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Gel-chromatographic Analysis of ^{99m}Tc-labeled Human Serum Albumin Prepared with Sn(II) as the Reductant

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The labeling of human serum albumin (HSA) with ^{99m}Tc using Sn(II) as the reductant has been reinvestigated in view of the possibility of formation of Sn–Tc colloids. Preparations of ^{99m}Tc–HSA and mixtures of ^{99m}Tc–TcO_4^- with $Sn(II)Cl_2$ have been analyzed by means of gel chromatography using various gel matrices and eluents. No Sn–Tc colloids could be eluted from samples of ^{99m}Tc–HSA preparations. The percentage of labeling was about 90%. A comparative investigation of a number of gel-chromatographic systems for the detailed analysis of ^{99m}Tc–HSA has been made. It is found that a long column with Sephadex G-200 gives the best results.

Introduction

In 1976 De Ligny *et al.*[1] reported the results of a detailed investigation of the influence of experimental conditions on the efficiency of labeling human serum albumin (HSA) with ^{99m}Tc, using Sn(II) as the reductant. The radiochemical purity was determined by gel chromatography with Sephadex G-25. In a comment on this work Pettit *et al.*[2] questioned the conclusions of De Ligny's work. They pointed out that gel chromatography with Sephadex G-25 is inadequate to distinguish ^{99m}Tc–HSA from reduced Tc species, e.g. tin–technetium colloids. They recommended the use of Sephadex G-200 or Sephacryl S-200 as gel chromatography matrices for the analysis of the reaction mixtures since these gels have a greater resolving capability. Pettit *et al.*[3] reinvestigated the ^{99m}Tc–HSA system using Sephacryl S-200 for the gel-chromatographic analysis. They observed that two radioactive components other than ^{99m}Tc–HSA were eluted from the column.

One component appeared in the void volume and the other component was eluted just after the ^{99m}Tc–HSA fractions. Both components, also obtained in preparations without HSA, were proposed to be Sn–Tc colloids, i.e. reduced technetium species intimately associated with hydrolyzed tin. De Ligny *et al.*[1] also mentioned the possibility of the formation of such a colloidal component (at high pH and a high Sn(II) concentration), consisting of a hydrated technetium–tin hydroxide colloid, which eventually appears in the albumin fraction at chromatography with a Sephadex G-25 column. However, this was not further investigated. The ^{99m}Tc–HSA preparations of De Ligny *et al.*[1] and Pettit *et al.*[3] were made under very different experimental conditions, *viz.* pH and albumin concentration. Therefore we have investigated to see if Sn–Tc colloids are also formed when the labeling of HSA is performed at the optimum experimental conditions as recommended by De Ligny, i.e. a low pH and a high concentration of albumin. At the same time the gel-chromatographic analysis of the ^{99m}Tc–HSA system was investigated further.

Experimental

Chemicals

^{99m}Tc generator (DRN 4332 Stercow 99*m*): Byk-Mallinckrodt CIL B.V., Petten, The Netherlands. ^{113}Sn ($SnCl_6^{2-}$ in 6 M HCl): Radiochemical Centre, Amersham, Great Britain. ^{137}Cs (CsCl): Philips-Duphar, Petten, The Netherlands. Human serum albumin solutions, HSA (200 g l^{-1}): Red Cross Laboratory, Amsterdam, The Netherlands. Normal serum albumin solution, NSA (human; 25%): Cutter Biological, Berkeley, U.S.A. Gel-chromatographic media (Sephadex G-25 fine, G-200, G-200 superfine; Sephacryl S-200 superfine; Sepharose 6B) and Blue Dextran 2000: Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were commercial reagent grade materials.

Apparatus

Chromatographic columns (K16/100, K16/40), flow adaptors (A16), laboratory valves, tubing connectors, polyethylene capillary tubing and gel and eluent reservoirs: Pharmacia Fine Chemicals, Uppsala, Sweden. Peristaltic pump (Minipuls 2) and fraction collector (Mini MTDC): Gilson, Villiers-le-Bel, France.

Two-channel, well-type NaI (Tl)-detector, counting system (Gamma 8000): Beckman Instruments, Irvine, U.S.A. Spectrophotometer (Modular Photometer System): Vitatron, Dieren, The Netherlands.

