

Search for an optimal Colloid for Sentinel Node Imaging

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Abstract:

This study aims at finding a cost-effective and stable colloid of appropriate size to replace antimony sulfide colloid which is now in routine use in Australia for sentinel lymph node (SLN) imaging. For this reason we evaluated three colloids; namely phytate, hepatate and stannous fluoride (SnF₂). As colloids of particle size of 100-200 nm seem to be appropriate for sentinel node imaging, the three radiolabelled colloid preparations were filtered through 0.1 and 0.22 µm filters and then studied on electron microscope. Electron microscopy showed that unlike phytate, the particle size of the hepatate and SnF₂ colloids did not increase beyond the size limit of 200 nm over a period of as long as 26 hours. Instead, they remained well within the size limits chosen. The stability of particle size is required for intra-operative gamma probe lymphatic mapping that sometimes may be performed on the following day. Hepatate and SnF₂ colloids appeared to be more suited for sentinel lymph node imaging, the latter being an in-house product is more cost-effective. Further studies based on nodal uptake and the behavior of these two radiopharmaceuticals in animals is suggested in order to evaluate their potential for future wide-spread application in human sentinel node imaging

Key Words: Sentinel lymph node imaging, stannous fluoride, hepatate, phytate, colloid

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Introduction

The concept of sentinel lymph node imaging is one of the most interesting recent developments in clinical oncology

that holds the promise of a major breakthrough in the detection and treatment of early lymphatic metastases from solid tumors, mainly melanoma and breast cancer. The sentinel node is the first lymph node to receive lymphatic drainage from a tumor site and therefore, it is the most suitable tissue for histological examination. In 1993, Morton et al (1) introduced the sentinel node concept to cutaneous melanoma using vital blue dye to visualize lymphatic drainage. They reported that non-sentinel nodes were the site of metastasis in only 2 out of 3079 nodes from 194 lymphadenectomy specimens, further strengthening the need for sentinel node imaging (2). Ariyan and co-workers (3) reported radioactive colloid to be more reliable (100%) in identifying the SLN than lymphazurin blue dye (51%). The concept of orderly progression of lymph node metastasis has been demonstrated by Reintgen et al (4). No micro-metastases were reported in any node in a lymph node basin whose sentinel node was negative for micro-metastasis. That study strongly supports the evidence of absence of skip metastasis, or in other words the orderly progression of lymph node metastasis, i.e., sentinel node will be the first to contain metastasis, and biopsy will accurately predict regional nodal status.

Multidisciplinary enthusiasm for the sentinel node concept has revived the interest in lymphoscintigraphy. Its growth is coupled with the availability and expanding role of hand-held surgical gamma probes, capable of precisely localizing radiolabelled nodes in the operation theatre. A substantial amount of efforts have been put forth to develop the best radiopharmaceutical preparation to achieve accurate and reproducible images of lymphatic drainage (5). Radiotracers have become a routine technical component of the new procedure of intra-operative lymphatic mapping and different colloids have different physicochemical properties. The biokinetics of these agents strongly depends on their particle size. Particles with small diameters (less than a few nanometers) mostly get exchanged through the blood capillaries, whereas, large particles of up to a few tens of nanometers are absorbed into the lymph capillaries. Larger particles (50-200 nm) are transported with the lymph and mostly trapped and phagocytosed in the lymph nodes. The commercially available colloids, namely, gold-198, Tc-99m -albumin, Tc-99m sulfur, and Tc-99m -antimony trisulfide (Sb₂S₃) colloids are of smaller size and may possibly penetrate the capillary membranes or rapidly disappear from the interstitial space into the lymphatic

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vessels in due course, whereas, we need to localize the colloid particles in sentinel nodes, only. The Sb_2S_3 is routinely used for lymph node scanning, but due to its small size of 3-30 nm, it may not be the most appropriate colloid for imaging the sentinel nodes. A number of radiocolloids (e.g., Tc-99m labeled ALBU-RES, TCK-17, TCK-1, Stannous sulfide, AlbuColl, Sb_2S_3 , and Tc-99m -S₇) from different manufacturers have been studied by Bergqvist and co-workers but none of the colloid particles studied were close to the ideal size mentioned above (6).

