2 ml of 1.5% solution of ferric chloride in 2 *N* hydrochloric acid were then added and the solution allowed to stand for 10 min. The pH was raised to between 6 and 7 with 1 *N* sodium hydroxide and the solution allowed to stand for 30 min.

RESULTS

To set up a quality-control system it is necessary to determine the likely contaminants. As all technetium labeling procedures involve the reduction of pertechnetate, there is a possibility of pertechnetate and hydrolyzed-reduced technetium being present in any ^{99m}Tc-labeled compound. Other contaminants may also be present depending on the specific labeling procedure used. For example, if the iron ascorbic acid reduction system is used, then ^{99m}Tc-iron ascorbate should be included in the list of possible contaminants, or if the stannous chloride method is used, then the possibility of colloidal tin being formed and incorporating some of the technetium must be considered.

^{99m}Tc-pertechnetate. Tables 1 and 2 show the paper and thin-layer chromatographic and paper electrophoretic data for pertechnetate. On Sephadex gel columns, pertechnetate is eluted with the ionic fraction which is the final fraction to be eluted.

^{99m}Tc-human serum albumin. The results obtained in quality-control studies on selected preparations are shown in Tables 1, 2, and 3. This preparation was chosen to demonstrate significant quantities of contaminants.

In studying the chromatographic data, it is obvious that, with the exception of paper chromatography in saline, the labeled serum albumin remains at the origin, and it is therefore necessary to select systems in which the various possible contaminants will migrate. The paper saline system was found to be unsuitable for routine use because nitrogen flushing of the developing solvent and of the drying chamber as well as careful attention to technique was necessary to obtain a satisfactory resolution of the constituents to allow quantitation. A careful study of the chromatography data of the various likely

TABLE 2. PAPER ELECTROPHORESIS DATA AS PERCENTAGE OF TOTAL COUNTS

Buffer	Integrated current (mamp-hr)	Migration distance (cm)	$^{99m}TcO_4^-$	^{99m}Tc-HSA	^{99m}Tc-DTPA	^{99m}Tc-poly (X)	^{99m}Tc-poly (Y)
		0	0	94	6	28	41*
Acetate	2	4	0	0	94	72	0
		8	100	6	0	0	59*
		0	0	88	5	86	76*
Barbiturate	2	4	0	12	95	14	0
		8	100	0	0	0	24*
		1	0	88	5	65	33*
Phosphate	2	5	0	12	95	35	0
		7	100	0	0	0	67*

* Standard deviations $> \pm 3$ and $< \pm 20$.
All other values have standard deviations of less than ± 3.

TABLE 3. SEPHADEX COLUMN DATA AS PERCENTAGE OF TOTAL COUNTS

Column type	Elution fraction	^{99m}Tc-HSA	^{99m}Tc-DTPA	^{99m}Tc-poly (X)	^{99m}Tc-poly (Y)	^{99m}Tc-FeAs
G50 (0.9 × 60 cm)	Void	74	1	1	1	0
	Small complexes* + ionic	14	95	57	67†	86
	Bound to column	12	4	42	32†	14
G25 (0.9 × 30 cm)	Void	74	95	67	77‡	74
	Ionic	14	0	0	11‡	13
	Bound to column	12	5	33	12‡	13

* On Sephadex G50 small complexes with molecular weights below 2,000 are not separated from ionic material. On this column complete resolution between the ionic and polyphosphate fractions were not obtained and they are therefore recorded as one peak.
† Standard deviations $> \pm 20$.
‡ Standard deviations $> \pm 3$ and $< \pm 20$.
All other values have standard deviations of less than ± 3.

contaminants shows that the hydrolyzed-reduced technetium also remains at the origin in all thin-layer and paper systems examined. Therefore the most successful quality-control system for labeled human serum albumin would seem to be a short column of Sephadex G50 from which the albumin is eluted at void volume and the pertechnetate and labeled complexes of smaller molecular size are eluted later, with the hydrolyzed-reduced technetium remaining bound to the gel column. We have found that a 0.9 × 15-cm column is satisfactory for this purpose and 1 ml is a convenient size fraction to collect.

^{99m}Tc-DTPA. The results obtained in quality-control studies on typical preparations are shown in Tables 1, 2, and 3. When these chromatographic results are examined, it becomes readily apparent that the quality control of the DTPA preparations may be carried out on silica gel thin-layer chromatography strips. The hydrolyzed-reduced technetium may be quantitated with saline as the solvent while the free pertechnetate may be quantitated with butyl acetate or acetone as the developing solvent. Other media may be used to analyze the preparation; however, the silica gel is both faster and easier to handle, and therefore is the method of choice, especially since it offers as good or better resolution than any of the other systems.

