Chemical Aspects of Labeling Sucralfate with $^{99m}TcO_4$

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Two formulations of [^{99m}Tc]sucralfate have been used to image gastric and duodenal ulcers and inflammatory bowel disease. One formulation is a complexation of [^{99m}Tc]HSA with sucralfate. The second is prepared by directly labeling sucralfate with [^{99m}Tc]pertechnetate in the presence of stannous ion. An in vitro study of the factors affecting the production and stability of these labeled sucralfate preparations was conducted. Both formulations were stable at the acidic pH likely encountered in the stomach. However, at pH > 6 the albumin-sucralfate complex began to dissociate while directly labeled sucralfate was stable to a pH of 9. Conversely it was shown that directly labeled sucralfate was more susceptible to loss of ^{99m}Tc to other chelating species. Sucralfate complexed with [^{99m}Tc]HSA was radiochemically stable up to a specific activity of 26 GBq (700 mCi) per gram while directly labeled sucralfate showed decreased 24-hr stability at specific activities >837 mCi (31 GBq) per gram.

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Sucralfate, the basic aluminum salt of sucrose octasulfate (Fig. 1) is used in the treatment of peptic ulcers. Sucralfate binds to the ulcerated surface by chemically complexing with the exposed protein. Since the molecule has a high molecular weight and has eight potentially hydrolyzable alumina groups, it protects the ulcerated surface from contact with the stomach acid and pepsin, thus reducing the immediate discomfort and facilitating the healing process.

Vasquez et al. (*1*) conceived the idea that the natural affinity of sucralfate for proteins could be used to attach a radioactive marker to the sucralfate. Provided the sucralfate molecule retained its ability to bind proteins after the radiolabeled protein is attached to it, it would then be possible to use this sucralfate labeled protein complex to localize and image peptic ulcers. In their first clinical report of this technique, Vasquez et al. (*1*) used technetium-99m-labeled human serum albumin ([^{99m}Tc]HSA) to complex with sucralfate ([^{99m}Tc]HSA-sucralfate). They were able to image ulcers in their patients following oral administration of the resultant complex. Since that time, there have been a number of literature reports of the use of labeled sucralfate in the clinical imaging of gastric and duodenal ulcers (*2*) and inflammatory bowel disease (*3*). In 1985 Pera et al. (*4*) reported a direct in vivo technique for labeling sucralfate in the detection of gastric ulcers. This technique involved the oral administration of a sucralfate/stannous ion suspension followed 2 hr later by oral administration of pertechnetate. In developing this technique, Pera et al. (*4*) demonstrated that sucralfate could be directly labeled with $^{99m}TcO_4$ in the presence of stannous ion. In 1987, Vasquez et al. (*5*) reviewed the clinical uses of ^{99m}Tc-labeled sucralfate, however, there is still very little data available on the chemical nature and stability of the bond between ^{99m}Tc and the localizing entity, sucralfate. It is known (*6*) that the affinity of sucrose octasulfate for albumin drops off rapidly at a pH above 4. Since sucralfate is a basic aluminum salt of sucrose octasulfate, there is reason to be concerned that at a high pH, such as that encountered in the duodenum and ileum, the albumin sucralfate complex may not exhibit very good stability. Thus, a loss of the radioactive marker, i.e., the [^{99m}Tc]HSA, might occur when the [^{99m}Tc]HSA-sucralfate is used to localize duodenal ulcers or inflammatory bowel disease. Since the attachment of the [^{99m}Tc]HSA and the localization of the sucralfate to the denuded mucosa share the same binding site on the sucralfate molecule, the possibility that labeling of sucralfate with [^{99m}Tc]HSA may reduce the affinity of sucralfate for the site of biological localization must be considered.

This current work represents an in vitro study of the characteristics of ^{99m}Tc-labeled sucralfate. Factors affecting the labeling and stability of both [^{99m}Tc]HSA-sucralfate and directly labeled sucralfate ([^{99m}Tc]sucralfate) have been investigated.

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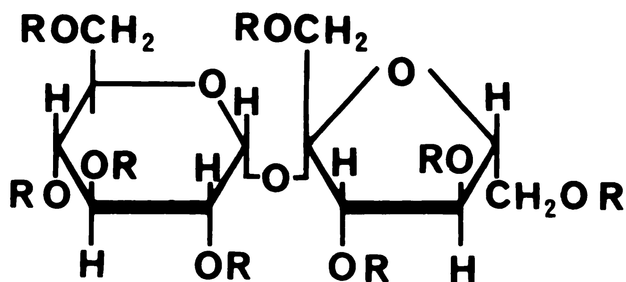

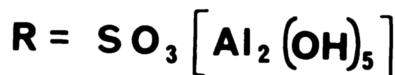

FIGURE 1
Structure of sucralfate.