In the present study our efforts were focused on finding a colloid of appropriate-size which could be readily labeled with Tc-99m, remains stable in vivo for 24-48 hours, localizes in sentinel nodes, stays there (and does not go further into the lymphatic channel) until intra-operative imaging is completed. In several studies, a mean colloid particle size of 100 nm (range 50-200 nm) has been suggested for subdermal and peritumoral injections (7,8). Currently, there are no such agents available for lymphoscintigraphic studies that have an optimal particle size, i.e., small enough to be rapidly removed from the intradermal injection site into lymphatics and swiftly transported to lymph nodes, yet large enough to be retained in sentinel nodes for many hours enabling detection by gamma probes. In this study, we have evaluated the possibility of separating phytate, hepatate, or SnF_2 colloid of lower particle-size (50-200 nm) and using them for sentinel lymph-node imaging as an alternative to Tc-99m - Antimony tri-sulphide (Sb_2S_3) which is currently available. So, we are looking for a colloid of relatively bigger particle-size of about 100-200 nm which could stay in the sentinel nodes up to the next day for the intra-operative scanning by a gamma probe.

Materials and Methods

Rationale for using Phytate, Hepatate or SnF_2 :

The calcium phytate colloid (Radpharm Scientific, Australia) is inositol hexaphosphate, which is being used routinely for liver-spleen scanning. Its particle size is 100-300 nm, whereas, we need 100-200 nm size which is suitable for sentinel lymph node scintigraphy.

Hepatate II (SnF_2 colloid; Amersham Healthcare, UK) contains more than 80% particles in the range of 50-600 nm with a majority (90%) being <400 nm. The chemical used to prevent change in particle size is a detergent known as Poloxamer 188.

Our in-house SnF_2 colloid (routinely used for white cell labeling) has also been studied for determination of its particle size and its in vitro stability.

Materials used:

1. Radpharm's calcium phytate colloid
It comprises of Inositol hexaphosphate (PHYTATE Radpharm) = 23.4 mg; $SnCl_2$ = 1.0 mg; Potassium hydrogen phthalate = 1.25 mg and $CaCl_2$ (CALCIUM

Radpharm) = 3.42 mg

2. Amersham's hepatate colloid
It comprises of NaF = 1.0 mg, SnF_2 = 0.125 mg and Poloxamer-188 (detergent) = 0.50 mg
3. In-house SnF_2 colloid
The final preparation contains NaF = 0.50 mg and SnF_2 = 0.0644 mg
4. Millipore filters (0.1 μ m sterile Millex-VV filter, 0.22 μ m sterile Millex-GS filter)
5. For ITLC: Gelman ITLC-SG strips and acetone.

Equipment used:

ZEISS transmission electron microscope model 109T (Department of Anatomical Pathology, SWAPS, South Western Sydney Area Health Service) was used to measure the size and spectrum of the particles. An LKB gamma counter was used for QA. The particle-size distribution study was done at the Biomedical Electron Microscope Unit of the University of New South Wales, Sydney.

Procedures:

Tc-99m Phytate was made as per routine procedure by adding the required amount (maximum 10 ml) of Tc-99m pertechnetate in saline into the 'PHYTATE Radpharm' vial followed by the addition of 0.25 ml of 'CALCIUM Radpharm'. Mixed for 20 seconds and left it for 10 min at room temperature (RT) before use. The stability of the preparation was found to be 6 hrs at room temperature.

The Amersham's hepatate colloid was made by adding 3-9 ml of Tc-99m into the vial followed by a gentle mixing for 20 sec. The stability of the preparation was found to be 6 hrs at room temperature.

In-house SnF_2 kit was prepared as per the following method: Four ml of sterile water for injection was added into vial A and mixed. Transferred 4 ml from this solution into vial B and mixed. Then 0.5 ml from vial B was transferred (through a 0.22 μ m filter) into a vial containing 2.5 ml of Tc-99m pertechnetate. The vial was placed on a rotator for 1 hr at room temperature.

Electron microscopy was performed on preparations left to incubate for 30 min, 6 hours, and overnight for 27 hours at 37°C post-reconstitution. Grids were prepared by placing a droplet of each solution on a Formvar coated 400 mesh copper grid, blotting off the excess with filter paper and allowing the remaining solution to air dry. Examination was carried out at 80 Kv.

