^{99m}Tc-HUMAN SERUM ALBUMIN

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The most important uses of ^{99m}Tc-human serum albumin (HSA) are for placental and cardiac scanning and for cisternography. Widespread use for these purposes has not been realized, especially in the case of placental scanning, because preparation of the ^{99m}Tc-HSA is plagued by long procedures and/or by multicomponent products.

In developing radiopharmaceuticals our ultimate goal is to prepare ^{99m}Tc compounds which contain a single component, as demonstrated by various analytical techniques, and which can be easily prepared, ideally by adding pertechnetate to a prepared solution. Such a one-step, single-component product would enable the widespread use of this compound, especially in those hospitals and clinics which have limited nuclear medicine facilities. One example of this is the recently published procedure for instant ^{99m}Tc-DTPA (*1*), a compound for brain tumor localization and kidney studies.

In the course of developing a red blood cell labeling procedure (*2*), we had been impressed by the effectiveness of stannous ion in enabling the objectives mentioned above to be attained. We therefore attempted to formulate a one-step, single-component product of ^{99m}Tc-HSA using stannous ion as the reducing agent.

There have been two recent publications dealing with ^{99m}Tc labeling of HSA using stannous ion as the reducing agent (*3,4*). Although both are more convenient than the Fe Tc-HSA* procedures, the authors present no evidence that the ^{99m}Tc-HSA is a single component.

The Fe ^{99m}Tc-HSA procedures are apt to require multi-pH manipulation to obtain a high-yield, single-component product. One procedure which achieves this objective (*5*) requires the preparation of a Fe ^{99m}Tc-ascorbate complex and three subsequent pH steps.

Binding of metals to albumin has been investigated in particular detail (*6,7*). With metals such as tin and technetium, hydrolysis occurs below pH 7 (*8*) and therefore interference in protein binding by hydrolysis will occur in the pH region where proteins are stable. Therefore, to achieve single-component ^{99m}Tc-HSA the hydrolysis of the technetium will have to be minimized.

A number of variables were studied to obtain the conditions of minimal hydrolysis of technetium and maximum binding to albumin using stannous ion as the reducing agent.

METHODS AND MATERIALS

The standard procedure used was to dissolve an amount of $SnCl_2 2H_2O$ in concentrated HCl (12 *N*) and then dilute it with distilled water to give the desired molarity and pH. To 0.5 ml of this solution was added 1 ml of 250 mg/ml HSA (from either Hyland Lab or Cutter Lab). The technetium was then added in a known volume and the pH adjusted to 6 with sodium hydroxide.

The preparative and analytical chromatography was performed on a 35-cm gel chromatographic column (Sephadex G25) eluted with N_2 purged saline. With gel chromatography ^{99m}Tc-HSA is eluted at the void volume and pertechnetate in 38–46 ml. Another peak was observed which apparently contained stannous and technetium colloidal particles. It appeared in the early fractions eluted from Sephadex G25 indicating a species size smaller than 75 Å. This peak was especially evident in preparations in which a large amount of neutral pH HSA solution was added to a small volume of acidic stannous solution, a standard procedure for producing small colloidal particles. The technetium adsorbed to the column represents the hydrolyzed fraction of

* Fe Tc-HSA indicates the species present and does not imply a proposed chemical formula.

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TABLE 1. EFFECT OF CONCENTRATION OF HSA ON YIELD OF ^{99m}Tc-HSA FOR A SOLUTION OF Sn^{2+} HSA AND 3 ml TcO_4^- MIXED AT pH 1.5

Conc of HSA	Yield of ^{99m}Tc appearing in HSA fraction (%)	Yield of ^{99m}Tc appearing in second fraction (%)	^{99m}Tc adsorbed on Sephadex (%)
250 mg HSA, 55 mg/ml	96	—	4
125 mg HSA, 25 mg/ml	80	—	18
125 mg HSA, 25 mg/ml	75	—	22
100 mg HSA, 20 mg/ml	78	—	19
75 mg HSA, 15 mg/ml	75	—	18
50 mg HSA, 10 mg/ml	81	—	18
25 mg HSA, 5 mg/ml	79	—	19
25 mg HSA, 5 mg/ml	84	—	13
25 mg HSA, 12 mg/ml (0.1 ml TcO_4^-)	90	—	7

technetium as shown in previous work (*9*). The adsorbed technetium was removed from the Sephadex G25 as pertechnetate by eluting with 2 ml of 0.1% H_2O_2 solution followed by isotonic saline. A standard was also counted to assure activity balance of ^{99m}Tc. Yields and identity of peaks were confirmed by Whatman 3 MM paper chromatography in saline as well as in 85% methanol.