MATERIALS AND METHODS

Preparation of Labeled Sucralfate

Technetium-99m-labeled sucralfate was prepared both by chemical binding of [^{99m}Tc]HSA to sucralfate and by the direct reaction of pertechnetate with sucralfate in the presence of stannous ion. These investigational studies used only 38.5 mg of sucralfate as opposed to clinical levels of 250 to 1,000 mg.

Technetium-99m HSA-sucralfate

(a) *Technetium-99m HSA*

This product was prepared in a similar manner to that used routinely in our nuclear medicine department. However, the final pH adjustment and the addition of dextrose were omitted since the binding of albumin to sucralfate occurs preferentially at acidic pH. The procedure used was as follows.

Human serum albumin (12.5 mg) was added to 4 ml of $^{99m}TcO_4$ (3 GBq, 8 mCi) in saline and 2 ml of 1N HCl in a 10-ml sterile multidose vial into which two tin wire electrodes had been inserted (electrolytic vial). These two electrodes were connected to a constant current power supply and the vial placed in an ultrasonic bath to provide continuous mixing. A current of 2 mA was then applied for 10 min. The vial was disconnected from the power supply, removed from the ultrasonic bath and allowed to stand for 5 min. The resultant [^{99m}Tc]HSA was diluted with 18 ml of saline and filtered through a 0.2-micron sterilizing filter unit (Nylon 66 membrane; Micron Separations Inc.).

(b) *Linkage of [^{99m}Tc]HSA with sucralfate*

The [^{99m}Tc]HSA-sucralfate complex was prepared using a modified version of Vasquez et al. (*1*). Sucralfate (38.5 mg) was suspended in 1.4 ml of [^{99m}Tc]HSA and the suspension diluted to 10 ml with sterile water. The suspension was rotated for 30 min to allow complexation of [^{99m}Tc]HSA with the sucralfate.

Direct labeling of sucralfate with [^{99m}Tc]pertechnetate ([^{99m}Tc]sucralfate)

Sucralfate (38.5 mg) was suspended in 6 ml of saline containing 0.2 GBq (5.5 mCi) of $^{99m}TcO_4$ in a 10-ml sterile electrolytic vial. A current of 4 mA was applied for 0.5 min.

Analysis of the Labeling Efficiency of the Sucralfate Complexes

The sucralfate suspension was rotated to ensure thorough mixing, then 0.2 ml was withdrawn and applied to a 0.2-micron (25 mm diameter) disposable filter unit (MSI). This aliquot was then washed through the filter with 2 ml of saline.

The filter and the filtrate were counted separately on a sodium iodide crystal coupled to a pulse height analyzer set for ^{99m}Tc. The labeling efficiency was calculated as follows:

$$\text{labeling efficiency} = \frac{\text{counts on filter}}{\text{counts on filter + counts in filtrate}}.$$

Optimization of [^{99m}Tc]HSA-Sucralfate Preparation

Effect of time of incubation. After the addition of the [^{99m}Tc] HSA to the sucralfate, the suspension was rotated for various time intervals (5, 10, 15, and 30 min). The suspension was sampled and the labeling efficiency was determined.

Effect of pH. The pH of the 10 ml of diluted [^{99m}Tc]HSA solution was controlled by adjusting the pH of the diluent with either 0.1N HCl or 0.1N NaOH. The appropriately pH adjusted diluent (8.6 ml) was added to 1.4 ml of [^{99m}Tc]HSA prior to addition of sucralfate powder.

Effect of stannous ion concentration on [^{99m}Tc]HSA-sucralfate binding. A stock solution of stannous chloride was prepared by dissolving 0.5 g of tin metal in 4 ml of concentrated HCl. Upon complete dissolution, the volume was made up to 50 ml with physiological saline, filtered through a 0.2-micron filter (MSI) into sterile multidose vials. The vials were then flushed with nitrogen prior to storage. Immediately before use, 0.1 ml of this stannous solution was diluted with 10 ml of nitrogen flushed saline resulting in a final concentration of 100 μg stannous ion per ml at pH 4.3. The freshly prepared stannous ion solution was then added to the [^{99m}Tc]HSA-sucralfate mixture at the time of mixing. A series of these mixtures with varying pHs were prepared and analyzed for the efficiency of [^{99m}Tc]HSA binding to the sucralfate.

Effect of specific activity on [^{99m}Tc]HSA-sucralfate complex stability. Two preparations of stannous human serum albumin were prepared, the first as outlined in section 1a. The second was prepared in an identical manner except that the $^{99m}TcO_4$ was replaced with saline. Varying proportions of each of these solutions, such that their sum was always 1.4 ml, were then incubated with 38.5 mg of sucralfate. Analysis was carried out after 1-hr rotation and after 24-hr storage at ambient temperature.