Preparation of reagents and columns

All solutions were prepared with N_2-purged double distilled water. A stock solution of 4×10^{-2} M Sn(II)Cl_2 in 1 M HCl was made weekly. The concentration of this solution was determined by biamperometric titration with 0.1 M $KBrO_3$. A few grains of Sn metal were added to prevent oxidation. A solution of 4×10^{-4} M Sn(II) of pH 2 was prepared just prior to use. The pretreatment of the gels and the packing and equilibration of the columns were performed as recommended by the manufacturer.[4 6] The dimensions of the gel beds in the columns were 30 × 1.6 cm (experiments with Sephadex G-200 superfine and Sepharose 6B), 35 × 1.6 cm (experiments with Sephadex G-25 fine) and 90 × 1.6 cm (experiments with Sephadex G-200, G-200 superfine and Sephacryl S-200). Two eluents were prepared: physiological saline (0.9% NaCl) containing 0.02% sodium azide as antimicrobial agent and a 0.04 M sodium phosphate buffer, pH 7. The void volume of the columns (V_0) was determined by eluting 1 ml 0.2% Blue Dextran 2000 with 0.9% NaCl and measuring the absorption of the fractions at 620 nm. A spike of ^{137}Cs (2.5 kBq) in 1 ml 0.9% NaCl was eluted in order to derive a value for the inner volume (V_i). The elution volume of this spike was determined by γ-scintillation counting of the fractions.

Procedures

The ^{99m}Tc-HSA was prepared as follows: One ml of the HSA or NSA solution was adjusted to the desired pH (2–4.9) with a solution of 1 M HCl after which the volume was made up to 2 ml with physiological saline of the appropriate pH. To this solution 1 ml of the 4×10^{-4} M Sn(II) Cl_2 solution and a spike of ^{113}Sn (10 kBq) was added. This mixture was allowed to equilibrate for at least 10 min for the isotopic exchange between Sn(II) and ^{113}Sn(IV) to occur. Then 1 ml of a physiological saline eluate of a 14–24 days old ^{99m}Tc generator (2–22 MBq) was introduced and after gentle agitation for another 10 min the pH was adjusted to the desired value (2–7) with 1 M NaOH. A reaction mixture consisting of ^{99m}Tc-TcO_4^- and Sn(II)Cl_2 was prepared similarly to that used for the labeling of serum albumin. In this case the starting-solution was 2 ml 0.9% NaCl (pH 2). For some chromatographic experiments (with Sepharose 6B) a solution was prepared consisting of 1×10^{-4} M Sn(II)Cl_2 spiked with ^{113}Sn (10 kBq) in 0.9% NaCl (pH 2).

The procedure for the chromatographic experiments was as follows: Aliquots of 0.5–0.75 ml of the samples to be analyzed were introduced into the column. The elutions were performed with physiological saline adjusted to the desired pH or with the phosphate buffer. In all cases the applied flow rate was about 15 ml h^{-1} except in the case of Sephadex G-200 superfine where it was 3 ml h^{-1}. Fractions of about 1.5 ml were collected. In the experiments with serum albumin the ultraviolet absorption at 280 nm of the fractions was measured.

The radioactivity of the collected fractions was measured using the Gamma 8000 counting system. Before counting sufficient waiting time was taken in order to avoid artefacts caused by the ^{113m}In daughter activity of ^{113}Sn. (This may occur when In and Sn species are separated during chromatography). The ^{99m}Tc and ^{113}Sn activities were counted simultaneously in the energy ranges 112.5–175 keV and 350–450 keV, respectively. A correction was made for the contribution of ^{113}Sn (^{113m}In) activity in the lower energy range by application of the "dual label percent bound" measuring mode using a vial with ^{113}Sn (5 kBq) as tag tube.[7] For this purpose the prescribed loading sequence of the sample conveyor train was slightly modified whilst at the same time the irrelevant machine error notes were ignored. A correction was made for ^{99m}Tc decay during the radioactivity measurements. The elution volumes of the various compounds in the reaction mixtures were determined from a graph of count rate or u.v. absorption vs elution volume. The percentage of ^{99m}Tc or ^{113}Sn activity in the various peaks was determined by comparing the integrated count rate of the fractions of the peak with the count rate of 0.5–0.75 ml samples of the reaction mixture.