Particle-size distribution studies to determine the mean diameter and standard deviation were then carried out using representative micrographs from each sample. Particles were traced from each micrograph onto a digitizing tablet which then transferred the data to an Excel spreadsheet. Routine statistical analysis of the data was performed within this application.

Colloid type		Min dimension (nm)	Max dimension (nm)	Mean (nm)	Std. Dev.
Phytate	0.1 μm 2 hr	30	240	140	0.08
Phytate	0.2 μm 2 hr	190	270	100	0.06
Phytate	0.1 μm 27 hr	150	350	250	0.05
Hepatate	0.2 μm , 6 hr	80	130	110	0.02
Hepatate	0.2 μm , 26 hr	50	240	140	0.06
SnF ²	0.2 μm , 6h	70	210	150	0.04
SnF ²	0.1 μm , 26h	80	130	100	0.01

Table-1. Summary of particle-size distribution studies



Figure 1 Phytate colloid filtered through 0.1 μm filter (Left panel). The size increased to >200 nm in 2 hours time. The figure in the middle panel shows Phytate colloid filtered through 0.1 μm filter. The size was increased to about 350 nm in 27 hours. The figure on the right panel shows Phytate colloid filtered through 0.22 μm filter. The colloid size was increased to about 220 nm in 2 hrs.

Results

Based on our electron microscopic examination and the analysis of the particle-size spectrum, the overall impression of the preliminary series of experiments was as follows:

Phytate: When filtered through 0.1 and 0.22 μm filters, a mean colloid particle size of 140 x 190 nm was obtained which in 26-27 hours grew up to 350-410 nm (probably due to opsonin coating) rendering this colloid unsuitable for our purpose (Figure 1)

Hepatate: Using 0.1 and 0.22 μm filters, particles of about 80-110 nm were obtained which grew to a mean dimension of 140 nm in 26 hours which seems to be suitable for the study of sentinel node (Figure 2)

In-house SnF₂: Using a 0.1 μm filter a mean size of 150 nm was obtained which did not show any increase in size with time (Figure 3).

The particle-size distribution studies on some selective images from different types of colloids were performed and outcome is summarized in the table 1.

Discussion

A strong relationship exists between the colloid particle size and its bio-kinetics (6). Small-sized colloid particles like Tc-99m antimony trisulfide (Tc-99m Sb₂S₃) with a

range of 3-30 nm (used in Australia) rapidly migrate through the sentinel nodes to higher levels. Tc-99m albumin colloid (Nanocoll, Nycomed Amersham, UK) appears to have more appropriate size (95% <80 nm), combining rapid absorption and transport with a prolonged selective sequestration in the sentinel node allowing accurate detection up to 24 hours post-injection. It is used in Europe and other countries, however, it is not a particulate by nature and shows a poor retention within lymph nodes and therefore, delayed imaging or gamma probe intra-surgical explorations may miss the sentinel nodes (6).

The absorption of large particle-sized Tc-99m S-colloid (not available in Australia) with an average size of 200 nm (range 200-1000 nm) as quoted by Paganelli et al (9) is slow leading to low nodal uptake that may possibly result in failure to visualize all sentinel nodes. Secondly, its size depends also on heating time and the age of Tc-99m eluate, thus jeopardizing the validity of the procedure. Sodium thiosulfate is the source of sulfur along with various types and amounts of stabilizing agents used by various manufacturers resulting in a variety of particle size ranges with different degrees of stability (10). However, in a clinical comparison of Tc-99m Sb₂S₃ and Tc-99m Sulfur colloid in 28 patients who underwent internal mammary lymphoscintigraphy at 0.5, 1.0, and 3.0 hr; Kaplan and associates have reported similar biological and clinical parameters in terms of number of nodes visualized and