Evaluation of the sucralfate capacity for binding albumin. Technetium-99m-labeled HSA prepared as described in 1a was mixed with varying quantities of unlabeled HSA so that the total albumin content varied. This solution was then used to form the [^{99m}Tc]HSA-sucralfate complex. The pH of the [^{99m}Tc]HSA and HSA solutions was kept at 4.3. Following the 1-hr incubation the suspension was removed from the rotator and an aliquot analyzed for the efficiency of complexation.

Optimization of the [^{99m}Tc]Sucralfate Synthesis

Effect of stannous ion concentration. The amount of stannous ion generated is directly proportional to the total electrical charge transmitted (*7*). In this series of experiments the current used to generate the stannous ion in the sucralfate suspension was reduced to 1 mA and the time varied from 15 sec to 2 min. Upon completion of electrolysis, the sample was rotated for 1 hr and then sampled for quality control analysis. A second quality control analysis was done 24 hr after the initial preparation.

Effect of specific activity on complex stability. A series of

experiments were conducted to determine at what specific activity the 24-hr stability of [^{99m}Tc]sucralfate would be compromised. Technetium-sucralfate preparations using 0.01 μg of stannous ion (1 mA, 15 sec) were prepared with varying amounts of ^{99m}Tc. Analysis was carried out after 1 hr and 24 hr incubation at ambient temperatures.

Comparison of [^{99m}Tc]HSA Sucralfate and [^{99m}Tc]Sucralfate Stability

Stability at various pHs. The stability of each of the ^{99m}Tc-labeled sucralfate complexes at various pH values was compared. Both products were prepared under optimum conditions, centrifuged, and the supernatant in which the labeling had been conducted was removed by aspiration. The labeled sucralfate was then resuspended in 10 ml of aqueous saline solution at the appropriate pH. The pH of the saline solution had been previously adjusted with either HCl or NaOH. The resulting suspensions were rotated for 20 hr and samples were removed for quality control analysis at 1 and 20 hr. The analysis consisted of the evaluation of the ^{99m}Tc binding efficiency to the sucralfate and the determination of the pH of the suspension.

Stability with respect to the exchange with albumin. The ^{99m}Tc-labeled sucralfate complexes were centrifuged, the supernatant removed and resuspended in saline solution containing various amounts of HSA. These suspensions were rotated for 20 hr and analyzed at 1 hr and 20 hr.

RESULTS

Optimization of the Conditions for Preparation of [^{99m}Tc]HSA Sucralfate

Effect of incubation time. Quality control data taken at various times after the initial mixing of [^{99m}Tc]HSA to sucralfate are shown in Table 1. These clearly indicate that the binding of [^{99m}Tc]HSA to sucralfate is a rapid process. In further studies, a 30-min rotation time was adopted as a matter of convenience.

Effect of pH. The results shown in Figure 2 indicate that quantitative binding of [^{99m}Tc]HSA to sucralfate required a pH of <5. Therefore, the 1.4 ml of [^{99m}Tc]HSA in all further studies was diluted to 10 ml with water which resulted in a pH of 4.1.

TABLE 1
[^{99m}Tc]Sucralfate Labeling Efficiency as a Function of Incubation Time of [^{99m}Tc]HSA and Sucralfate

Incubation time* (min)	^{99m}Tc bound to sucralfate (%)†
5	97.1
10	98.3
15	98.7
30	98.8

* 0.73 mg [^{99m}Tc]HSA and 38.5 mg sucralfate were rotated at ambient temperature for the appropriate time.

† % Binding efficiency was calculated as the % of the total activity bound to the filter after passing an aliquot of the suspension through a 0.2-μ membrane filter (MSI).

Effect of stannous ion concentration on [^{99m}Tc]HSA sucralfate binding. Additional stannous ion solution ($SnCl_2$) containing 10 μg of stannous ion was added to a series of samples prepared as in 1B above. The results, also shown in Figure 2, clearly indicate that the addition of $SnCl_2$ to the suspension does not improve the efficiency with which the ^{99m}Tc is bound to sucralfate.

Effect of the specific activity of the [^{99m}Tc]HSA-sucralfate on complex stability. The results of the 1- and 24-hr stability studies conducted on samples of [^{99m}Tc]HSA-sucralfate containing various amounts of radioactivity are shown in Table 2. No breakdown due to the radioactivity was observed over the range studied [up to 1 GBq (27 mCi) per 38.5 mg sucralfate (26 MBq/mg, 0.7 mCi/mg)]. The experiment was limited at this point due to technical aspects associated with the radioactive concentration of the available pertechnetate solution. Since this upper level was above the envisaged for clinical use (i.e., it corresponds to 26 GBq (700 mCi) per g of sucralfate) it was felt unnecessary to pursue this further.

Evaluation of the sucralfate capacity for binding HSA. Figure 3 shows the results of the study involving increasing quantities of albumin incubated with 38.5 mg of sucralfate. The data indicates that albumin was bound up to a molar ratio of 1:400 before there was any reduction in the binding efficiency of [^{99m}Tc]HSA. Thus the quantity of albumin used in this labeling technique was ~2% of the binding capacity of the sucralfate.