All experiments, inclusive of the preparations, were performed in duplicate.

Results

First the labeling of serum albumin performed at the experimental conditions as recommended by De Ligny *et al.*[1] was reinvestigated. The labeling was performed at pH 2, [NSA] = 62.5 mg ml^{-1} and [Sn(II)] = 10^{-4} M, i.e. approximately at the optimum conditions. The reaction mixtures were analyzed using Sephadex G-25 fine. A typical example of the results is shown in Fig. 1. In Table 1 the chromatographic distribution coefficients and the percentages of radioactivity of the various chromatographic peaks are presented. In this Table also the results are shown of the analysis of a reaction mixture of Sn(II) Cl_2 (10^{-4} M) and ^{99m}Tc-TcO_4^- incubated at pH 2. The latter preparation was also analyzed on a Sepharose 6B column (gel bed 30 × 1.6 cm) using 0.9% NaCl or 0.04 M phosphate buffer (pH 7) as eluents. These results are presented in Table 2. For comparison a solution of 10^{-4} M Sn(II) Cl_2 in 0.9% NaCl (pH 2) spiked with ^{113}Sn was analyzed on the same column. Next, a number of experiments was carried out to investigate the utility of the gel matrices Sephadex G-200, G-200 superfine and Sephacryl S-200 for the analysis of the ^{99m}Tc HSA system. For this purpose aliquots of ^{99m}Tc-labeled serum albumin (HSA or

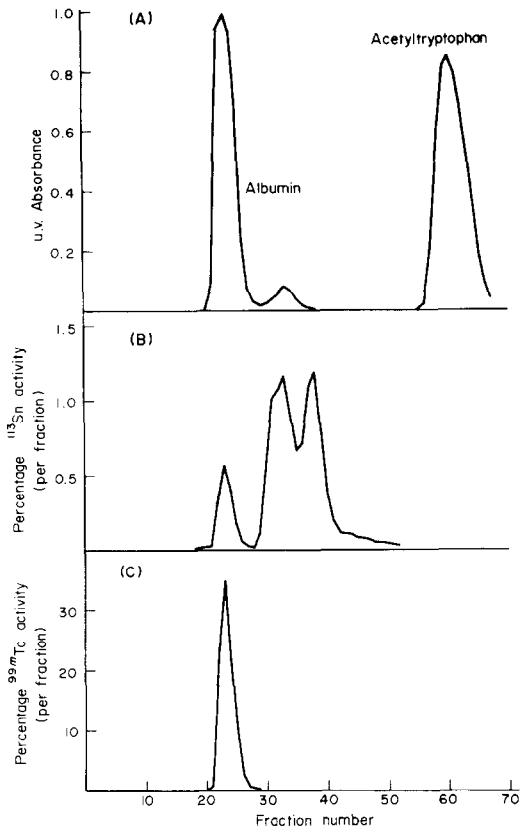

FIG. 1. Gel chromatography of ^{99m}Tc–HSA on Sephadex G-25 fine (gel bed 35 × 1.6 cm); eluent 0.9% NaCl (pH 7) (15 ml h^{-1}). The labeling was performed at pH 2, [NSA] = 62.5 mg ml^{-1} and [Sn(II)] = 10^{-4} M. (A) Ultraviolet absorption at 280 nm. (B) Percentage ^{113}Sn radioactivity. (C) Percentage ^{99m}Tc radioactivity.

NSA) or HSA were chromatographed on these gels using columns with various bed lengths. Illustrative examples of the observed elution profiles are shown in Fig. 2. In all cases the ^{99m}Tc radioactivity coincides with the elution curves of albumin (e.g. see Figs 2C and 2D). For the sake of simplicity the radioactivity distribution has been omitted in Fig. 2A.

In Table 3 the distribution coefficients and the percentages of radioactivity of the various chromatographic peaks obtained on Sephadex G-200 and Sephadex G-200 superfine (column II) are presented. Finally, the ^{99m}Tc–TcO_4^-/Sn(II)Cl_2 reaction mixture ([Sn(II)] = 10^{-4} M, incubation at pH 2) was also investigated on the Sephadex G-200 column using various eluents. The results are shown in Table 4. For comparison a mixture of ^{99m}Tc–TcO_4^- and serum albumin was analyzed on the same column.

