
Evaluation of Zeta-Potential and Particle Size of Technetium ^{99m}Tc -Sulfur Colloid Subsequent to the Addition of Lidocaine and Sodium Bicarbonate

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The use of ^{99m}Tc -sulfur colloid lymphoscintigraphy for the determination of lymph flow patterns from a tumor site and localization of the sentinel node has been widely adopted. However, the effects of multiple injections of the radiopharmaceutical can range from mild discomfort to pain. pH-adjusted lidocaine HCl coadministered with ^{99m}Tc -sulfur colloid presents a risk of introducing instability of the radiopharmaceutical, which could lead to aggregation, possibly impeding the kinetics of lymphatic drainage from the tumor site. **Methods:** In the present study, lidocaine pH-adjusted with 4.2%, 6.3%, or 8.4% sodium bicarbonate was added to the ^{99m}Tc -sulfur colloid radiopharmaceutical to monitor effects on radiochemical purity, zeta-potential, particle size, and pH. These parameters were then used to evaluate the short-term stability of the preparation. **Results:** The study revealed that the formulation of lidocaine pH-adjusted with 8.4% sodium bicarbonate added to ^{99m}Tc -sulfur colloid demonstrated a similar change in zeta-potential (-4.09 ± 2.90 mV) and particle size (10–330 nm) to that of control filtered ^{99m}Tc -sulfur colloid (-5.09 ± 1.68 mV and 11–343 nm, respectively). However, the 4.2% preparation showed a zeta-potential of -3.01 ± 2.24 mV and a particle size range of 10–351 nm. The pH of the 8.4% buffered preparation, at 7.1, was closer to physiologic pH than was the control, at 6.0. The 6.3% pH-adjusted lidocaine– ^{99m}Tc -sulfur colloid preparation failed radiochemical purity; thus, it was not included in the analysis. **Conclusion:** Compared with other ^{99m}Tc -sulfur colloid test formulations of 4.2% and 6.3% pH-adjusted lidocaine, the 8.4% sodium bicarbonate pH-adjusted lidocaine– ^{99m}Tc -sulfur colloid preparation, taken as a whole, yielded superior quality-control parameters. This formulation would be an acceptable alternative to the control.

Key Words: ^{99m}Tc sulfur colloid; lymphoscintigraphy; breast cancer; melanoma; particle size

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Lymph flow from the tumor site follows a defined pattern that leads to one or more sentinel lymph nodes. In tumors that metastasize through the lymphatic system, it is important to identify the first lymph node, also known as the sentinel node, to determine the degree of metastasis. When excising a tumor, the goal is to remove as much of the cancer as possible with minimal morbidity. In melanoma, 1 technique is to remove the tumor and 10–30 lymph nodes, subjecting the patient to lymphedema (1). Once the physician identifies the sentinel node, determining the presence or absence of cancer cells can then direct the surgery, resulting in the excision of fewer lymph nodes. If cancer cells are found, the physician can follow the flow from the sentinel node and test the distal nodes for cancer.

The ^{99m}Tc -sulfur colloid radiopharmaceutical is approved for intravenous administration for liver or spleen imaging or for oral administration for gastrointestinal studies. Interstitially administered ^{99m}Tc -sulfur colloid is a medically accepted radiopharmaceutical for off-label use in lymphoscintigraphy and sentinel node mapping (2). ^{99m}Tc -sulfur colloid lymphoscintigraphy has proven to be useful in the delineation of lymphatic drainage from a tumor site and in the localization of sentinel nodes in breast cancer and melanoma (3). The sulfur colloid particles are localized in the lymph nodes when administered interstitially. Particle size is a concern in lymphoscintigraphy and sentinel node mapping, because the size of the particle affects the rate of migration from the interstitial space to the lymphatic channels (4), which in turn will determine the time standards for the surgery. This information is useful to surgeons for the evaluation of metastatic sites.

Whether the injections are subcutaneous, intradermal, or peritumoral, the discomfort can range from being irritating to quite painful (1,5). Different techniques have been used to minimize the discomfort. Some clinicians have chosen to anesthetize the area locally with pH-adjusted lidocaine before the injection of ^{99m}Tc -sulfur colloid. pH-adjusted lidocaine is chosen over just lidocaine HCl because lidocaine itself can be painful. Williams et al. reported on