Establishment of the Conditions for the Direct Preparation of [^{99m}Tc]Sucralfate

Required stannous content. The results shown in Table 3 indicate that for 8 mCi (300 MBq) of $^{99m}TcO_4$ as little as 0.01 μg of stannous ion was adequate for quantitative binding of ^{99m}Tc to sucralfate.

Radioactivity limitation. The results in Figure 4 show the 1-hr and 24-hr radiochemical purity obtained with 0.01 μg of stannous ion, 38.5 mg of sucralfate and various amounts of $^{99m}TcO_4$. This data indicated that 0.01 μg of stannous ion, generated in situ by this electrolytic technique, was adequate to bind up to 32 mCi (1.2 GBq) ^{99m}Tc to sucralfate (31.2 MBq/mg; 0.83 mCi/mg). Good stability of the radioactive label was observed for at least 24 hr.

Factors Affecting the Stability of Both ^{99m}Tc-Labeled Sucralfate Preparations

pH. Figures 5A and 5B show the results of incubating the ^{99m}Tc labeled sucralfate complexes in a pH range from 2 to 10. The directly labeled [^{99m}Tc]sucralfate was stable throughout the pH range 2 to 9 (20 hr stability >95%). Limited dissociation of the radioactive label occurred between pH 9 and 10. In contrast, [^{99m}Tc]HSA sucralfate showed significant loss of the radioac-

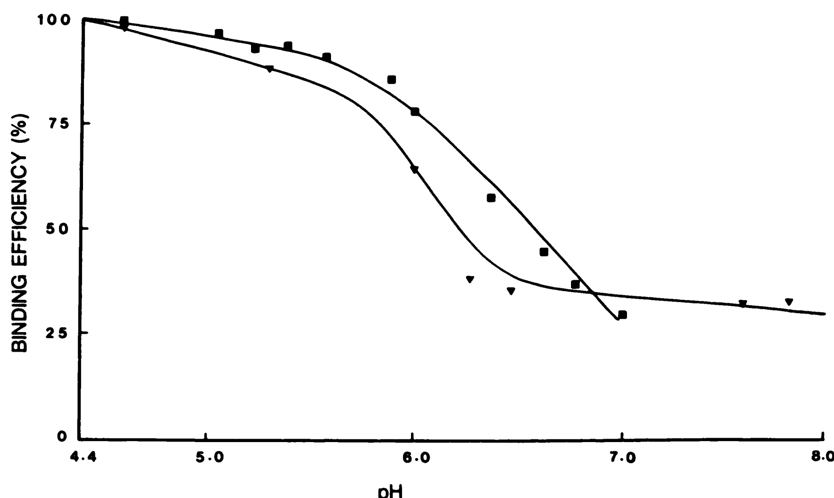

FIGURE 2
Effect of pH on the binding efficiency of [^{99m}Tc]HSA to sucralfate. (▼) 10 μg Stannous ion added to the suspension; (■) without added stannous ion. Sucralfate (38.5 mg) was added to [^{99m}Tc]HSA [200 MBq (5.5 mCi), 0.5 mg] pH adjusted and incubated at ambient temperature for 1 hr to the appropriate pH. The % binding efficiency was calculated as the % of the total activity bound to the filter after passing an aliquot of the suspension through a 0.2-μ membrane filter (MSI).

tive label when the pH of the suspending solution was above six.

Stability against exchange with HSA. The stability of the radiolabel on ^{99m}Tc-labeled sucralfate was evaluated by competition with HSA. The results shown in Table 4 indicate that for [^{99m}Tc]HSA-sucralfate there was no significant loss of the label. In contrast, the fraction of ^{99m}Tc bound to sucralfate decreased nominally from 98% to 83% within 1 hr and to 72% after 24 hr incubation. These suspensions were then centrifuged and the supernatant analyzed by instant thin layer chromatography (ITLC) using methyl ethyl ketone (MEK) and saline as independent solvents. The radioactivity which dissociated from the sucralfate was ~30% pertechnetate and 70% [^{99m}Tc]HSA.