Stabilizers used in commercial preparations of HSA, acetyl tryptophan, and octanoic acid did not bind ^{99m}Tc as shown in separate experiments.

All preparations were carried out in a N_2 purged glove box, and all stock solutions were stored under N_2.

RESULTS

The hydrolysis of the stannous ion seemed to play a part in the distribution of technetium, perhaps by irreversibly binding the technetium. Tin-113 distribution studies showed that only 6% of 1 mg of stannous chloride was bound to the HSA; the rest was present in the hydrolyzed form. In experiments with 1 mg $SnCl_2 2H_2O$ and 250 mg HSA, where the technetium was added at pH 6, higher yields of ^{99m}Tc-HSA were obtained when the stannous hydroxide was removed before addition of the pertechnetate. This same effect has been observed in other uses of stannous ion (*10*). It is known that stannous particles can adsorb technetium (*11*), and the elimination of this binder either by adding the technetium at low pH, by removal on Sephadex, or by using less initial stannous ion raises the yield of ^{99m}Tc-HSA. These three methods form the basis of our approach.

Low pH procedure. Table 1 shows a study of the effect of original HSA concentration on the yield. As seen, the yield does not seem to increase with increasing HSA concentration between 5 and 25 mg/ml. However, variability seems to be high at low HSA concentration, and it appears that the use of 250 mg/ml HSA protects against this problem, probably by increasing the technetium HSA kinetics to a point at which variability in colloid formation is not a factor. These results are consistent with the data of Richards et al (*5*) concerning the variability of the ^{99m}Tc-HSA yield if the pH is not sufficiently low to prohibit colloidal formation.

Table 2 shows the ^{113}Sn distribution data for 1 mg and 60 μg $SnCl_2 2H_2O$. Decreasing the amount of stannous ion did not increase the yield of ^{99m}Tc-HSA significantly. At the 60-μg level stability was affected because of air oxidation of the relatively small amount of stannous ion.

The procedure which gave the most consistent high yields at this $SnCl_2 2H_2O$/HSA ratio involved adding the technetium at low pH. In this procedure the technetium is added to a solution of $SnCl_2 2H_2O$ and HSA below pH 3. The important factor here does not seem to be the volume of the pertechnetate per se but rather the resulting pH of the solution as

TABLE 2. ^{113}Sn DISTRIBUTION STUDIES FOR $SnCl_2 2H_2O$/250 mg HSA FOR A SOLUTION OF Sn^{2+}/HSA AND 0.1 ml TcO_4^- MIXED AT pH 2–3

$SnCl_2 2H_2O$	Yield of ^{99m}Tc appearing in HSA fraction (%)	Yield of ^{113}Sn appearing in HSA fraction (%)	Yield of ^{99m}Tc appearing in second fraction (%)	Yield of ^{113}Sn appearing in second fraction (%)	^{99m}Tc adsorbed on Sephadex (%)	^{113}Sn adsorbed on Sephadex (%)
60 μg	88	23	—	38	12	39
60 μg (5 hr later)	94	25	1	45	5	30
1 mg	81	5	—	47	19	44
0.5 mg	92	—	—	—	8	—
2 mg	95	—	—	—	5	—
1 mg	97	3	—	63	3	34

TABLE 3. DATA FOR PROCEDURE FOR PREPARING ^{99m}Tc-HSA AT LOW pH

Condition	Yield of ^{99m}Tc appearing in HSA fraction (%)	Yield of ^{99m}Tc appearing in second fraction (%)	^{99m}Tc adsorbed on Sephadex (%)
60 mg/ml HSA	93*	—	5
60 mg/ml HSA	94	—	5
12 mg/ml HSA diluted 5-fold	90	—	10
6 mg/ml HSA diluted 10-fold	75	—	22
12 mg/ml HSA	97†	—	2
	93	—	6
	97	—	3
	95	—	—
	97	—	3