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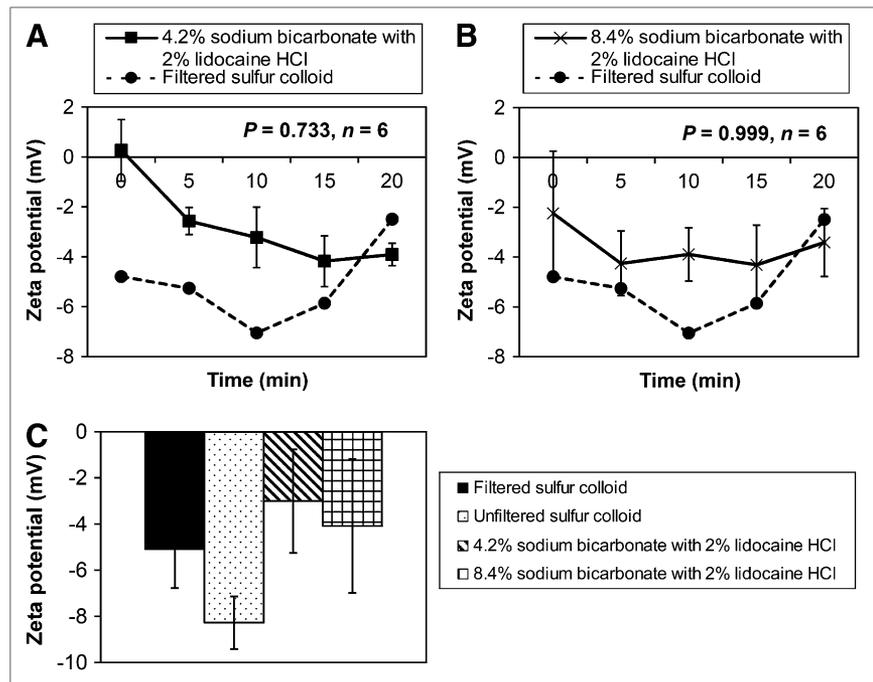


FIGURE 1. (A and B) Comparison of zeta-potential over time for different preparations. (C) Comparison of cumulative data for different preparations.

their success with injecting a mixture of lidocaine with ^{99m}Tc -sulfur colloid for the purpose of minimizing the discomfort of the injection (1). Dura and Hinkle found that the addition of 1% lidocaine HCl to ^{99m}Tc -sulfur colloid resulted in no substantial change in pH, radiochemical purity, or radioactivity retention on a 0.1- μm filter (6). Stokes et al. reported that buffering ^{99m}Tc -sulfur colloid did not alter the quality of the radiopharmaceutical and reduced injection pain (5). None of these authors addressed the issue of zeta-potential, which could affect the particle size of the colloid preparation (4–6). A larger aggregate could result in altered lymph flow kinetics of the ^{99m}Tc -sulfur colloid. As compared with unfiltered ^{99m}Tc -sulfur colloid, particles 220 nm or smaller have the propensity to penetrate lymphatic vessels quickly and in greater concentrations (7). The immediate objective of our study was to determine whether mixing pH-adjusted lidocaine with ^{99m}Tc -sulfur

colloid would adversely alter the zeta-potential, particle size, radiochemical purity, and pH.

MATERIALS AND METHODS

The ^{99m}Tc -sulfur colloid kit was prepared according to the package insert using ^{99m}Tc -pertechnetate eluate obtained from a ^{99}Mo - ^{99m}Tc generator (Lantheus). The filters were from Millipore, the 4.2% and 8.4% sodium bicarbonate injections (United States Pharmacopeia [USP] grade) and the 2% lidocaine HCl injection (USP grade) were from Hospira, Inc., and the Hydriion paper pH strips (Micro Essential Laboratory) were commercially available. All particle size and zeta-potential analyses were performed using the Zetasizer (Malvern). Experiments were divided into 5 study groups: unfiltered sulfur colloid, filtered sulfur colloid, filtered sulfur colloid with 4.2% sodium bicarbonate and 2% lidocaine HCl, filtered sulfur colloid with 6.3% sodium bicarbonate and 2% lidocaine HCl, and filtered sulfur colloid with 8.4% sodium bicarbonate and 2% lidocaine HCl. Using a new sulfur colloid kit

TABLE 1
Cumulative Data

Sample name	Zeta-Potential \pm SD* (mV [†])	Particle size (d.nm [‡])	Radiochemical purity (%)	pH
Unfiltered sulfur colloid	-8.27 \pm 1.14	396–531	99.5	5.8
Filtered sulfur colloid	-5.09 \pm 1.68	11–343	98.5	6.0
4.2% sodium bicarbonate with 2% lidocaine HCl	-3.01 \pm 2.24	10–351	94.1	6.3
8.4% sodium bicarbonate with 2% lidocaine HCl	-4.09 \pm 2.90	10–330	94.0	7.1

*SD = standard deviation.

[†]mV = millivolts.

[‡]d.nm = diameter in nanometers.

