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# Labeling of new formulation of tin–sucralfate freeze-dried kit with technetium-99m and its biological evaluation

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**Abstract** The present investigation deals with a simple preparation of new formulation of tin–sucralfate freeze-dried kit (F.D.K.), to be directly labeled with $^{99m}$Tc at optimal pH value of 7.0. The lyophilized form containing 100 mg sucralfate and 11.3 mg dihydrated stannous chloride. Other optimal pH values of the preparation were found to be from 4.0 to 11.0. The range of sucralfate amount studied (50–500 mg) not affected the radiochemical purity of the labeled complex. The radiochemical purity and the stability of the labeled preparation that assessed by filtration were more than 95%. $^{99m}$Tc sucralfate was radiochemical stable up to a specific activity of 1,000 mCi per gram which was more stable than earlier published value (700 mCi per gram) without any radiolytic decomposition. The biological behavior of $^{99m}$Tc-pertechnetate was evaluated in two groups of animals, the first group (neither fasted nor ulcerated) and the second group (fasted and ulcerated mice). The data of organ distribution of $^{99m}$Tc–sucralfate in ulcerated fasted mice showed that more than 99% of the administered dose was accumulated in the stomach (87.92%) and intestine (11.43%). The radioanalytical results together with the in vivo-biological behavior of the labeled preparation demonstrate it's stability, efficacy and usefulness in medical applications for the detection of gastrointestinal ulcers.

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## Introduction

Sucralfate, the aluminum hydroxide complex of sulfated sucrose (Fig. 1), which have remarkable adherence ability to the ulcers forming a barrier against acid, pepsin, and bile acid penetration and promotes the healing of peptic and duodenal ulcers [1]. This unlabeled drug has been studied by many authors showed its various actions of mechanisms in animals and humans [2–5]. Many investigators employed $^{99m}$Tc–sucralfate complex to diagnose few types of ulcers, such as esophageal, peptic, and duodenal ulcers [6–17]. Among them was Pera et al. [10], who reported a direct in vivo technique for labeling sucralfate used for the diagnosing gastric ulcers. The technique involve oral administration of a sucralfate/stannous ion suspension followed by oral administration of radioactive pertechnetate after 2 h. Vasquez et al. [12], successfully imaged gastric ulcers with sucralfate complex with $^{99m}$Tc-human serum albumin ($^{99m}$Tc-HSA–sucralfate). Billinghurst et al. [13], studied extensively two formulations, the first one was $^{99m}$Tc–sucralfate (by direct in vitro method), and the other one was $^{99m}$Tc-HSA–sucralfate (by using modified version of Vasquez method) provided studying the various factors affecting its stability. Naumovski et al. [15], prepared $^{99m}$Tc-DTPA–Suc (by modification of the method presented by Scopinaro et al. [18]) and suggested that scintigraphy with $^{99m}$Tc-DTPA–sucralfate can be considered as additional method, complementary to routine investigations in detecting mucosal lesions.

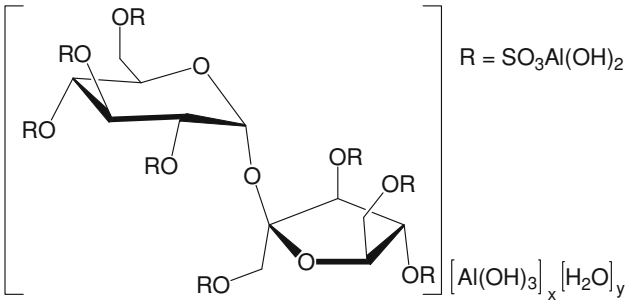


**Fig. 1** Structure of sucralfate

The present work aims to test and improve the validity of the intended freeze-dried kit production process of the formulation introduced by Billinghurst et al. [13] for the benefit of Iraqi nuclear medicine centers. The work is to be supported by radioanalytical studies with the in vivo biological behavior in laboratory animal to demonstrate the stability extent of the in vitro labeled formulation and its usefulness in medical applications for the detection of gastrointestinal ulcers.