Discussion

The data in Table 1 show that 93.5% of the Tc was eluted with the main fraction of serum albumin (cf. Fig. 1) in the void volume of the column. Only a small amount of Sn (1.4%) was eluted in the void volume whereas about 3.5% was eluted in a small (non-identified) albumin fraction (cf. Fig. 1) at $K_d' = 0.26$. The total recoveries of Tc and Sn were 95.2% and 10%, respectively. Table 1 also shows that when a mixture of ^{99m}Tc–TcO_4^- and Sn(II)Cl_2 was chromatographed on Sephadex G-25 no radioactivity was eluted in the void volume. In this case only small amounts of Tc and Sn were recovered from the column. The results of these experiments clearly demonstrate that none or practically no so-called Sn–Tc colloids are eluted in the void volume of Sephadex G-25 when the labeling is performed at pH 2. On the contrary, PETTIT *et al.*[2] observed that a mixture of ^{99m}Tc–TcO_4^- and Sn(II)Cl_2 formed a radioactive component that eluted in the void volume of Sephadex G-25. An explanation for these different findings cannot be given since the experimental conditions were not reported.

The analysis of the ^{99m}Tc–TcO_4/Sn(II)Cl_2 mixture on Sepharose 6B using two eluents gave remarkable

TABLE 1. Gel chromatography of ^{99m}Tc–HSA and of a [^{99m}Tc]pertechnetate/Sn(II)Cl_2 reaction mixture on Sephadex G-25 fine

		Distribution coefficients and percentages of radioactivity					
	Detected component	K_d':0	0.12	0.26	0.39	0.43	1.00
^{99m}Tc–HSA†	^{99m}Tc	93.5					
	^{113}Sn	1.4		3.5	3.5		
	HSA	*		*			
	Acetyltryptophan						*
[^{99m}Tc]pertechnetate/Sn(II)Cl_2	^{99m}Tc		0.3				
	^{113}Sn					4	

The distribution coefficient, K_d', is calculated as follows:

$$K_d' = (V_e - V_0)(V_{Cs} - V_0)^{-1}$$

where

V_e = elution volume of the component
V_0 = void volume
V_{Cs} = elution volume of a ^{137}Cs-spike in NaCl.

Accuracy of K_d' is 0.02.
* No quantitative analysis performed. † [NSA] = 62.5 mg ml^{-1}. Eluent: 0.9% NaCl (pH 7).

TABLE 2. Gel chromatography of a [^{99m}Tc]pertechnetate/Sn(II)Cl$_2$ reaction mixture and of a Sn(II)Cl$_2$ solution on Sepharose 6B

	Eluent	Detected component	Distribution coefficients and percentages of radioactivity	
			K'_d:0	0.9
^{99m}Tc-pertechnetate/Sn(II)Cl$_2$	A	^{99m}Tc	3	0.8
		^{113}Sn	4	0.9
	B	^{99m}Tc	41	4
		^{113}Sn	54	17
Sn(II)Cl$_2$	A	^{113}Sn	4	0.3
	B	^{113}Sn	67	15

A: 0.9% NaCl (pH 7). B: 0.04 M sodium phosphate buffer (pH 7).