DISCUSSION

Sucralfate may be labeled using the original approach of Vasquez et al. (*1*) involving the binding of labeled protein to sucralfate or by the direct labeling of ^{99m}Tc to sucralfate in the presence of stannous ion. It was the purpose of this study to evaluate each production technique and to characterize the two ^{99m}Tc-labeled preparations. Neither method required significant incubation time to achieve maximum binding efficiency. However, in terms of technologist time, it must be recognized that the preparation of [^{99m}Tc]HSA is an additional step and complicates the overall labeling procedure. In addition, it is important to note that the binding of labeled protein to sucralfate requires that the protein solution have a pH <5. This is more acidic than most labeled protein solutions prepared for nuclear medicine and therefore necessitates either a modification in the production technique or a postpreparation acidification. The protocol of Puttemans et al. (*2*), for the preparation of [^{99m}Tc]HSA-sucralfate adds stannous ion to the sucralfate in addition to the labeled protein. Our studies showed that this additional stannous ion did not improve the labeling efficiency and unnecessarily complicated the technique. The amount of stannous ion necessary to provide good stability of directly labeled [^{99m}Tc]sucralfate with up to 32 mCi (1.2 GBq) of ^{99m}Tc is very low (0.01 μg). No significant labeling (~10%) was achieved in the absence of stannous ion. A simple protocol utilizing a reasonable low stannous ion content which provided assurance of consistently good labeling and was insensitive to trace oxidants or dissolved oxygen was developed. This eliminated the need for nitrogen flushing of production vials. The radiolytic decomposition observed at greater than 1.2 GBq (32.4 mCi) of ^{99m}Tc (Fig. 4) was not investigated further since this level was considerably higher than that required for clinical use. However, the radiochemical stability of higher specific activity products would be possible by increasing the stannous ion concentration (*8*).

TABLE 2
Stability of [^{99m}Tc]HSA-Sucralfate Versus Total Radioactivity*

Radioactivity				^{99m}Tc bound to sucralfate† %	
MBq	MBq/mg	(mCi	mCi/mg)	1 hr	24 hr
1050	27.5	(28.4	0.74)	98.7	98.7
900	23.5	(24.3	0.63)	97.7	98.6
800	20.5	(21.6	0.56)	98.8	98.5
600	15.5	(16.2	0.42)	98.8	98.5
550	13.5	(14.9	0.38)	98.0	97.2
350	9.5	(9.5	0.24)	98.3	97.7

* Concentration of HSA in each preparation was kept constant (0.5 mg) while the amount of ^{99m}Tc was varied. 38.5 mg sucralfate used in each experiment.

† % Binding efficiency was calculated as the % of the total activity bound to the filter after passing an aliquot of the suspension through a 0.2-μ membrane filter (MSI).

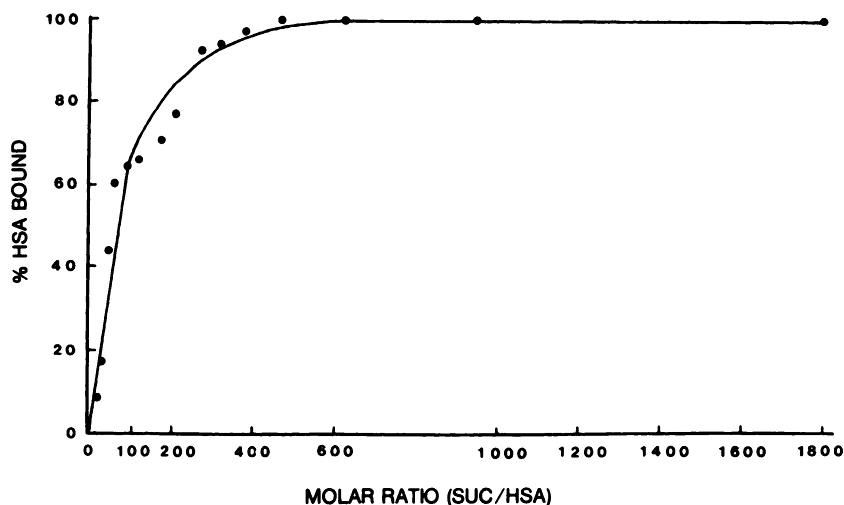

FIGURE 3
HSA binding efficiency of sucralfate. [^{99m}Tc]HSA was diluted with increasing concentrations of unlabeled HSA and incubated with a constant concentration of sucralfate (38.5 mg) at pH 4.3 for 1 hr. The % binding efficiency was calculated as the % of the total activity bound to the filter after passing an aliquot of the suspension through a 0.2-μ membrane filter (MSI).

The original publications on labeled sucralfate as an ulcer imaging agent based the formation of the labeled species on the affinity of sucralfate for HSA. This affinity for protein is also the basis for the use of sucralfate in ulcer therapy. Therefore, the same feature is used for both the labeling and the in vivo localization. Thus, it is possible that if the molecular ratio of the HSA to sucralfate used for labeling was too high then the in vivo binding to the ulcer site would be compromised. The study of the binding efficiency of [^{99m}Tc] HSA to sucralfate as a function of HSA content shows that quantitative binding occurs up to a molar ratio of 1:400 HSA to sucralfate (i.e., sucralfate will bind up to ~7% of its own weight of albumin). It must be remembered however, that sucralfate is an insoluble powder. This means that the affinity of sucralfate both for HSA and the protein of the denuded mucosa is not a function of the total sucralfate mass but of the surface area. In vitro, at pHs above 3, this surface area is a factor of the powder size (i.e., the finer the powder, the greater the surface area and hence the larger the HSA capacity). Thus, the molar ratio reported here of 1:400 represents only a rough guide and it would be wise to keep the HSA content in clinical preparations well below this value. It should also be remembered that upon exposure to the acidity of the stomach, the sucralfate powder forms a sticky amorphous substance which will have substantially less surface area than the relatively finely divided powder. Although some of the HSA molecules attached to the sucralfate in the powder form will certainly be contained within the matrix of this glutenous mass it is also realistic to expect that the sucralfate molecules which make up the surface of the mass will largely be molecules which were on the surface of the powder particles. Therefore it is unlikely that the ratio of the sucralfate molecules to which an HSA molecule is bound to those which are not associated with an HSA molecule will change favorably. Since the binding limit in the powder form is ~7% on a weight basis, it would be prudent to keep the mass of HSA to <1% of the sucralfate weight in a clinical preparation.