## Experimental

### Preparation of new formulation of tin–sucralfate freeze-dried kit (F.D.K.)

The preparation of developed formulation of tin–sucralfate (F. D. K.) was carried out by using chemicals of commercial sources without further purification (sucralfate was imported by The State Company for Marketing Drugs & Medical Appliances/Iraq, and dihydrated stannous chloride was obtained from Riedel-De-Haen, AG-Germany). This developed formulation was prepared by mixing acid solution of a constant concentration of hydrated stannous chloride (11.3 mg/5 mL) with different amounts of sucralfate powder at various pH values. The suspension mixture was incubated at 37 °C for 1 h then centrifuged to separate the precipitate from the supernatant. The precipitate was resuspended in normal saline (0.9% NaCl solution) after each vigorous shaking a homogenized sample of 2.0 mL suspension dispensed in each vial (10 mL vial), then lyophilized in freeze dryer for 48 h.

The labeling method proceeded by resuspension of the F. D. K. with a suitable volume (2.0–5.0 mL) of technetium eluate ($Na^{99m}TcO_4$) taken from $^{99}Mo$–$^{99m}Tc$ generator (CIS–biointernational, France) of certain radioactivity (0.5–10 mCi). For the determination of the radiochemical purity of labeled preparations the labeled sucralfate suspension were rotated for homogenization then different sample volumes (0.1–0.2 mL) were withdrawn at various incubation time (5, 15, 30, 60 and 120 min) and filtered through 3 mm filter paper (25 mm in diameter). Each precipitate was then washed twice with 1 mL normal saline. The filter and the filtrate were counted separately in well-type scintillation counter (sodium iodide crystal). The labeling efficiency was calculated according to the published formula by Billinghurst et al. [13].

### Parameters studied related to the preparation of the new formulation

#### *Effect of pH*

The pH of the preparation suspension was adjusted by using either 0.1 N HCI or 0.1 N NaOH to get different pH values (2, 4, 6, 7, 8, 9, 10, and 11). Samples were taken for assessment at various incubation time intervals (5, 15, 30, 60 and 120 min) after $^{99m}Tc$-eluate addition. These samples were incubated and analyzed at ambient temperature.

#### *Effect of sucralfate amount*

Various quantities of sucralfate amounts (50, 100, 150, 200, 250 and 500 mg) were selected with fixed amount of hydrated stannous chloride (11.3 mg) at optimal pH value (7.0). These preparations were directly labeled with $^{99m}Tc$-eluate and analyzed by filtration for the detection of the radiochemical purity.

#### *Effect of specific activity on complex stability*

A series of experiments were conducted to determine at what specific activity the 24 h stability of $^{99m}Tc$–sucralfate complex would be compromised. $^{99m}Tc$ sucralfate preparations using one tenth of our developed formulation (1.13 mg hydrated stannous chloride and 10 mg sucralfate) were incubated with various values of $^{99m}Tc$-radioactivity concentrations (0.5, 1.0, 3.0, 5.0, 8.0 and 10.0 mCi). Analyses were carried out after 3 incubation time intervals (1, 4 and 24 h) at ambient temperature.

### Biological studies

White *Swiss albino* mice weighing 15–25 gram were applied for biological comparative studies. The animals were grouped into three groups (according to the presence of ulcer and nature of the radioactivity to be orally administered) as follows:

1. *First group (control group)* The animals of this group were none fastend nor ulcerated and orally administered with $^{99m}Tc$-eluate ($Na^{99m}TcO_4$).
2. *Second group (ulcerated group)* The animals received nothing orally 15 h and orally administered with $^{99m}Tc$-eluate ($Na^{99m}TcO_4$).

3. *Third group (ulcerated group)* The animals received nothing orally for 15 h and orally administered with $^{99m}$Tc–tin–sucralfate.

Ulceration could be made by successive multidoses of glacial acetic acid to the fasted animals. The animals of all groups were administered orally 0.1 mL sample of radioactivity (50–100 μCi $^{99m}$Tc-eluate or $^{99m}$Tc–sucralfate preparation). The animals were let to drink water then killed 90 min post-administration. The organs of interest and blood samples were taken from the dissected animals and counted in a well-type scintillation counter. The total blood activity was calculated assuming that blood constitutes 7% of the total body weight.

## Results and discussion

### Labeling kinetics and labeling yield of the new formulation of tin–sucralfate

Our good method of preparation of the developed formulation based on the mixing of an acid solution of hydrated stannous chloride (11.3 mg/5 mL) with suitable amount of sucralfate (100 mg) at optimal pH value (7.0).The best kit contents leads to obtaining a very high value of radiochemical purity (>95%) as illustrated in Fig. 2. The high labeling yield obtained at early time as indicated in Fig. 2 was very fast which is may be due to rapid process of binding of $^{99m}$Tc with sucralfate and optimal kit contents.