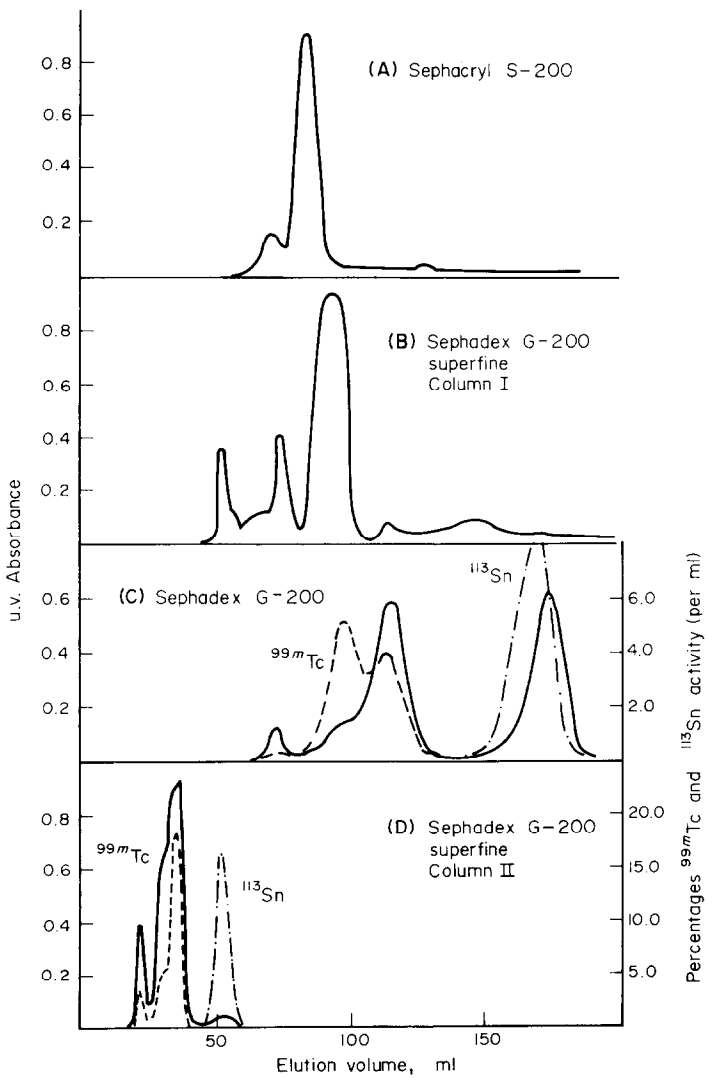

FIG. 2. Comparison of various gel-chromatographic systems in the analysis of ^{99m}Tc–HSA. (A) ^{99m}Tc HSA on Sephacryl S-200 (gel bed 90 × 1.7 cm): eluent 0.9% NaCl (pH 4) (16 ml h^{-1}); Labeling conditions: [HSA] = 50 mg ml^{-1}, [Sn(II)] = 10^{-4} M, pH 4. (B) HSA on Sephadex G-200 superfine (gel bed 90 × 1.6 cm); eluent 0.9% NaCl (pH 7) (3 ml h^{-1}); [HSA] = 100 mg ml^{-1}. (C) ^{99m}Tc-HSA on Sephadex G-200 (gel bed 90 × 1.6 cm); eluent 0.04 M phosphate buffer (pH 7) (15 ml h^{-1}); Labeling conditions: [NSA] = 62.5 mg ml^{-1}, [Sn(II)] = 10^{-4} M, pH 2. (D) ^{99m}Tc–HSA on Sephadex G-200 superfine (gel bed 30 × 1.6 cm); eluent 0.9% NaCl (pH 7) (3 ml h^{-1}); Labeling conditions: [HSA] = 50 mg ml^{-1}, [Sn(II)] = 10^{-4} M, pH 4.

TABLE 3. Gel chromatography of ^{99m}Tc–HSA on Sephadex G-200 and Sephadex G-200 superfine

	Detected Component	Distribution coefficients and percentages of radioactivity					
		K'_d:0	0.27	0.27 0.43	0.43	0.96	1.00
Sephadex G-200 (gel bed 90 × 1.6 cm); Various eluents†; Preparation A	^{99m}Tc	2		87			
	^{113}Sn					93	
	HSA-p	*					
	HSA-d		*				
	HSA-m				*		
	Acetyltryptophan						*
Sephadex G-200 superfine (gel bed 30 × 1.6 cm); Eluent 0.9% NaCl (pH 7); Preparation B	^{99m}Tc	12		84			
	^{113}Sn					95	
	HSA-p	*					
	HSA-d		*				
	HSA-m				*		
	Mandelate						*

Labeling conditions: Preparation A: [NSA] = 62.5 mg ml^{-1}, [Sn(II)] = 10^{-4} M, pH 2; Preparation B: [HSA] = 50 mg ml^{-1}, [Sn(II)] = 10^{-4} M, pH 4.

† Eluents: 0.04 M phosphate buffer (pH 7) or 0.9% NaCl (pH 4.9 or 7).

* No quantitative analysis performed.