* 250 mg HSA, 1 mg $SnCl_2 2H_2O$, 2 ml TcO_4^- at pH 2–2.5.
† 250 mg HSA, 60 μg $SnCl_2 2H_2O$, 2 ml TcO_4^- at pH 2.0; terminal dilution to 20 ml.

a result of the volume. If 0.5 ml of 1 *N* HCl–$SnCl_2$ solution is used with 1 mg of 250 mg/ml HSA, up to 2 ml of TcO_4^- solution will give yields because the resultant pH is still below 3. After the ^{99m}Tc-HSA is formed a five-fold dilution does not alter the yield. Such a dilution would enable a resultant concentration of ~12 mg/ml, a value sufficiently low for cisternography. The results of eight experiments with this procedure gives an average yield of 96% as ^{99m}Tc-HSA. Table 3 contains data pertaining to this procedure. Attempts at storing the tin-HSA solution in 1 *N* HCl resulted in gel formation after 24 hr.

Instant procedures. If daily use of ^{99m}Tc-HSA is contemplated, preparation of a number of aliquots of nonradioactive stannous HSA to which technetium is added is most convenient. As mentioned earlier, the ^{99m}Tc-HSA yields using 1 mg $SnCl_2 2H_2O$ 250 mg HSA seemed to be higher and more reproducible if the stannous hydroxide was removed before reaction with pertechnetate at pH 6. With this in mind the stannous hydroxide was removed by eluting a stannous HSA mixture across Sephadex G25 before addition of pertechnetate. The stannous HSA solution at pH 6 can then be subdivided into 1-ml aliquots under nitrogen. As ^{99m}Tc-HSA is needed, pertechnetate can be added to an aliquot. Again the concentration of HSA is important. If the Sn-HSA is eluted to >80 mg/ml on Sephadex, 0.1–1 ml of pertechnetate can be added to 1 ml of the stock HSA solution. A stability study of eluted stannous HSA is given in Table 4.

Attempts at minimizing the effect of stannous hydroxide by reducing the total stannous ion concentration also led to a high reproducible yield of ^{99m}Tc-HSA. If 0.5 mg $SnCl_2 2H_2O$/250 mg HSA is used, a stable stock solution at pH 6 can be prepared without removal of the excess stannous hydroxide. Apparently because of the reduced stannous colloidal formation, interference in the ^{99m}Tc-HSA binding is less, and high yields are obtained. Reduction to 60 μg $SnCl_2 2H_2O$/250 mg HSA resulted in a solution unstable to air oxidation, and sizable yields of TcO_4^- were obtained. The 60-μg $SnCl_2 2H_2O$ samples were also much more susceptible to day-to-day variations in the oxidative ability of the pertechnetate solution. Table 5 shows a stability study using 0.5 mg $SnCl_2 2H_2O$/250 mg HSA without removing excess stannous hydroxide.

CONCLUSION

Thus three procedures have been presented which result in satisfactory preparations of ^{99m}Tc-HSA depending on the user's requirements. The low pH procedure produces very-high-yield preparations but

TABLE 4. DATA FOR PREPARATION OF ^{99m}Tc-HSA BY INSTANT METHOD*

	Yield of ^{99m}Tc appearing in HSA fraction (%)	Yield of ^{99m}Tc appearing in second fraction (%)	^{99m}Tc adsorbed on Sephadex (%)
Day 0	92*	—	6
Day 1	93	—	6
Day 1	92	—	6
Day 6	88	—	5
Day 6 (1 ml TcO_4^-)	82	—	13

* 4 ml HSA (250 mg/ml), 2 ml $SnCl_2 2H_2O$ (1 mg/ml), pH raised to 6 with 1 *N* NaOH, eluted to 135 mg/ml HSA, 0.1 ml TcO_4^- added to 1-ml stock solution.