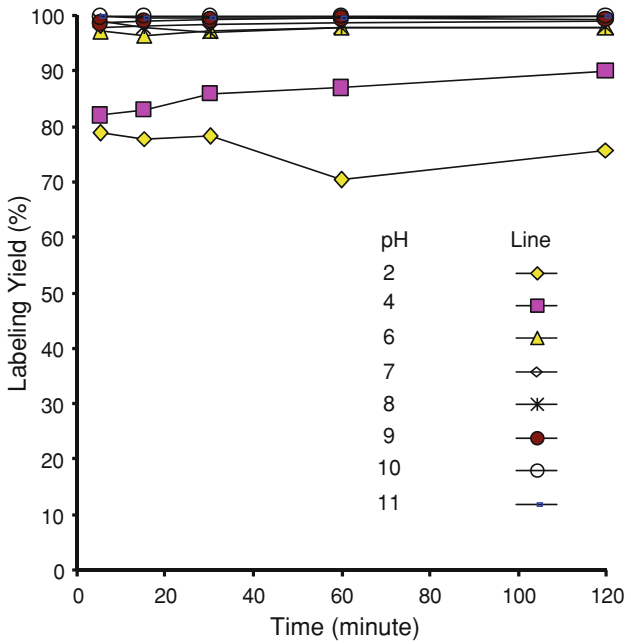


**Fig. 2** Represents the formation rate of the labeled complex ($^{99m}$Tc–sucralfate) as a function of pH

The labeled complex is consistent and stable as shown in Table 2. The labeling kinetics of the labeled complex was achieved by direct labeling without any successive steps. These results were comparable with one of the author's results [13] and better than other investigators followed in vivo and transchelating methods [9, 10, 14, 15].

### Parameter studied related to the preparation of the new formulation

#### *Effect of pH*

Figure 2 shows the results of the formation rate of the $^{99m}$Tc–tin–sucralfate complex as a function of pH (from 2.0 to 11.0). The results showed high labeling yield (>95%) in the range studied except pH 2.0 the labeling yield showed significant lower percentage (70–80%). Better labeling yield was obtained in pH range upper than 9.0 when compared with results reported by Bilinghurst et al. [13].

#### *Effect of sucralfate amount*

The results shown in Table 1 indicate that the amounts of the chelating agent (sucralfate) were not affected the radiochemical purity of the labeled complex. High labeling yield (>94%) obtained in a wide range of chelate amounts (50–500 mg) at a fixed amount of dihydrated stannous chloride (11.3 mg) at optimal pH value 7.0.

#### *Effect of specific activity on complex stability*

Data of stability studies conducted on samples of the labeled preparations ($^{99m}$Tc–tin–sucralfate) containing various concentrations of radioactivity were shown in Table 2. No breakdown due to the radioactivity was seen in the range studied [up to 10 mCi/10.0 mg sucralfate (1.0 mCi/1.0 mg)]. Since this new upper level is above the need for clinical use (it corresponds to 1,000 mCi per gram of sucralfate). This value is higher than that published earlier (700 mCi/gram sucralfate) [13].

#### *Biological studies*

Organ distribution data of free pertechnetate ($Na^{99m}TcO_4$) in none fasting nor ulcerated mice (control group-first group) are summarized in Table 3. The results have shown that high radioactivity uptake was observed in the stomach (41.30%) and intestine (33.73%). Lower radioactivity uptake was seen in the other organs at 90 min post-administration. These high percentages of radioactivity accumulation were attributed to the adherence of the radioactivity with food present (non fastened animals).

**Table 1** Represents the data of radiochemical purity of $^{99m}$Tc–sucralfate as a function of sucralfate amount at optimal pH value (pH = 7.0)