TABLE 3
Effect of Stannous Ion Concentration on [^{99m}Tc] Sucralfate Labeling Efficiency and Stability*

Stannous ion† concentration (μg)	Labeling efficiency‡ (%) 1 hr	24 hr
0.00	10.8	—
0.01	94.6	96.0
0.02	97.8	98.8
0.03	97.8	98.3
0.04	98.8	98.6
0.06	98.6	98.2
0.08	97.5	98.2

* 38.5 mg Sucralfate in normal saline at pH 4.3 and 300 MBq (8 mCi) $^{99m}TcO_4$.

† Stannous ion ($SnCl_2$) was prepared by dissolution of 0.5 g tin metal in 4 ml concentrated HCl and diluted to 50 ml with nitrogen flushed saline.

‡ % Binding efficiency was calculated as the % of the total activity bound to the filter after passing an aliquot of the suspension through a 0.2-μ membrane filter (MSI).

In considering the suitability of each of these labeled sucralfate products for ulcer imaging, there are a number of items of potential concern. One is the possibility of the transfer of ^{99m}Tc onto other species in vivo. The imaging procedure is normally carried out on fasting patients and administration of the radiopharmaceutical is normally followed by a flushing drink of water or oral doses of mannitol to encourage gastric emptying and remove unbound material. In this way, the labeled sucralfate should not be exposed to significant quantities of competing chelating agents in the stomach contents. To evaluate the relative product stabilities, unlabeled HSA was incubated with each formulation of labeled sucralfate. Very little ^{99m}Tc was removed from [^{99m}Tc]HSA at concentrations of HSA well above

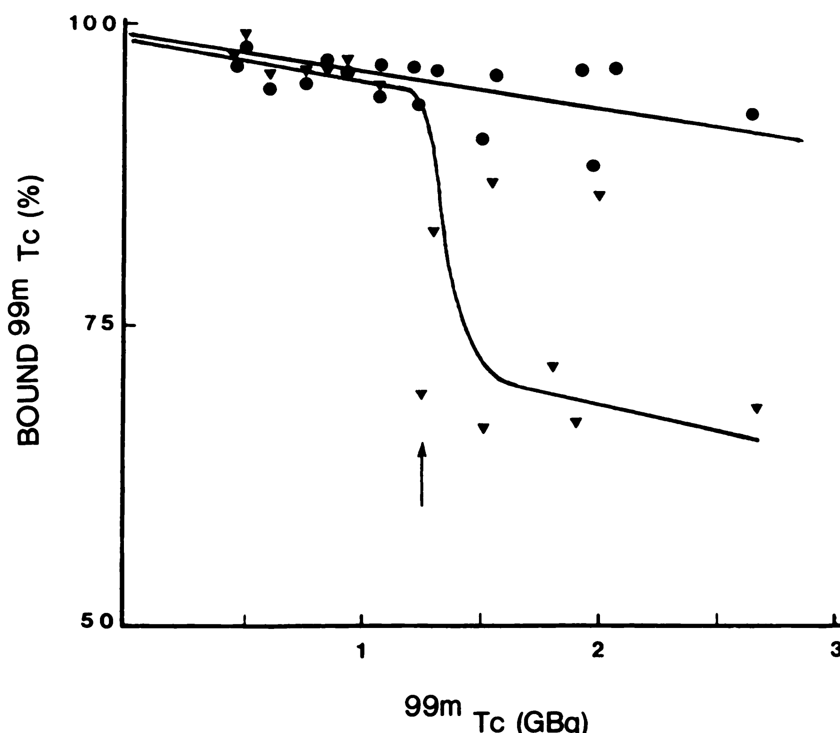

FIGURE 4
Effect of ^{99m}Tc concentration on [^{99m}Tc]sucralfate stability. (●) 1 hr incubation; (▼) 24 hr incubation. Sucralfate (38.5 mg) was labeled with increasing concentrations of ^{99m}Tc in the presence of 0.01 μg stannous ion. The % binding efficiency was calculated as the % of the total activity bound to the filter after passing an aliquot of the suspension through a 0.2-μ membrane filter (MSI). The arrow indicates the ^{99m}Tc concentration at which [^{99m}Tc]sucralfate demonstrated 24 hr instability.

