

# <sup>99m</sup>Tc-Labeled Microsized Liposomes

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### SUMMARY

Respiratory diseases can cause the death of the patient if not diagnosed at the early stages. Imaging techniques in nuclear medicine are frequently preferred for diagnosing of these diseases. Radiopharmaceuticals can be used to evaluate the perfusion or ventilation capacity of the lung. Liposomes, formed by lipid layers and an aqueous core, are micro- or nanometer-sized vesicular systems. They can be passively accumulated in lung capillaries due to their surface and physicochemical properties. In light of this information, in our study, liposome formulations with micrometer particle sizes containing DTPA-PE chelating agent for radiolabeling with <sup>99m</sup>Tc were designed for the imaging of lung diseases. After the preparation of liposomes, the optimum formulation exhibits proper particle size (~5 µm) and zeta potential (~-6 mV) values, and the vesicular integrity was imaged with SEM in characterization studies. Liposomes were dried by lyophilization to obtain cold-kit formulations for radiolabeling. The effect of the lyophilization process on the characterization properties of the vesicles was evaluated. All liposome formulations were radiolabeled with high efficiency in the optimal radiolabeling conditions comprising 1 mCi of radioactivity, 250 µg.ml<sup>-1</sup> of stannous chloride, and 15 min incubation period. In addition, the radiolabeled liposomes were found to be stable, with radiolabeling efficiencies of over 90% for 6 hours. As a result, the developed Tc-<sup>99m</sup> labeled formulation has the potential to be used as a lung perfusion imaging agent following in vitro and in vivo studies and clinical trials.

**Key Words:** Technetium-99m, liposome, lung perfusion imaging, capillary blockade

## <sup>99m</sup>Tc İşaretli Mikro Boyutlu Lipozomlar

### ÖZ

Solunum yolu hastalıkları erken teşhis edilmediği takdirde hastanın ölümüne neden olabilir. Nükleer tıp görüntüleme teknikleri bu hastalıkların tanısında sıklıkla tercih edilmektedir. Radyofarmasötikler akciğerin perfüzyon veya ventilasyon kapasitesinin değerlendirilmesinde kullanılabilir. Lipid katmanlar ve sulu bir çekirdekte oluşan lipozomlar, mikro veya nanometre boyutuna sahip veziküler sistemlerdir. Yüzey ve fizikokimyasal özellikleri nedeniyle akciğer kılcal damarlarında pasif olarak birikebilirler. Bu bilgiler ışığında çalışmamızda, akciğer hastalıklarının görüntülenmesinde <sup>99m</sup>Tc ile radyoışaretleme için DTPA-PE şelatör ajanı içeren, mikrometre partikül büyüklüğünde lipozom formülasyonları tasarlanmıştır. Lipozomların hazırlanmasından sonra, karakterizasyon çalışmalarında, optimum formülasyon uygun partikül boyutu (~5 µm) ve zeta potansiyeli (~-6 mV) değerlerini sergilerken, veziküler bütünlük SEM ile görüntülenmiştir. Radyoaktif işaretleme uygun soğuk kit formülasyonları elde etmek için lipozomlar liyofilizasyon yoluyla kurutulmuş ve liyofilizasyon işleminin veziküllerin karakterizasyon özellikleri üzerindeki etkisi değerlendirilmiştir. Tüm lipozom formülasyonları, 1 mCi radyoaktivite, 250 µg.ml<sup>-1</sup> kalay klorür ve 15 dakikalık inkübasyon süresi içeren optimal radyoaktif işaretleme koşullarında yüksek verimle işaretleştirilmiştir. Ayrıca radyoaktif lipozomların 6 saat boyunca %90'ın üzerinde radyoışaretleme verim değerlerine sahip olması nedeniyle stabil olduğu bulunmuştur. Sonuç olarak geliştirilen Tc-<sup>99m</sup> işaretli formülasyon, in vitro ve in vivo çalışmalar ve klinik deneylerin ardından, akciğer perfüzyon için potansiyel bir görüntüleme ajanı olma potansiyeline sahiptir.

**Anahtar Kelimeler:** Teknesyum-99m, lipozom, akciğer perfüzyon görüntüleme, kapiller blokaj

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## INTRODUCTION

Respiration begins from the nostril, passes through the pharynx and larynx, and continues to the main bronchi leading to the two lungs and from the bronchi to the bronchioles, at the ends of which are the alveoli. Airflow through the bronchial system is called ventilation. The blood flow in the pulmonary arterial system is called pulmonary perfusion. Respiratory diseases, the leading causes of death in the world, comprise serious diseases such as chronic obstructive pulmonary disease (COPD), pneumonia, lung cancer, bronchitis, tuberculosis, or asthma (Labaki & Han, 2020). These diseases, affecting lung ventilation or perfusion, can cause the death of the patient if not diagnosed at the early stages.