HSA-p, HSA-d and HSA-m represent poly-, di- and monomeric serum albumin, respectively.

results (see Table 2): with physiological saline (pH 7) small amounts of Tc (3%) and Sn (4%) were eluted in the void volume; with phosphate buffer (pH 7) large amounts of Tc (41%) and Sn (54%) were eluted in the void volume. In the latter case also appreciable amounts of both metals were eluted at $K'_d = 0.9$. Analysis of a Sn(II)Cl_2 solution on the same column using the same eluents gave analogous results. PETTIT *et al.*[3] investigated the same reaction mixture on Sepharose 6B using a phosphate buffer (pH 7.4) as eluent. They observed two elution peaks, which were designated α and β Sn–Tc colloids. The first peak (α) was eluted in the void volume. The relative quantity of each peak was found to be pH-dependent. From our results and those of PETTIT *et al.* it appears that the eluent plays an important role in the gel-chromatographic analysis of Sn and Tc colloids on Sepharose 6B. In the case of saline (pH 7) the recovery from the column was very low. Hydrolysis of $SnCl_2$ might result in coprecipitation of reduced Tc, and this Sn–Tc colloid may or may not appear in the eluate (depending on its particle size). In the case of a phosphate buffer (pH 7–7.4) apparently a considerable amount of colloidal particles is formed with such particle size that it elutes in the void volume, while the larger amount of activity at $K'_d = 0.9$ might be the result of the formation of complexes of Sn and/or Tc with phosphate anions.

PETTIT *et al.*[3] investigated the ^{99m}Tc–HSA system using a long (gel bed 87.8 × 2.4 cm) Sephacryl S-200 column. It was found that also in the presence of serum albumin α and β colloids could be eluted. The α colloid appeared again in the void volume whereas the β colloid was eluted after the albumin peak. On

TABLE 4. Gel chromatography of a [^{99m}Tc]pertechnetate/Sn(II)Cl_2 reaction mixture and of a [^{99m}Tc]pertechnetate/HSA mixture on Sephadex G-200

	Eluent	Detected component	Distribution coefficients and percentages of radioactivity					
			K'_d:0	0.27	0.45	1.00	1.03	1.2
^{99m}Tc-pertechnetate Sn(II)Cl_2	A	^{99m}Tc					18 ± 15	
		^{113}Sn						
	A′	^{99m}Tc					90	
		^{113}Sn						21
	B	^{99m}Tc					11	
		^{113}Sn						
^{99m}Tc-pertechnetate/HSA†	B	^{99m}Tc				97		
		HSA-p	*					
		HSA-d		*				
		HSA-m			*			
		Acetyltryptophan				*		

A: 0.9% NaCl (pH 7). A′: 0.9% NaCl (pH 2). B: 0.04 M sodium phosphate buffer (pH 7). * No quantitative analysis performed. † [NSA] = 62.5 mg ml^{-1}.

the basis of these observations and of the results of our experiments discussed hitherto we reinvestigated the ^{99m}Tc–HSA system using various gel matrices and column lengths. In order to compare the results with Pettit's work and our previous work[1] the experiments have been performed with NSA and/or HSA (see chemicals). From the elution profiles presented in Fig. 2 the following conclusions can be drawn:

1. The resolving power of Sephacryl S-200 is less than that of Sephadex G-200 and G-200 superfine (cf. Fig. 2A–D) because the dimer and monomer serum albumin cannot be distinguished (cf. Fig. 2A). The inferior performance of Sephacryl S-200 compared to that of Sephadex G-200 is confirmed by the study of MORGAN and RAMSDEN[8] who compared the performance of Sephacryl S-200 with that of Sephadex G-150. They found that for proteins within the effective fractionation range of 5000–250,000, the separating power of Sephacryl S-200 approximated that of Sephadex G-100.

2. The resolving power of a long column with Sephadex G-200 superfine (Column I) for serum albumin fractionating is very good (cf. Fig. 2C). However, since the maximally attainable flow rate is very low[4] the time needed for the analysis is inconveniently long. Additionally, samples with high ^{99m}Tc radioactivity are then required.

3. Application of a relative short column with Sephadex G-200 superfine (Column II) gives a reasonable separation of the albumin components (cf. Fig. 2D). Again, the low flow rate involves a long analysis time.

4. The use of a long column with Sephadex G-200 for the analysis of the ^{99m}Tc HSA system is the best choice (cf. Fig. 2C). The resolving power is good and the time needed for complete elution is reasonable (about 13 h).