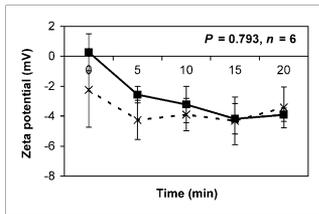


FIGURE 2. Changes in zeta-potential with time.

for each experiment, the zeta-potential determinations were run in triplicate ($n = 3$). The zeta-potential determination for the filtered sulfur colloid study group was performed only once for reference.

The ^{99m}Tc -sulfur colloid was prepared using the Pharmaluce sulfur colloid kit (8). Each kit was reconstituted with 1 mL of ^{99m}Tc -sodium pertechnetate containing up to approximately 37 GBq (100 mCi). The final volume of 4 mL for the prepared kit included the contents from vial A (1.5 mL) and vial B (1.5 mL) and the 1 mL of ^{99m}Tc -sodium pertechnetate. A 0.8-mL sample was removed before the kit was passed through a 0.22- μm filter. Four samples were then taken from the filtered kit. Each sample consisted of 0.6 mL of the final kit preparation drawn up in a 1-mL syringe with a 21-gauge needle after mixing to ensure adequate dispersion of the particles.

From each filtered kit, 4 formulations were evaluated: filtered sulfur colloid, 2% lidocaine HCl buffered with 4.2% sodium bicarbonate, 2% lidocaine HCl buffered with 6.3% sodium bicarbonate, and 2% lidocaine HCl buffered with 8.4% sodium bicarbonate. The pH-adjusted lidocaine samples were prepared by adding 0.5 mL of sodium bicarbonate with a concentration of 4.2%, 6.3%, or 8.4% to 2.5 mL of 2% lidocaine HCl. To prepare the samples for zeta-potential analysis, we combined 0.6-mL samples of ^{99m}Tc -sulfur colloid with 0.2 mL of the pH-adjusted lidocaine to yield the 0.8 mL of total volume. For the filtered and unfiltered sample, a 0.8-mL aliquot was used. The final samples were stored in lead-lined syringe shields at room temperature until analysis was performed. Preparation of the pH-adjusted lidocaine samples required approximately 15 min.

All zeta-potential data points and particle sizes were determined using the Zetasizer Nano ZS at ambient temperature, with the first data point acquisition beginning at approximately 40 min after preparation of the pH-adjusted samples. The analysis proceeded in the following order: unfiltered sulfur colloid, filtered sulfur colloid with 4.2% sodium bicarbonate-buffered lidocaine HCl, filtered sulfur colloid with 6.3% sodium bicarbonate-buffered lidocaine HCl, and filtered sulfur colloid with 8.4%

sodium bicarbonate-buffered lidocaine HCl. On the occasions that the kit did not contain an adequate volume to supply separate samples for zeta-potential determination and particle sizing, then the zeta-potential was determined first and the volume was extracted from the folded cuvette and reused for particle sizing. The pH was determined via color chart comparison using the Hydriion paper pH strips. The radiochemical purity was determined by instant thin-layer chromatography (ITLC) using ITLC-silica gel. A small amount was spotted on an ITLC-silica gel plate and separated using a mobile phase consisting of normal saline (9,10). The resulting purity was accepted only if it met the minimal acceptable purity of 92% as defined by the USP monograph (11).

For each comparison, a one-way ANOVA was used to identify statistical significance with a P value less than or equal to 0.05 (SPSS, edition 17.0; SPSS Inc.).

RESULTS

Lymphoscintigraphy can be painful to the patient because the ^{99m}Tc -sulfur colloid is an irritant due to its pH (1,5). To reduce the irritation, we proposed bringing the pH closer to physiologic pH without altering the quality of the product. During our study, we found no significant difference in the zeta-potential when combining pH-adjusted lidocaine with ^{99m}Tc -sulfur colloid, compared with the standard filtered ^{99m}Tc -sulfur colloid preparation (Figs. 1A–1C). Particle size and radiochemical purity were also comparable, except for the radiochemical purity of the lidocaine pH-adjusted using 6.3% sodium bicarbonate, which was below the minimum acceptable radiochemical purity of 92%. Our results show an appreciable difference in pH, with the 8.4% formulation being closest to physiologic pH. The cumulative data for each formulation are reported in Table 1. No significant difference in zeta-potential between the 4.2% and the 8.4% sodium bicarbonate and lidocaine formulations was observed ($P = 0.793$, $n = 6$) (Fig. 2). A single outlier data point was removed from both the 4.2% and the 8.4% sodium bicarbonate and lidocaine formulations. When examining the particle size of the varying concentrations, we found that the primary peak of the formulation using the 8.4% sodium bicarbonate pH-adjusted lidocaine yielded particles approximately 36 nm (57.2%) in diameter, which is a more