saturation (Fig. 3), yet, loss of ^{99m}Tc from [^{99m}Tc]sucralfate was observed (Table 4). Analysis of the unbound ^{99m}Tc revealed that this radioactivity was predominantly [^{99m}Tc]HSA suggesting that transchelation had taken place. However, the lowest level of HSA used was four times the HSA saturation level for sucralfate. It would seem that this is unlikely to be a problem when the radiopharmaceutical is given to fasting patients. However, this should be considered in unexpected findings and does represent a significant difference with respect to the properties of the two formulations.

Since there is interest in imaging sites of ulceration in not only the stomach but also the duodenum and ileum, it must be recognized that not only must the radioactive label on the sucralfate be stable at the low pH of the stomach but it must also be stable at the significantly higher pH encountered in the duodenum and ileum. In his report on the characteristics of sucralfate, Nagashima (*6*) noted that the binding of sucrose octasulfate to HSA dropped rapidly at a pH over 4. We have demonstrated that for satisfactory labeling, a pH below 5 was required. That it was possible to have a pH as high as 5 and still have satisfactory labeling was almost certainly a result of the excess sucralfate over HSA in these preparations. In addition, it would be anticipated that the [^{99m}Tc]HSA-sucralfate formed at lower pH may well dissociate if the pH of the environment is raised. Indeed, the data in Figure 5 indicates that above pH 6 significant decomposition occurs dependent on the pH. Thin layer chromatographic analysis of the radioactivity released from sucralfate at these higher pHs indicated that it was [^{99m}Tc]HSA. This confirmed that the loss of radioactivity from sucralfate was due to rupture of the HSA-sucralfate bond. These facts would suggest that if [^{99m}Tc]HSA-sucralfate is used to image the duodenum and ileum we should expect to see a small percentage of activity due to [^{99m}Tc]HSA besides that associated with sucralfate. No release of ^{99m}Tc from [^{99m}Tc]sucralfate at a pH below 9 was noted in a similar study. Therefore, if the diagnostic test includes imaging of the duodenum or ileum there may be less chance of artifacts due to radioactivity not associated with sucralfate if the directly labeled [^{99m}Tc] sucralfate is employed.

Pera et al. (*4*) first reported the direct labeling of sucralfate with ^{99m}Tc in the presence of stannous ion. They showed that it worked as an in vivo labeling process. In this approach, the sucralfate, mixed with stannous ion was administered to the patient, then 2 hr later, $^{99m}TcO_4$ was administered. In the empty stomach at low pH, the stannous ion will likely remain as stannous ion and therefore successfully bind the ^{99m}Tc when administered orally. However this technique may not be useful for evaluation of inflammatory bowel disease since the pH rises in the bowel and as a result the stannous ion will be increasingly subject to hydrolysis. This will result in the lack of binding of ^{99m}Tc to the sucralfate that has left the stomach prior to exposure to pertechnetate. In vivo animal studies are necessary