In routine clinics, to diagnose these diseases, lung and respiratory system diseases can be noninvasively imaged through radiological (chest radiography, computed tomography (CT), magnetic resonance imaging (MRI)), or nuclear medicine [Positron Emission Tomography (PET), single-photon emission computed tomography (SPECT)] imaging techniques (Hollings & Shaw, 2002). Chest radiography is used to diagnose or monitor the prognosis of the diseases affecting the thorax.

Thorax CT is a superior imaging modality to plain chest radiography due to its much higher contrast resolution and ability to prevent tissue superposition. Thorax CT examinations are performed with multi-slice spiral CT (MDCT) systems today. Although MRI is not a primary method of choice in the evaluation of chest diseases, it is preferred for the assessment of lesions in chest wall and mediastinal tumors thanks to its main advantages such as high soft tissue contrast, ability to perform multiplanar examination and image tumors and vessels without contrast agent. Compared to other methods, nuclear medicine techniques are often preferred because they can obtain three-dimensional images containing metabolic information with high precision.

Radiopharmaceuticals, containing pharmaceutical and radionuclide parts, are used for diagnostic or therapeutic purposes in nuclear medicine. The pharmaceutical part is responsible for delivering the radionuclide to the target tissue and is not responsible for any pharmacological activity. Radiopharmaceuticals accumulate in the desired organs or tissues depending on the physical, chemical, and biological properties of the pharmaceutical part. Radionuclide part is responsible for imaging or treatment thanks to the rays emitted by the radionuclide. Alpha- and beta-emitting radionuclides are used for therapeutic applications. Radiopharmaceuticals are used in SPECT or PET imaging depending on gamma or positron emission. Radiopharmaceuticals can be used to determine the perfusion or ventilation capacity of the lung. Lung perfusion imaging, used in the diagnosis of pulmonary embolism, pulmonary artery stenosis, hepatopulmonary syndrome, is based on the capture of particles larger than 10  $\mu\text{m}$  in the capillary bed of the lung. Particles larger than 10  $\mu\text{m}$  size accumulate in capillaries during the first pass through the pulmonary artery following the intravenous administration. Radionuclide ventilation studies of the lungs can be performed to determine any airway obstruction using aerosols (Elgazzar, 2011). In clinics, lung capacity is determined by performing perfusion and ventilation imaging using radiopharmaceuticals such as  $^{99\text{m}}\text{Tc}$ -labeled macroaggregate albumin particles ( $^{99\text{m}}\text{Tc}$ -MAA),  $^{99\text{m}}\text{Tc}$ -labeled DTPA aerosols,  $^{67}\text{Ga}$ -citrate, and  $^{18}\text{F}$ -labeled 18-Fluoro-2-deoxy-D-glucose ( $^{18}\text{F}$ -FDG).  $^{99\text{m}}\text{Tc}$ -MAA and  $^{99\text{m}}\text{Tc}$ -DTPA aerosols are used for lung perfusion and ventilation imaging, respectively, while  $^{67}\text{Ga}$ -citrate and  $^{18}\text{F}$ -FDG are used for imaging tumor or infection in the lung (Jacobson et al., 2015; Saha, 2018).  $^{99\text{m}}\text{Tc}$ -MAA is one of the most frequently utilized radiopharmaceuticals in nuclear medicine clinics. Its applications include the evaluation of lung perfusion and the determination of patient characteristics before the administration of selective internal radiation therapy (Sancho et al., 2017). Nevertheless, in certain instances, in addition to minor adverse

effects such as hypersensitivity, dyspnea, dizziness, and rash, various types of serious adverse events, including angioedema, cardiac arrest, bradycardia, and respiratory arrest, have been documented in patients undergoing  $^{99m}\text{Tc}$ -MAA imaging (Schreuder et al., 2019). Therefore, the development of alternative agents to  $^{99m}\text{Tc}$ -MAA is a necessity.

Liposomes are spherical vesicles formed by one or more biological membrane-like lipid layers with an aqueous phase in between. The phospholipid molecules that form liposomes are amphiphilic and consisting of a hydrophilic head and hydrophobic hydrocarbon chains. Liposomes are biocompatible, non-pyrogenic, non-antigenic and biodegradable systems due to their similarity to the cell membrane. They are used in the diagnosis and/or treatment of many diseases and offer advantages such as slow and controlled drug release, entrapment of drugs with different physicochemical properties, surface modification and conjugation of different ligands or radioisotopes. Liposomes can be classified as multilamellar vesicles (MLV), small unilamellar vesicles (SUV), large unilamellar vesicles, and multivesicular liposomes (MVL) depending on the number of layers and particle sizes (Šturm & Poklar Ulrih, 2021). MVL, containing MLV and/or SUV liposomes in its structure, have particle sizes between 500 nm and 100  $\mu\text{m}$ . They have a high encapsulation capacity due to their high water content (95%) and large particle size.