| Ref. | Incubation time (min) | Quantity of sucralfate/mg | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 50 mg | | 100 mg | | 150 mg | | 200 mg | | 250 mg | | 300 mg | |
| | | A | B | A | B | A | B | A | B | A | B | A | B |
| 1 | 5 | 99.6 | 0.4 | 99.3 | 0.7 | 97.4 | 2.6 | 94.6 | 5.4 | 94.4 | 5.6 | 98.9 | 1.1 |
| 2 | 15 | 99.9 | 0.1 | 99.6 | 0.4 | 99.3 | 0.7 | 95.6 | 4.4 | 95.6 | 4.4 | 98.8 | 1.2 |
| 3 | 30 | 99.7 | 0.3 | 99.8 | 0.2 | 99.2 | 0.8 | 95.5 | 4.5 | 95.5 | 4.5 | 98.4 | 1.6 |
| 4 | 60 | 99.8 | 0.2 | 99.3 | 0.7 | 97.2 | 2.8 | 96.1 | 3.9 | 94.2 | 5.8 | 97.9 | 2.1 |
| 5 | 120 | 99.8 | 0.2 | 99.0 | 1.0 | 98.8 | 1.2 | 94.3 | 5.7 | 96.1 | 3.9 | 98.1 | 1.9 |

A = Percentage of labeled complex ($^{99m}$Tc–sucralfate)

B = Percentage of free pertechnetate (Na $^{99m}TcO_4$)

**Table 2** Represents the stability of $^{99m}$Tc–sucralfate versus total radioactivity

| Ref. | Radioactivity (mCi) | Activity mCi/ sucralfate mg | Incubation time (h) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 h | | 4 h | | 24 h | |
| | | | A | B | A | B | A | B |
| 1 | 10.0 | 1.0 | 98.6 | 1.4 | 97.4 | 2.6 | 97.5 | 2.5 |
| 2 | 8.0 | 0.8 | 99.6 | 0.4 | 99.1 | 0.9 | 98.3 | 1.7 |
| 3 | 5.0 | 0.5 | 98.5 | 1.5 | 98.2 | 1.8 | 97.2 | 2.8 |
| 4 | 3.0 | 0.3 | 97.1 | 2.9 | 97.0 | 3.0 | 96.6 | 3.4 |
| 5 | 1.0 | 0.1 | 99.5 | 0.5 | 99.1 | 0.9 | 98.6 | 1.4 |
| 6 | 0.5 | 0.05 | 97.3 | 2.7 | 97.1 | 2.9 | 96.7 | 3.3 |

A = Percentage of labeled complex ($^{99m}$Tc–sucralfate)

B = Percentage of free pertechnetate (Na $^{99m}TcO_4$)

**Table 3** Represents the biodistribution data of labeled complex ($^{99m}$Tc–sucralfate) 90 min post-oral administration in the fasted and ulcerated mice

| Organ | % Dose/Organ | | |
|---|---|---|---|
| | First group Mean ± S.D. | Second group Mean ± S.D. | Third group Mean ± S.D. |
| Blood | 2.66 ± 0.680 | 6.08 ± 4.823 | 0.12 ± 0.058 |
| Liver | 3.26 ± 0.503 | 3.38 ± 1.636 | 0.07 ± 0.060 |
| Lungs | 0.70 ± 0.200 | 0.54 ± 0.207 | 0.03 ± 0.012 |
| Spleen | 0.20 ± 0.100 | 0.18 ± 0.109 | 0.03 ± 0.005 |
| Stomach | 41.30 ± 2.066 | 40.06 ± 9.494 | 87.89 ± 2.711 |
| Intestine | 33.73 ± 1.747 | 15.38 ± 4.054 | 11.43 ± 0.596 |
| Kidneys | 0.46 ± 0.152 | 0.48 ± 0.109 | 0.04 ± 0.015 |
| Carcass | 17.66 ± 1.350 | 33.90 ± 5.529 | 0.44 ± 0.399 |

The results of ulcerating and fasting (second group) effects on the biological behavior of free pertechnetate are presented in Table 3. Data clear that the stomach radioactivity uptake (40.06%) was the same as in the control group (41.30%) except that intestine showed lower radioactivity uptake (15.38%).

To demonstrate the variation in the biological behavior in a comparison with the previous groups (first and second groups), the results of organ distribution of the labeled preparation ($^{99m}$Tc–tin–sucralfate) in the ulcerated and fasted animals (third group) were given in Table 3. The data show that very high radioactivity uptake were accumulated in both stomach and intestine (87.92 and 11.43%) respectively. This mean that about more than 99% of administered activity was accumulated in the gastrointestinal tract with trace amounts in the other listed organs.

The radioanalytical results together with the biological findings of our developed formulation of $^{99m}$Tc–tin–sucralfate, demonstrate its stability, efficacy and usefulness in clinical applications specially in the detection of gastro duodenal ulcers.

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