The data in Table 3 show that the gel-chromatographic analysis of two different ^{99m}Tc–HSA preparations on Sephadex G-200 and G-200 superfine (Column II) gave, within experimental error, the same results. The labeling conditions (pH and concentration of reagents) differed only slightly.

For both preparations the percentage of labeling of the dimer plus monomer albumin was about 85%.

In the case of preparation B a higher labeling percentage (12%) of the polymer albumin has been found than with preparation A (2%). This might be caused by a difference in the quantity of polymer albumin present in NSA and HSA. In both cases Sn was eluted at $K'_d = 0.96$. The recovery was about 94%. These results are different from those obtained with the Sephadex G-25 column (cf. Table 1). In that case only 10% Sn was recovered from the column. These different findings show that the gel matrices can have a strong interaction with the Sn species in the chromatographic sample. The starting-materials NSA and HSA contain 0.02 M acetyltryptophan and 0.02 M sodium mandelate, respectively; both contain 0.02 M sodium caprylate which does not absorb at 280 nm. From test-tube experiments it was found that soluble Sn(II)-caprylate complexes are formed in the investigated pH range 2–7. Because our ^{99m}Tc–HSA preparations were made with relatively high albumin concentrations, and consequently with high caprylate concentrations (viz. 5×10^{-3} M), it is possible that Sn-caprylate complexes were formed. When the sample is chromatographed on Sephadex G-25 these complexes might decompose by interaction with the gel matrix and insoluble colloidal Sn species will then remain on the column. In the case of the highly porous Sephadex G-200 matrix this interaction will be very small and Sn can be eluted from the column. Decomposition by interaction with dense gel matrices has also been found with weak Tc complexes.[9,10] Obviously, no Sn–Tc colloids have been observed, neither in the void volume, nor in fractions following the albumin peaks. In contrast with the findings with Sepharose 6B (cf. Table 2) no influence of the eluent on the chromatographic results could be observed. In addition to the data presented in Table 3 it is worth mentioning that also a labeling has been performed using 10 times less NSA, *viz.* 6.25 mg ml^{-1}. It was found that the results are in good agreement with those given in Table 3 (labeling %: polymer albumin 1.9%, dimer plus monomer albumin 86%).

The data in Table 4 indicate that also in the case of a reaction mixture of ^{99m}Tc-TcO_4^- and $Sn(II)Cl_2$ no Sn–Tc colloids could be eluted from the Sephadex G-200 column. When the elution was performed with saline at pH 2 90% of the reduced Tc appeared at $K'_d = 1.03$. (TcO_4^- was found to be eluted at $K'_d = 1.00$, cf. Table 4).

OWUNWANNE *et al.*[11] suggested that at pH $\leqslant 2$ the reduced Tc is present as $Tc(OH)_2^{2+}$ or TcO^{2+} ions. Above this pH these species hydrolyze to $TcO_2 \cdot 2H_2O$, the colloidal form of this substance will then remain at the top of the chromatography column. Except for saline at pH 2 the Sn recovery was found to be negligible. Again, insoluble colloidal Sn species remain at the top of the column.

Recapitulating the results of the labeling of serum albumin with ^{99m}Tc (cf. Tables 1 and 3) it can be concluded that no Sn–Tc colloids can be eluted from Sephadex G-25 or G-200 columns when the labeling is performed under the stated experimental conditions. Thus high yields of ^{99m}Tc–HSA (about 90%) are obtained at low pH. This result confirms our previous work[1] and the findings of ECKELMAN *et al.*[12] The latter author pointed out that labeling procedures using Sn^{2+} at neutral pH may cause the formation of ^{99m}Tc colloids.[12,13] Therefore the labeling should be performed at low pH. From the detailed gel-chromatographic analysis of the ^{99m}Tc–HSA system it can be concluded that a long column (about 90 cm) with Sephadex G-200 is the best choice (cf. Fig. 2) because of its ability to separate the various ^{99m}Tc-labeled compounds and the other components present in the reaction mixture. One should be aware of the influence of the eluent (composition, pH) and of the

gel matrix on the results of the gel-chromatographic analysis of ^{99m}Tc–TcO_4^-/Sn(II)Cl_2 systems (cf. Tables 2 and 4).

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