Some studies have been carried out in the literature using MVL-type liposome formulations for a variety of diseases. Jain et al. prepared acyclovir-loaded MVL-type liposomes with a particle size of 7-16  $\mu\text{m}$  for the treatment of diseases caused by the herpes simplex virus. They reported that the drug encapsulation efficiency of MVL was 3 to 6 times higher and the drug release lasted longer compared to MLV-type liposomes (Jain et al., 2005). Another MVL type liposome formulations with particle sizes ranging from 1-10  $\mu\text{m}$  were prepared and radiolabeled with  $^{177}\text{Lu}$  (a beta-emitting radionuclide) by Cvjetinovic et al. for the radiosynovectomy, which is a treatment method

applied by intra-articular injection of therapeutic radiopharmaceuticals. According to the scintigraphy images obtained from the knee joints of rats, liposomes were localized in the joint until the 30<sup>th</sup> day. Additionally, it was reported in the biodistribution study that no free Lu-177 or Lu-177 labeled liposome formulation was detected outside the joint tissue (Cvjetinovic et al., 2021).

Targeting drug delivery systems is essential to overcome the shortcomings of conventional imaging techniques and treatment methods, especially in complex diseases such as cancer. Targeting increases the concentration of the drug in the diseased area and reduces the concentration of the drug in other parts of the body. This reduces the risk of side effects on healthy tissue and allows better pharmacological effects to be achieved with the same or lower drug concentration. Additionally, drug can be delivered more effectively to intracellular areas and hard-to-reach organs such as the brain. Passive targeting is achieved by altering the particle size and/or surface properties of liposomes. In contrast, specific ligands or polymers or lipids that are sensitive to physical changes in the disease are used for active targeted liposomes (Karpuz et al., 2018). Particle size affects the biodistribution, the half-life, the drug encapsulation efficiency, the drug release behavior, the toxic profile, the stability properties, and the tissue to reach the formulation. After intravenous injection, drug carrier systems with particle sizes between 5-15  $\mu\text{m}$  are captured by the lung's capillary network and distributed widely in the lung tissue. Therefore, it is possible to passively target drug carrier systems to the lungs by using particles of this size (SreeHarsha et al., 2019).

In the light of the above information, in our study, MVL-type liposome vesicles, as an alternative to the  $^{99m}\text{Tc}$ -MAA, are designed and formulated to radiolabel with  $^{99m}\text{Tc}$ , for the imaging of lung diseases by gamma camera or SPECT. It is designed to passively accumulate vesicles in the lungs thanks to their 5-15  $\mu\text{m}$  particle size. After preparation, the liposomes were lyophilized and the effect of lyophilization on

characterization profiles of the vesicles was evaluated. Last but not least, the optimum radiolabeling conditions were determined. Our study has a novelty due to preparation and radiolabeling of liposome vesicles with micrometer particle size for imaging of lung diseases.

## MATERIALS AND METHODS

### Materials

Lecithin from soybean 90 (PC) was obtained from PanReac Applichem. Cholesterol (Chol), stannous chloride (SnCl<sub>2</sub>), diethylenetriaminepentaacetic acid anhydride (DTPA), and Tris(hydroxymethyl) aminomethane, and 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (PE) to synthesize DTPA-PE were purchased from Sigma-Aldrich.

### Methods

#### Preparation of Liposomes

After obtaining DTPA-PE, a chelating conjugate for radiolabeling with <sup>99m</sup>Tc, based on our previous studies (Karpuz et al., 2024; Karpuz et al., 2020), liposome formulations were prepared by two different techniques (thin-film hydration and reverse-phase evaporation). In the Bangham technique (Bangham, Standish, & Watkins, 1965), a lipid mixture containing PC, Chol, and DTPA-PE was dissolved in chloroform.

After the evaporation of organic solvent at 45°C using vacuum and heating bath (BUCHI R100, Switzerland), dried film was hydrated using Tris buffer (20 mM, pH:7.4). In reverse-phase evaporation technique (Cvjetinović et al., 2021), after the solving of lipid phase in chloroform-methanol (4:1, v/v), Tris buffer (20 mM, pH:7.4) was added dropwise on the lipid-organic solvent mixture. The organic solvent was evaporated at 80°C using a vacuum and heating bath (BUCHI R100, Switzerland), and after evaporation of the organic solvent, the liposome dispersion was kept in a fume hood for 1 h at room temperature to remove residual organic solvents. The rate for lipids in the preparation of liposomes is given in Table 1. After the preparation, optimum liposome dispersions were lyophilized to obtain cold-kit formulations. To that end, the vials were sealed with parafilm to prevent contamination and loss of substance, and a needle was used to puncture the parafilm to allow for lyophilization. The formulations were subjected to a freezing process at -80 °C for 24 hours. Subsequently, the formulations were subjected to freeze-drying using a Christ Alpha 1-2 LD Freeze Dryer apparatus, with a pressure of 1 Pa maintained at -45 °C for 48 hours (Glavas-Dodov et al., 2005).