^{99m}Tc-polyphosphate. The results of the quality-control studies on the kits supplied by Manufacturers X and Y are shown in Tables 1, 2, and 3. The most noticeable difference between these two kits is the standard deviation of the results obtained from different preparations. It was observed that the product prepared from the kit supplied by Manufacturer X gave consistent analytical data while that from Manufacturer Y showed a very large standard deviation. A recent paper by Valk, et al (7) reports this type of nonreproducible gel chromatographic data with ^{99m}Tc-Sn-gluconate preparations. They were unable to observe any corresponding variation in the in vivo behavior of the product and so concluded that the phenomenon was related to the fact that ^{99m}Tc-Sn-gluconate is a weak chelate while reduced technetium will bind strongly to Sephadex. If such interactions also occur on the thin-layer and paper chromatography media, then this large standard deviation of the quality-control results with the kit from Manufacturer Y would imply that it is a much weaker chelate than that formed with the kit from Manufacturer X. It is clear from an examination of the quality-control results that only a small and consistent amount of decomposition of ^{99m}Tc-polyphosphate from Manufacturer X takes place on Sephadex columns.

The quality control of the product prepared from the kit supplied by Manufacturer X is readily accomplished by several combinations. For example, a silica gel ITLC in saline is used to quantitate the hydrolyzed-reduced technetium and a silica gel ITLC in acetone or butyl acetate to quantitate the free pertechnetate. In clinical work with this polyphosphate kit, we have found the silica gel ITLC in butyl acetate to be a very significant result. On occasions, when the initial ITLC showed more than 3% free pertechnetate, scans done later in the day all showed high levels of radioactivity in the stomach of the patients. For the kit supplied by Manufacturer Y it would seem that the best quality control would be a combination of a silica gel ITLC in saline to quantitate the hydrolyzed-reduced technetium and a Sephadex G25 column to evaluate the free pertechnetate, the remaining fraction being assumed to be ^{99m}Tc-polyphosphate.

^{99m}Tc-iron ascorbate. The results obtained in the quality-control studies on this material are shown in Tables 1 and 2. No results are shown for the electrophoretic studies because the resolution obtained was unsatisfactory. These tables provide both the information necessary to select a suitable quality-control system for ^{99m}Tc-iron ascorbate or to help in the selection of a quality-control system for other radiopharmaceuticals in which the ferric ion-ascorbic acid couple is used as a reductant. Of considerable interest in this latter respect is the mobility of this complex in 85% methanol on both paper and silica gel. This shows that, if the iron ascorbate system is used as a reductant, then the 85% methanol solvent may be used to quantitate both the ^{99m}Tc-pertechnetate and the ^{99m}Tc-iron ascorbate in most of the preparations examined in this study. The mobility of ^{99m}Tc-iron ascorbate on polyamide 6 suggests that this type of strip may have applications in the quality control of ionic complexes.

DISCUSSION

The R_f values of the compounds studied in the various solvents and chromatographic media investigated in this study are summarized in Table 1.

The silica gel and saline system is very rapid and easily handled. It is possibly the rapidity that allows the chromatography to be carried out with microliter amounts of the various labeled compounds without any indication of significant oxidation and consequent smearing of the chromatogram. The paper-saline system is slower and is seriously affected by atmospheric oxidation, so much so, that I feel it is not really suitable for routine quality control in smaller facilities where the quality control must nec-

essarily be carried out by technicians without extensive training and experience in paper chromatography.

The 85% methanol, 15% water system with both silica gel and paper media also shows some smearing due to atmospheric oxidation of the microliter amounts of the preparation which are spotted on the thin-layer chromatogram. The amount of smearing on the silica gel chromatogram is less than that on the paper, as would be expected from the shorter developing time. Thus, the silica gel-85% methanol system is more practical than the corresponding paper system.

The acetone and butyl acetate silica gel and paper systems are all easily handled, developing relatively quickly and showing no indication of the effects of atmospheric oxidation. However, the paper butyl acetate system is of little use as none of the compounds investigated in this study show any mobility in it. The other three systems show good potential for the evaluation of free pertechnetate.

The alumina thin-layer chromatography is of limited use as it required several hours to develop in all the solvents which were tried and therefore does not lend itself to the routine quality control of short half-life radiopharmaceuticals. However, alumina does have very good resolution and may be of some use in the research and development of new radiopharmaceuticals or the routine quality control of some longer half-life compounds.

The polyamide 6 thin-layer chromatograms developed in 85% methanol, acetone, and butyl acetate have little application for the compounds studied in this report, as all remain at the origin. Those developed in saline tend to take nearly 1 hr and appear to provide a good assay of the technetium chelate species, ^{99m}Tc-DTPA and ^{99m}Tc-iron ascorbate.

Small discrepancies which can be observed between various procedures are probably due to the effects of such uncontrollable variables as atmospheric oxidation, trace impurities in the solvents, and minor packing faults in the Sephadex columns. Such problems must always be considered under the conditions in which routine quality control would be carried out. However, normal care should prevent them from becoming too significant if suitable media and solvents are chosen.

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