TABLE 5. DATA FOR PREPARING ^{99m}Tc-HSA BY pH 6 STOCK SOLUTION*

	Yield of ^{99m}Tc appearing in HSA fraction (%)	Yield of ^{99m}Tc appearing in second fraction (%)	^{99m}Tc adsorbed on Sephadex (%)
Day 0	—	—	—
Day 1	91	—	8
Day 2	95	—	7
Day 6	90	—	5
Day 6 (1 ml TcO_4^-)	89	—	10

* 4 ml HSA (250 mg/ml), 2 ml $SnCl_2 2H_2O$ (1 mg/ml), pH raised to 6 with 1 *N* NaOH, diluted to 135 mg/ml HSA, 0.1 ml TcO_4^- added for 1-ml stock solution.

TABLE 6. PROCEDURES FOR PREPARING ^{99m}Tc-HSA

Low pH method	Instant procedures	
	Excess tin not removed	Excess tin removed
1. 1 ml of 250 mg/ml HSA is added to 0.5 ml of 2 mg/ml $SnCl_2 2H_2O$*	1. 4 ml of 250 mg/ml HSA is added to 2 ml of 1 mg/ml $SnCl_2 2H_2O$*	1. 4 ml of 250 mg/ml HSA is added to 2 ml of 1 mg/ml $SnCl_2 2H_2O$*
2. 0.1–2 ml $^{99m}TcO_4^-$ is added	2. pH of solution is raised to 6 with 1 N NaOH†	2. pH of solution is raised to 6 with 1 N NaOH†
3. Solution is mixed for 1 min	3. 0.1–1 ml $^{99m}TcO_4^-$ is added per ml stock solution as needed‡	3. Entire solution is eluted across 35 cm. Sephadex G25 column with saline eluent
4. pH of solution is raised to 6 with 1 N NaOH†	4. Terminal dilution up to 20 ml total can be made if lower HSA concentration is desired‡	4. Protein fractions are collected and solution assayed for HSA concentration
5. Terminal dilution up to 20 ml total can be made if lower HSA concentration is desired		5. 0.1–1 ml $^{99m}TcO_4^-$ is added per ml stock solution as needed‡

* Preparation of $SnCl_2 2H_2O$ solution: The appropriate amount of $SnCl_2 2H_2O$ is dissolved in 2.5 ml concentrated HCl with heat. The solution is then diluted to 25 ml with N_2 purged distilled water.

† NaOH solution of ≤1 N NaOH should be used to neutralize the solution to prevent localized concentrations of strong base which lead to formation of hydrolyzed technetium. Phosphate buffer can also be used.

‡ All stock solutions are stored under nitrogen.

does require some chemical manipulation. The instant procedures may not be as reproducibly high in yield but do offer a quick method for daily preparation of ^{99m}Tc-HSA. The ^{99m}Tc-HSA prepared by all procedures has been tested for biological stability. The "low pH" procedure was compared with ^{99m}Tc-HSA prepared by the iron ascorbate procedure and shown to be similar in biological half-life in rabbits. The "instant procedure" which does not remove the stannous colloid produced a stable ^{99m}Tc label with slight variable liver uptake (<10%), whereas the other produced scans comparable to the low pH method. Complete animal and human data will be presented in a subsequent article. Table 6 contains a reiteration of the three procedures.

In this paper we have tried to study the factors controlling the production of single-component ^{99m}Tc-HSA. This empirical approach is necessitated by the fact that neither the ^{99m}Tc-HSA equilibrium constant nor the ^{99m}Tc hydrolysis constant is known. If these constants were available, inspection of the following equation

$$\frac{(^{99m}\text{Tc-HSA})}{(^{99m}\text{TcO}_2)} = \frac{K_1\,(\text{HSA})}{K_2\,(\text{OH}^-)^2} \qquad (1)$$

would indicate the HSA concentration needed to obtain a high yield at a given pH. We hope to determine these constants in the near future. In general, however, the hydrolysis of the reduced technetium seems to be a major factor in its reaction. As shown in this and in previous work (*1,8,9*) the hydrolysis of reduced technetium seems to compete strongly above pH 3, and the prevention of this reaction is the major factor in the binding to albumin.

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