**Table 1.** Liposomes and their lipid contents.

Formulations	Lipid Conc. (mM)	PC (% M)	Chol (% M)	DTPA-PE (mg.mL <sup>-1</sup> )	Preparation Technique	Lyophilization
Lipo-1	40	80	20	5	Bangham	Not implemented
Lipo-2	40	70	30	5	Bangham	Not implemented
Lipo-3	40	80	20	5	Reverse phase evaporation	Not implemented
Lipo-3L	40	80	20	5	Reverse phase evaporation	Implemented
Lipo-4	40	70	30	5	Reverse phase evaporation	Not implemented
Lipo-4L	40	70	30	5	Reverse phase evaporation	Implemented

#### Imaging and Characterization of Liposomes

The mean particle size and zeta potential of liposomes were measured by Malvern Mastersizer and Malvern Zetasizer, respectively. According to the particle size analysis, optimal formulations were selected and, the morphology of optimal liposome vesicles was imaged by scanning electron microscopy

(ZEISS Sigma 300 VP SEM, Germany) under high vacuum (Quorum Q150R ES, UK) after drying the formulations in a glass lamella at 37°C.

#### Radiolabeling of Liposomes with <sup>99m</sup>Tc

All formulations were radiolabeled with <sup>99m</sup>Tc using the chelating agent DTPA-PE, which provides a stabile complex between the bilayer of the liposome

and the radionuclide (Karpuz et al., 2020). The tin-reduction technique was used in the radiolabeling, and the optimum concentration of SnCl<sub>2</sub> solution in 0.01 N HCl (10, 250, 500 µg.ml<sup>-1</sup>) and incubation time (5 and 15 min) were evaluated to obtain the highest radiolabeling yield. For this purpose, 1 ml of formulations were radiolabeled with <sup>99m</sup>Tc solution after the reducing of Na<sup>99m</sup>TcO<sub>4</sub> with 1 mCi radioactivity using SnCl<sub>2</sub> solution, then this mixture was vortexed, and incubated for different periods at the room temperature. The radiation dose was determined according to the previous studies in the literature (Gharepapagh et al., 2021; Karpuz et al., 2024; Ozgenc et al., 2022). Radiolabeled liposomes were dialyzed by using a regenerated cellulose membrane with a 3500-Da cut-off size for 5h at 4°C against Tris buffer (20 mM, pH 7.4) to remove free <sup>99m</sup>Tc and hydrolyzed <sup>99m</sup>Tc, which are radiochemical impurities for <sup>99m</sup>Tc radiopharmaceuticals (Silindir-Gunay et al., 2019). A control sample containing Na<sup>99m</sup>TcO<sub>4</sub> solution was used to eliminate the decrease of radioactivity as a function time and dilution.

#### **Evaluation of Radiolabeling Efficiency and Stability**

For quality control of radiolabeling, the percentage of radiolabeling efficiency (RE %) was determined by radio-thin-layer chromatography (RTLC) through separation of radiochemical impurities. To this end, radiolabeled liposomes were applied to a silica gel plate, as a stationary phase. At the end of development, radioactivity values in the cut plates were measured by RTLC (Bioscan AR 2000) for 6 h. Hydrolyzed Tc-99m ( $R_f = 1.0$ ) and free Tc-99m ( $R_f = 1.0$ ) were identified as radioactive contaminants using pyridine: acetic-acid: water (3: 5: 1.5 v/v/v) and saline mobile phases, respectively (De Silva et al., 2019; Métayé & Desmarquet, 2001; Métayé et al., 2001). RE % was calculated using the following equation (Eq. 1):

$$RE \% = 100 - [\text{Free Tc-99m} (\%) + \text{Hydrolyzed Tc-99m} (\%)] \quad (\text{Eq. 1})$$

In the study of radiolabeling stability, after the radiolabeling of liposomes using optimum conditions,

100 µL of radiolabeled liposomes were incubated in 900 µL of saline at 37°C. The stability was checked using RTLC by calculating of RE % up to 6 h.

#### **Statistical analysis**

All measurements were performed at least in triplicates, and the data was shown using mean ± standard deviation (SD). Two-way analysis of variance with Tukey's or Sidak's multiple comparisons tests were used to determine whether there was a statistical difference between the groups. Differences were considered as significant when  $p < 0.05$  (\*).

### **RESULTS AND DISCUSSION**

#### **Preparation, Characterization, and SEM Imaging of Liposomes**

A literature review revealed the occurrence of anaphylactic and allergic reactions to <sup>99m</sup>Tc-MAA (Schreuder et al., 2019a; Schreuder et al., 2019b). Due to their cell-membrane-like structures, liposomes are biocompatible and non-allergic drug delivery systems. Accordingly, the objective of this study was to develop micro-sized liposome formulations as the pharmaceutical component of a radiopharmaceutical with the intention of emulating the <sup>99m</sup>Tc-MAA.

In the liposome formulations, lecithin and cholesterol were used to obtain bilayer vesicles and to increase the stability, respectively. In addition, DTPA-PE, a chelating agent between radioisotope and liposome bilayer, was added to the formulation for the radiolabeling procedure. The preparation technique is chosen according to the intended use of the vesicles. In this study, it is aimed that liposomes having in the range of 5-10 micrometer particle size are obtained as a passively targeted formulation to lung capillaries. Therefore, liposome formulations were successfully prepared using lipid-hydration and reverse phase evaporation techniques.