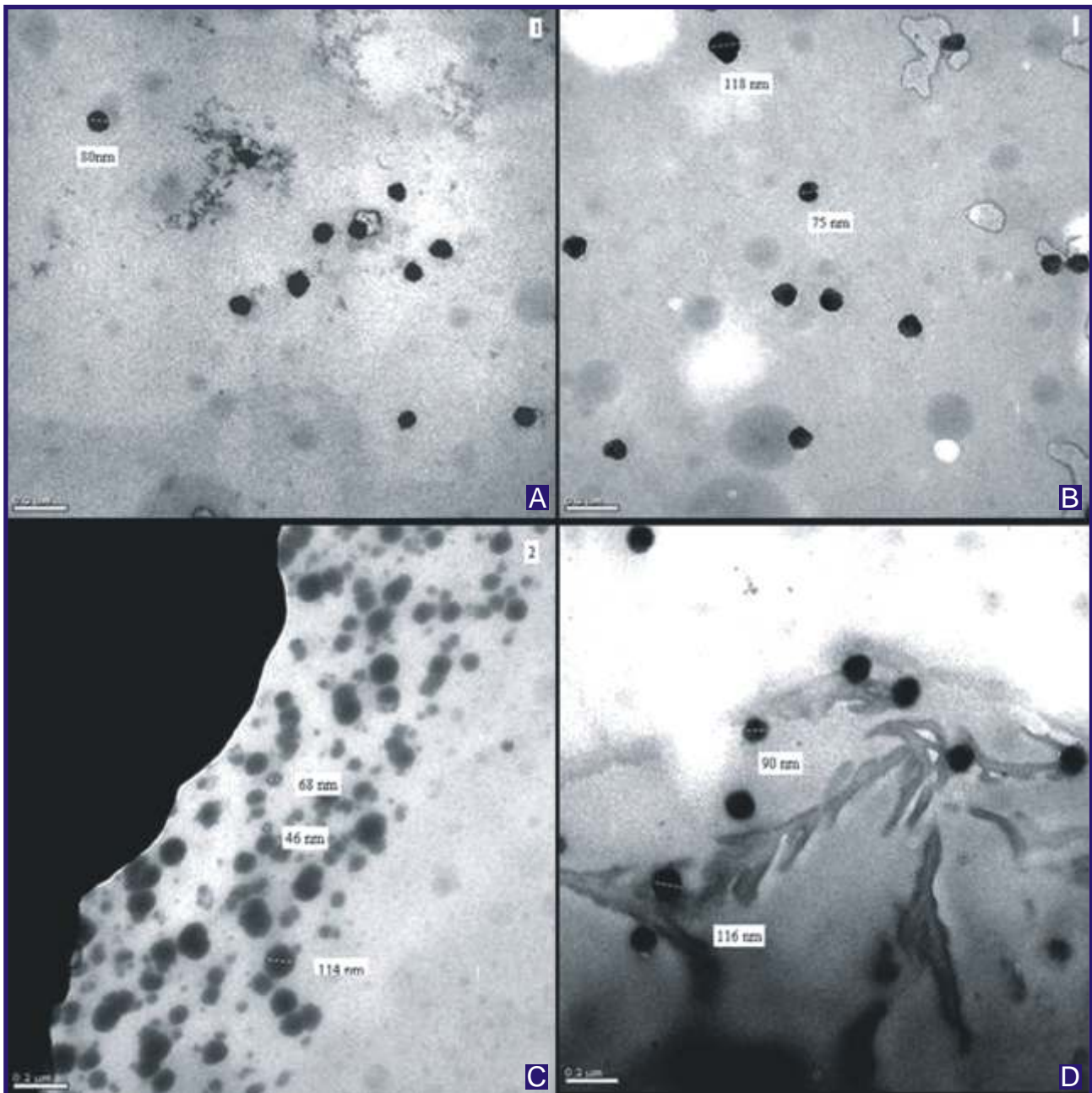


Figure 2 Hepatate colloid filtered through $0.1\mu\text{m}$ filter (Top left). The average colloid size was about 80 nm within 30 min of preparation. Top right figure shows Hepatate colloid filtered through $0.1\mu\text{m}$ filter. The maximum colloid size was about 118 nm after 26.5 hrs of preparation. Bottom left figure shows Hepatate filtered through $0.2\mu\text{m}$ filter. The maximum colloid size was about 114 nm within 30 min of preparation. Bottom right figure shows Hepatate filtered through $0.22\mu\text{m}$ filter. The maximum colloid size remained unchanged (116 nm) even after 26.5 hours of preparation.

extent of radiocolloid migration (11). The mean % injected dose of both radiotracers within visualized nodes was less than 1 at each time interval, the % dose remaining at the injection site at 3.0 hr was 83 for antimony sulfide and 76 for sulfur colloid (not statistically significant).