TABLE 2
Particle Size as Affected by pH-Adjusted Lidocaine

Sample name	Primary peak			Secondary peak			Tertiary peak		
	d.nm*	$\pm\text{SD}^\dagger$	%	d.nm	$\pm\text{SD}$	%	d.nm	$\pm\text{SD}$	%
Filtered sulfur colloid	10.55	0.03536	63.2	343.5	53.53	27.1	60.84	29.94	7.6
4.2% sodium bicarbonate with 2% lidocaine HCl	159.6	73.75	69.2	44.08	9.482	20	9.04	0.3309	10.8
8.4% sodium bicarbonate with 2% lidocaine HCl	36.13	32.66	57.2	178.1	6.152	26	7.318	2.656	16.9
Unfiltered sulfur colloid	466.7	0	100	0	0	0	0	0	0

*d.nm = diameter in nanometers.

$^\dagger\text{SD}$ = standard deviation.

desirable size than the approximately 159-nm (69.2%) particles yielded by the 4.2% formulation (Table 2). Particle size determination for the unfiltered sulfur colloid yielded a single peak with a range of 396–531 nm. Size was determined only once because it fell within the accepted range for the unfiltered product; therefore, no SD was calculated.

DISCUSSION

To benefit the patient, every nuclear medicine study must satisfy strict quality-control requirements as agreed on by the Food and Drug Administration and USP. For the ^{99m}Tc -sulfur colloid radiopharmaceutical, the minimum acceptable radiochemical purity is defined as binding of 92% of the ^{99m}Tc to the sulfur colloid (11). This level ensures that the biodistribution of the radiopharmaceutical will help identify the pattern of lymph flow from the tumor site; anything less could provide an altered biodistribution that may fail to identify the sentinel node. Other parameters that need to be addressed are zeta-potential, particle size, and pH. zeta-potential and particle size are interrelated in that when zeta-potential changes, particle size can be significantly affected. For example, like charges repel one another, preventing agglomeration of particles. If the zeta-potential approaches a net charge of zero, the surface charge on these particles dissipates and creates larger aggregates (12). Therefore, it is essential to maintain a net positive or negative charge to stabilize the particle size in the radiopharmaceutical.

To minimize discomfort to the patient from interstitial administration of ^{99m}Tc -sulfur colloid, the pH of the product should be as close to physiologic pH as possible. The pH reported in the current USP ranges from 4.5 to 7.5 (11). Several parameters were evaluated in our study. The prime consideration was whether the radiochemical purity was acceptable with the addition of the pH-adjusted lidocaine. The sample mixture of ^{99m}Tc -sulfur colloid and 6.3% sodium bicarbonate pH-adjusted lidocaine did not pass the quality-control test of radiochemical purity, forcing us to remove it from the study. Without acceptable radiochemical purity, biodistribution patterns for locating sentinel nodes are not reliable and can be misleading. The mixture of ^{99m}Tc -sulfur colloid and 4.2% sodium bicarbonate pH-adjusted lidocaine and the mixture of ^{99m}Tc -sulfur colloid and 8.4% sodium bicarbonate pH-adjusted lidocaine were comparable in radiochemical purity, zeta-potential, and particle size. The mixture of ^{99m}Tc -sulfur colloid and 8.4% sodium bicarbonate pH-adjusted lidocaine was determined to be the better choice and more appropriate in patient use because the pH was consistently closer to physiologic pH. On completion of product stability studies, this mixture has the potential to provide a safe and effective product with minimal discomfort to the patient.

CONCLUSION

In selecting an appropriate formulation for a radiopharmaceutical, one should give the primary consideration to

the radiochemical purity of the product. If the formulation does not meet the USP standards of radiochemical purity, then further pursuit is unnecessary. Because the 6.3% sodium bicarbonate pH-adjusted lidocaine did not satisfy the minimum acceptable level of radiochemical purity, we removed it from the selection process. The zeta-potential influences the particle aggregation of the formulation—an important factor in the kinetics of the ^{99m}Tc -sulfur colloid through the lymphatic system. The addition of either 4.2% or 8.4% sodium bicarbonate pH-adjusted lidocaine did not affect these parameters. Finally, to minimize discomfort and irritation from an injectable drug, a preparation approaching the physiologic pH of 7.4 is desirable. The 8.4% sodium bicarbonate pH-adjusted lidocaine- ^{99m}Tc -sulfur colloid, at an average pH of 7.1, was consistently closer to physiologic pH and would, therefore, provide an alternative formulation to the control filtered ^{99m}Tc -sulfur colloid because it showed better quality control parameters than the other test formulations.

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