As shown in Table 2, liposome vesicles with the desired particle size could not be obtained using the Bangham technique. This finding agrees with the literature where MLV type liposomes with a particle size of ~1 µm were obtained by the Bangham



technique (Has & Sunthar, 2020). However, the liposomes prepared by the reverse phase evaporation technique showed more suitable particle sizes. MVL type formulations in the range of 1-16  $\mu\text{m}$  particle size were prepared in the literature using a technique similar to that used in this study (Cvjetinovic et al., 2021; Fadel & Kassab, 2011; Jain et al., 2005). The reverse phase evaporation technique has been employed by various studies to formulate liposomes, with the resulting particle size of MVL-type liposome vesicles being found to be greater than 1  $\mu\text{m}$  (Chen et al., 2014; Shi & Qi, 2018). Our findings align with this observation. In the study performed by Ko et al., after obtaining liposomes by reverse phase evaporation technique, the particle size of vesicles was reduced using extrusion (Ko & Bickel, 2012). In addition, the histograms are given in Figure 1 to show the particle size distribution. The SEM images and the particle size distribution graphs agree in showing a wide distribution of particle sizes. Liposome vesicles have a broad particle distribution range because no process such as extrusion or sonication has been used to reduce the particle size and achieve uniform distribution. As seen in Figure 2, although Lipo-3 and Lipo-4 have a lower mean particle size than desired, the histograms show that there are liposomes with larger particle sizes in the formulation. Therefore, Lipo-3 and Lipo-4 were lyophilized to not only increase the stability of the liposomes but also to provide a ready-to-use cold kit for radiolabeling with Tc-99m (Yu et al., 2021). After the lyophilization process, no statistically significant change was observed in the particle size of the Lipo-4 formulation, while the particle size of Lipo-3 decreased. Chen et al. observed a similar decrease in particle size of liposomes after freeze-drying process (Chen et al., 2014). However, this difference was not found to be statistically significant. Similarly, no significant change in particle size of 5-fluorouracil-loaded liposomes after lyophilization has been reported in the literature (Glavas-Dodov et al., 2005). Furthermore, the use of a cryoprotectant is usually recommended to avoid potential adverse effects

such as disruption of vesicle integrity or changes in particle size that may occur during lyophilization (Arshinova et al., 2012). Therefore, for further studies, a cryoprotectant agent can be added to the liposome formulations.

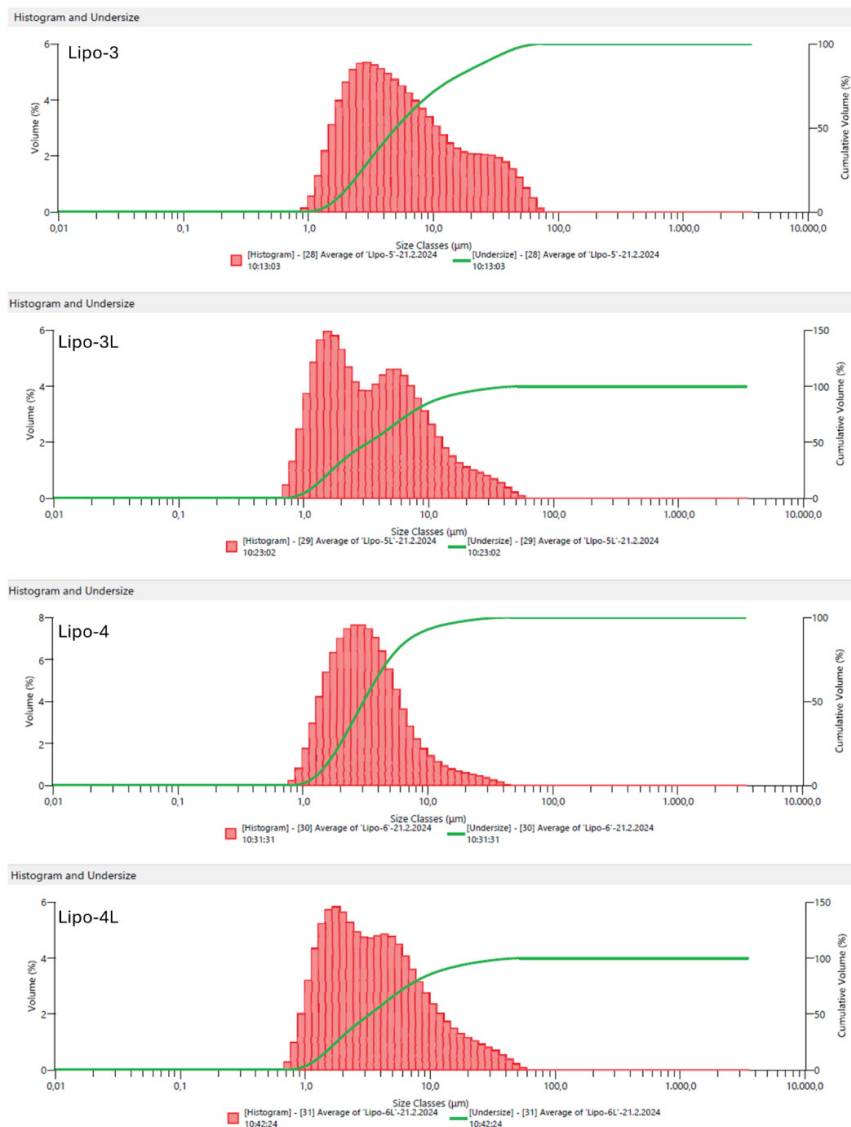
The zeta potential refers to the difference in electrical potential between the layer and the fluid surrounding a charged particle (Clogston & Patri, 2011). Formulations with a high zeta potential exhibit a more stable profile due to the repulsive forces between highly electrically charged particles, which reduces the possibility of aggregation. The zeta potential values are given in Table 2. Statistically essential differences were found between the zeta potential values of the formulations depending on the preparation technique, as shown in Table 2 ( $p < 0.05$ ). Some factors such as preparation technique, hydration medium, lipids influence the zeta potential of liposomes (Smith et al., 2017). In this study, the preparation technique may be the reason for this difference in zeta potential. Furthermore, a study evaluated the impact of particle size on zeta potential. It was reported that using ultrasonication and an elevated ultrasonic probe power contributed to an augmentation in zeta potential value, which was attributed to a reduction in particle size (Nakatuka et al., 2015). As the particle size of the vesicles decreases, the probability of aggregation in liposome dispersions decreases. Therefore, it can be said that another possible reason is differences in particle size. Although Lipo-1 and Lipo-2 formulations exhibited high stability due to zeta potential values higher than 30 mV, their small particle sizes were less suitable for accumulation in pulmonary capillaries. No statistically significant alterations were observed in the zeta potential values of the liposomes before and after the lyophilization process. In addition, it can be said that Lipo-3 and Lipo-3L formulations were more stable compared to Lipo-4 and Lipo-4L formulations due to their higher zeta potential values. Therefore, Lipo-3 and Lipo-3L formulations were chosen for SEM imaging. The vesicle integrity of Lipo-3 and Lipo-3L can be seen in