The current study aimed at finding a more cost-effective, appropriately sized and stable colloid to replace antimony sulfide colloid which is in routine use in Australia for sentinel lymph node (SLN) imaging. For this reason we studied three colloids; namely phytate, hepatate and stannous fluoride (SnF_2). As colloids of particle size of 100-

200 nm seem to be appropriate for sentinel node imaging, the three radiolabeled colloid preparations were filtered through 0.1 and $0.22\mu\text{m}$ filters and then studied on electron microscope. Electron microscopy showed that unlike phytate, the particle size of the hepatate and SnF_2 colloids did not increase beyond the size limit of 200 nm over a period of as long as 26 hours. Rather, they remained well within the size limits chosen. The stability of particle size is required for intra-operative gamma probe lymphatic mapping that some times may be carried out on the following day. Hepatate and SnF_2 colloids appeared to be

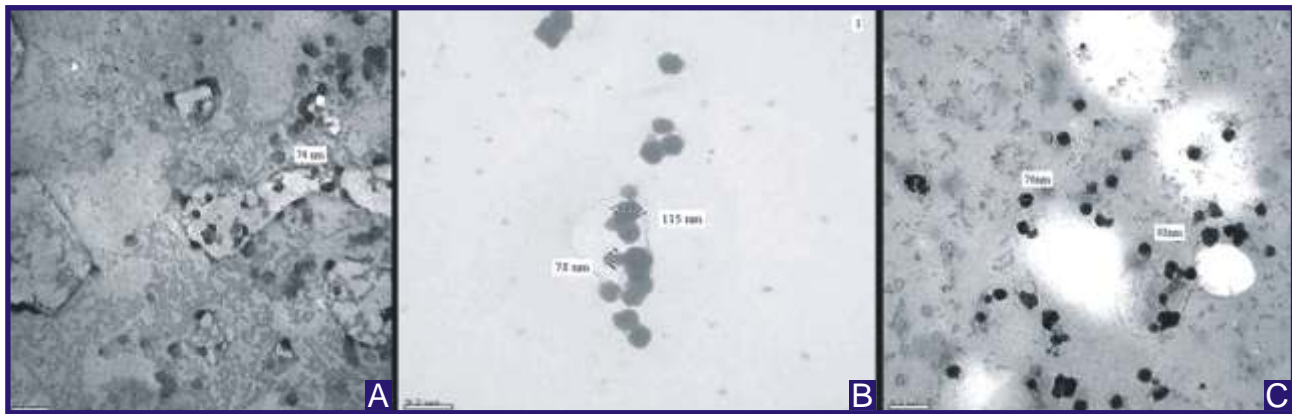


Figure-3 SnF₂ colloid filtered through 0.1 μ m filter (Left panel). The colloid size was in 70-80 nm range within 30 min of preparation. The middle panel shows SnF₂ colloid filtered through 0.1 μ m filter. The colloid size remained in the range of about 78-115 nm even after 26 hours of preparation. The right panel shows SnF₂ colloid filtered through 0.2 μ m filter. The colloid size remained unchanged even after 26.5 hours of preparation.

more suited, the latter being an in-house product is more cost-effective. Further studies based on nodal uptake and the behavior of these two radiopharmaceuticals in animal are suggested in order to evaluate their potential for future wide-spread human sentinel node imaging.

Conclusion

The radioactive technique has got definite advantages over vital blue dye mapping alone. The gamma probe-guided search is remarkably efficient and easy to learn, in contrast to the tedious and technically demanding wide dissection required during blue dye alone. The concept of radionuclide imaging of sentinel lymph-nodes provides a method of studying the early lymphatic spread of tumor cells allowing more insight into the intricate process of micrometastasis. In-house SnF₂ and Hepatate seem to be worthwhile to study further to explore their suitability for sentinel lymph node imaging, preferably the former with an added advantage of being more cost-effective, too.

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Disclaimer: The article is the authors' personal view and opinion about colloid particles for sentinel node imaging and may not necessarily reflect the opinion of the Department of Nuclear Medicine, PET and Ultrasound of Liverpool Hospital. The responsibility of the article rests solely upon the authors.

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