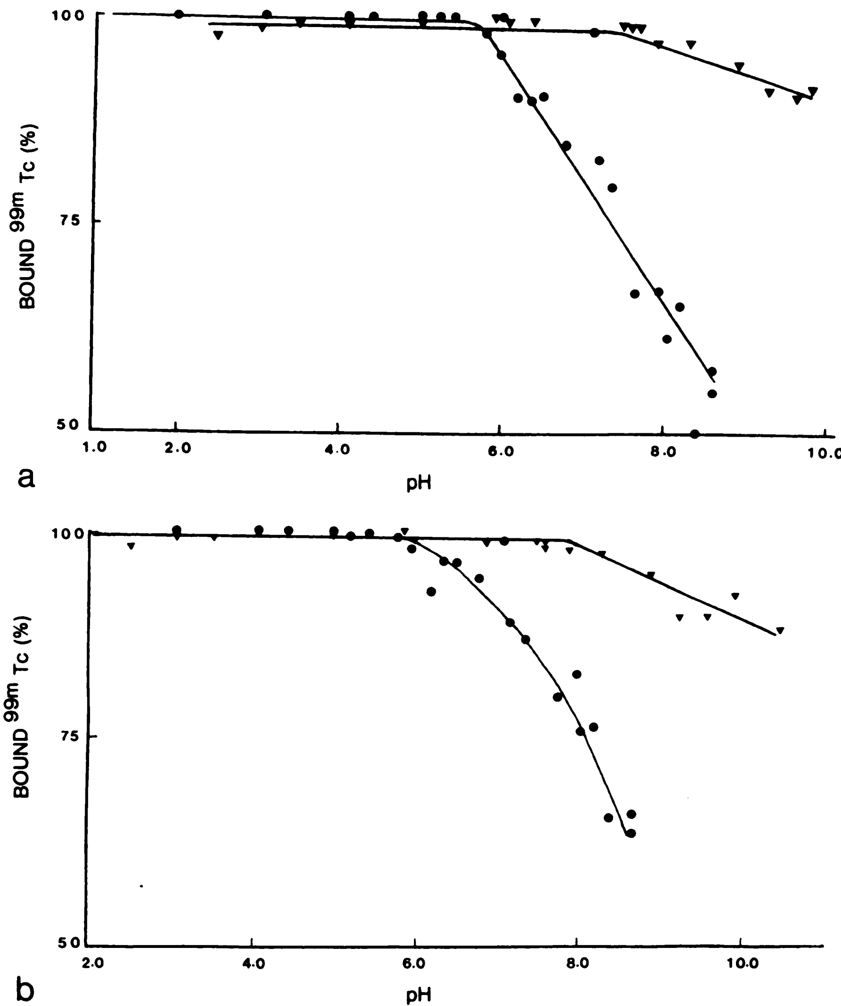

FIGURE 5
Effect of pH on the stability of [^{99m}Tc]HSA-sucralfate (●) and [^{99m}Tc]sucralfate (▼) 1 hr (A) and 24 hr (B) after preparation. Both [^{99m}Tc]HSA-sucralfate and [^{99m}Tc]sucralfate complexes were prepared under optimum conditions. The preparations were centrifuged, the supernatant was removed and the product was resuspended in 10 ml normal saline at the appropriate pH. The % binding efficiency was calculated as the % of the total activity bound to the filter after passing an aliquot of the suspension through a 0.2-μ membrane filter (MSI).

to confirm these suggestions. However, until such results are available it would be wise to avoid the use of the in vivo labeling approach when the purpose of the study is other than the detection of gastric ulceration.

TABLE 4
Competition of HSA with [^{99m}Tc]HSA-Sucralfate and [^{99m}Tc]Sucralfate

HSA concentration (μmol)	[^{99m}Tc] HSA-sucralfate* % Bound ^{99m}Tc‡		[^{99m}Tc] sucralfate† % Bound ^{99m}Tc‡	
	1 Hr	24 Hr	1 Hr	24 Hr
0.00	98.6	99.3	98.0	97.2
0.21	97.5	99.2	83.3	73.8
0.42	96.6	98.7	82.4	71.3
0.63	96.1	96.6	86.9	73.5
0.84	99.2	94.4	83.4	71.4
1.04	99.0	92.6	82.1	72.3

* 16 μmol Sucralfate labeled with 0.011 μmol [^{99m}Tc]HSA.
† 16 μmol Sucralfate labeled directly with $^{99m}TcO_4$.
‡ % Binding efficiency was calculated as the % of the total activity bound to the filter after passing an aliquot of the suspension through a 0.2-μ membrane filter (MSI).

CONCLUSION

Both formulations of ^{99m}Tc-labeled sucralfate provided a readily prepared product which was stable under acidic conditions such as encountered in the stomach. It is worth noting that some caution should be exercised with [^{99m}Tc]HSA sucralfate. It is recommended that the ratio of sucralfate to HSA be kept above 100 to 1 and a fine sucralfate powder be employed. Clinically, it is likely that the distinction with respect to the utility of these agents will be observed in the imaging of the bowel where the pH rises. Once the pH exceeds 6 there is a tendency for the HSA to dissociate from the sucralfate. This would be expected to give rise to artifacts and/or undesired background radioactivity in the imaging of the bowel, especially the lower bowel. On the other hand, [^{99m}Tc]sucralfate shows good stability as high as pH 9 and is therefore unlikely to experience dissociation in the bowel. Although under normal circumstances, transchelation is not likely to be a major concern, it should be remembered that [^{99m}Tc]HSA-sucralfate does show greater resistance to transchelation than does [^{99m}Tc]sucralfate (Table 5).

TABLE 5
Comparison of [^{99m}Tc]HSA-Sucralfate and [^{99m}Tc]Sucralfate

Factor	[^{99m}Tc]HSA-sucralfate	[^{99m}Tc] sucralfate
Labeling protocol		
Complexity	−	+
Time	−	+
Stannous ion	+	+
Preparation stability		
pH	−	+
Specific* activity	+	+
Label exchange	+	−

Summary

1. [^{99m}Tc]Sucralfate required less time and handling to prepare than [^{99m}Tc]HSA-sucralfate.
2. [^{99m}Tc]Sucralfate was more stable at the high pH that would be encountered in imaging of the bowel.
3. [^{99m}Tc]HSA-sucralfate was more resistant to loss of label to exogenous protein.

*No breakdown of the [^{99m}Tc]HSA-sucralfate complex was observed at the highest ^{99m}Tc concentration achievable under standard conditions.

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