Figure 2. The images of vesicles are compatible with particle size results. In the present study, the vesicles can be observed using the 1,000-magnification due to their micrometer particle sizes. Although more

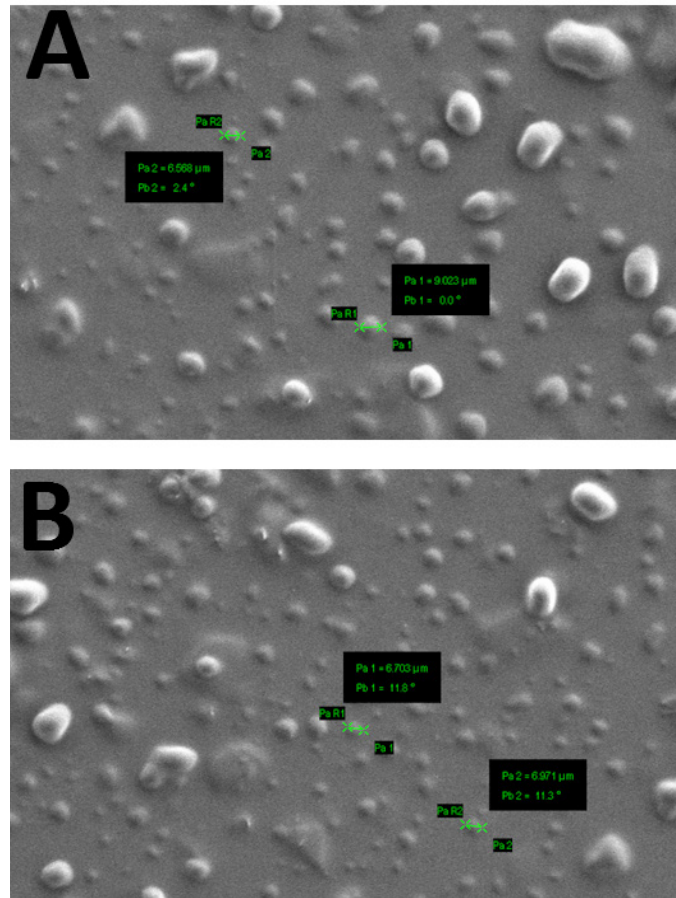
sensitive techniques are preferred, in some studies, SEM was used for this purpose (Karpuz et al., 2024; Lujan et al., 2019)

**Table 2.** Characterization properties of liposome formulations (n=6).

Formulation	Particle Size ( $\mu\text{m} \pm \text{SD}$ )	Zeta Potential ( $\text{mV} \pm \text{SD}$ )
Lipo-1	$1.48 \pm 0.49$	$-35.30 \pm 0.62$
Lipo-2	$1.06 \pm 0.26$	$-56.50 \pm 0.55$
Lipo-3	$5.00 \pm 1.34$	$-6.33 \pm 0.59$
Lipo-3L	$3.31 \pm 1.13$	$-8.42 \pm 1.24$
Lipo-4	$3.06 \pm 0.95$	$0.57 \pm 0.31$
Lipo-4L	$3.29 \pm 1.20$	$0.26 \pm 0.27$



**Figure 1.** Particle size distribution histograms of liposome formulations.



**Figure 2.** SEM images of Lipo-3 (A) and Lipo-3L (B) formulations in 1.00 KX magnification.

### Radiolabeling Efficiency and Stability

Liposomes prepared by reverse phase evaporation technique were radiolabeled with  $^{99m}\text{Tc}$  due to the DTPA-PE content in the formulations. Based on previous studies and radiation safety principles, a radiation dose of 1 mCi was chosen (Karpuz et al., 2023). Liposomes can be radiolabeled by different methods including chelation. The DTPA and PE parts of the chelator are bound to the radiometal and bilayer of liposome vesicles, respectively (Silindir Günay, 2020). The radiolabeling stability of this method is higher than other techniques such as radioisotope encapsulation or direct surface labeling (Bentivoglio et al., 2023). In this radiolabeling procedure, an inducing agent such as  $\text{SnCl}_2$ , ascorbic acid, or ferrous ions is essential for reducing the oxidation state of  $^{99m}\text{Tc}^{+7}$  to  $^{99m}\text{Tc}^{+5}$  (Mushtaq et al., 2021).  $\text{SnCl}_2$  was used as the inducing agent, and statistically significant

differences were found among experiment groups that used different amounts of inducing agent ( $p < 0.05$ ). As given in Figure 3, the highest RE % values were obtained when  $250 \mu\text{g.ml}^{-1}$  of  $\text{SnCl}_2$  was used. All formulations were radiolabeled with higher than 85% RE at this concentration. After determining the optimum inducing agent amount, the effect of incubation time on RE% was evaluated. As seen in Figure 4, statistically higher RE % values were obtained when a 15 min incubation time was applied compared to 5 min ( $p < 0.05$ ). This finding is similar to the study in which  $^{99m}\text{Tc}$ -MAA was prepared (Hunt et al., 2006). Therefore, the optimum  $\text{SnCl}_2$  concentration and incubation time were detected as  $250 \mu\text{g.ml}^{-1}$  and 15 min, respectively, to reach the highest RE %.

In addition, for radiopharmaceuticals, the bond between the radionuclide and the pharmaceutical parts should be stable until the purpose of application



in the desired area of the body is achieved. Therefore, the radiolabeling stability of radiolabeled liposomes was tested by evaluating RE % values during the physical half-life of <sup>99m</sup>Tc (6 h). As seen in Figure 5,

the RE % values of all liposome formulations were found to be higher than 80% during 6 h thanks to the use of optimal radiolabeling conditions and chelation technique.

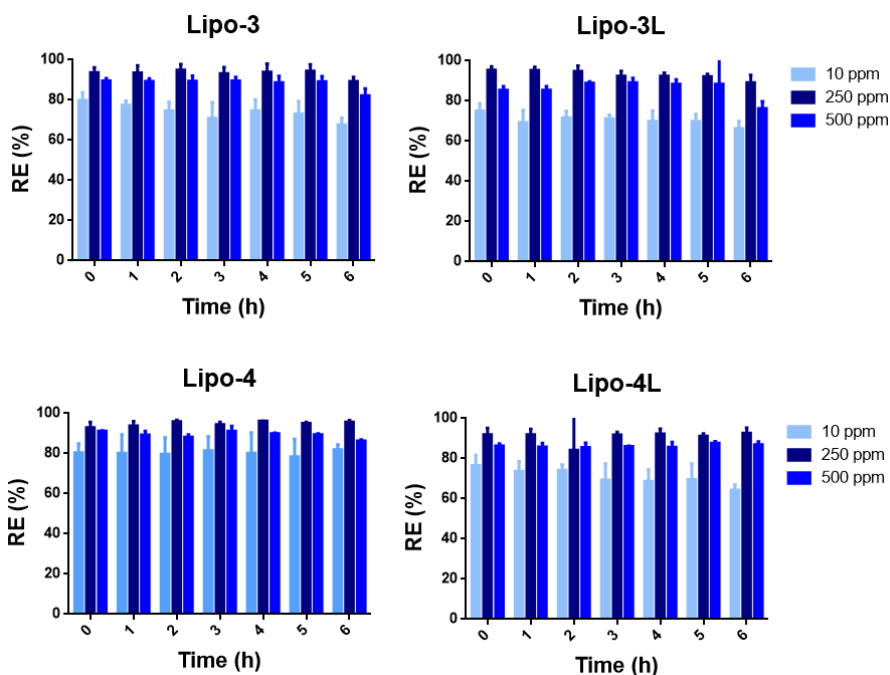


Figure 3. The effect of SnCl<sub>2</sub> concentration on RE % of liposome formulations (n=3) (ppm: parts per million, μg.mL<sup>-1</sup>).

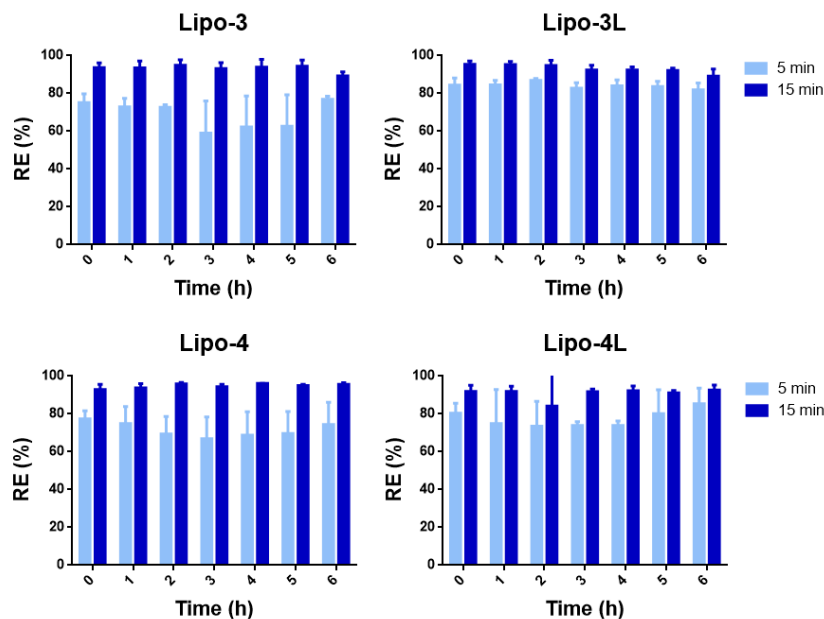
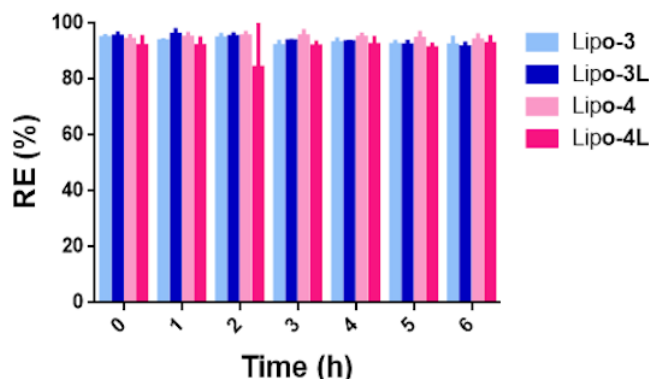


Figure 4. The effect of incubation time on RE % of liposome formulations (n=3). SnCl<sub>2</sub> was used at the concentration of 250 μg.mL<sup>-1</sup>



**Figure 5.** Stability of *in vitro* radiolabeling of formulations in PBS (n=3).

## CONCLUSION

Respiratory diseases such as chronic obstructive pulmonary disease, pneumonia, lung cancer, bronchitis, tuberculosis, or asthma are the leading causes of death in the world. These diseases can cause death of the patient because of their effect on lung ventilation or perfusion. Nuclear imaging techniques have some superiorities, such as their ability to obtain three-dimensional images containing metabolic information with high precision compared to radiological ones. Liposomes are the most preferred drug delivery system thanks to their biocompatible profiles. They may vary in number of bilayers and particle size due to preparation technique and lipid content. Liposomes with particle sizes in the range of 5-15  $\mu\text{m}$  can be passively targeted to the lung capillary.

In this study, micro-sized liposome formulations were prepared using two different techniques to radiolabel with  $^{99\text{m}}\text{Tc}$  due to their DTPA-PE contents. According to the results of characterization studies, formulations prepared by the reverse phase evaporation technique (Lipo-3 and Lipo-4) exhibit more proper particle size distribution compared to liposomes prepared by the Bangham technique. No significant change in physicochemical properties was observed in these liposomes after the lyophilization process to obtain cold-kit formulations. In addition, the highest values of RE % were obtained in the radiolabeling conditions comprising 250  $\mu\text{g}\cdot\text{ml}^{-1}$  of  $\text{SnCl}_2$  concentration and 15 min of incubation time. Also, the radiolabeling stability of formulations

remained for 6 h.

Therefore, it was concluded that the Lipo-3 formulation and its lyophilized form (Lipo-3L) are more suitable as the pharmaceutical part of a radiopharmaceutical for radiolabeling with  $^{99\text{m}}\text{Tc}$  in the lung perfusion imaging.

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## AUTHOR CONTRIBUTION STATEMENT

Conception and design (MK), data collection and processing (HH, MK, EO, EG), analysis and interpretation (MK, EO, EG) literature search (HH, MK), preparing the study text (HH, MK), critical reviews (EO, EG).

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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