Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Development of Tc-99m radiolabeled PLGA nanoparticles: Preparation, characterization and evaluation of radiolabeling parameters

Elif Tugce SARCAN [1] *, Humeyra BATTAL [1], Mine SILINDIR GUNAY [1], Suna ERDOGAN [1]

[1] Department of Radiopharmacy, Faculty of Pharmacy, Hacettepe University, Altındağ 06100, Ankara, Turkey.

* Corresponding Author. E-mail: tugce.sarcan@hacettepe.edu.tr (E.T.S.); Tel. +90-312-305 21 52.

Received: 5 July 2024 / Revised: 16 October 2024 / Accepted: 19 October 2024

ABSTRACT: Radiolabeled polymeric NPs are widely used drug delivery systems in cancer therapy and imaging due to their easy and rapid formation. These systems provide several advantages for imaging and therapy of many diseases and cancers, such as increased sensitivity, better image quality, etc. In this study, Tc-99m radiolabeled PLGA nanoparticles were prepared in different conditions and these conditions were investigated. The amount of reducing agent, incubation temperature and time, and pH were investigated to obtain the ^{99m}Tc-PLGA nanoparticles with higher RCP (%). NPs were formed well with the nanoprecipitation method between 180-200 nm. Then, the optimum formulation was obtained with 50 µl $SnCl_2$ at pH 7 and 25 °C conditions for 30 minutes of incubation time. This formulation was found stable at 25 °C for 12 hours while showing 3 hours of stability at 37 °C. The data proved that PLGA NPs can be radiolabeled with Tc-99m at high efficiency and showed relatively high radiolabeling stability.

KEYWORDS: Technetium-99m; PLGA nanoparticles; radiolabelling; radiopharmaceuticals.

1. INTRODUCTION

Polymeric systems are biodegradable and biocompatible systems that are easily synthesized [1]. Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable hydrophobic polymer that is approved by the Food and Drug Administration (FDA) and European Medicine Agency (EMA) and is considered an ideal material due to its biodegradability and biocompatibility [2,3]. These properties have made PLGA nanoparticles (NPs) increasingly popular in both clinical and preclinical stages [4].

Nuclear imaging modalities, Single Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) are non-invasive imaging techniques that provide anatomical and functional information at molecular and cellular levels [5]. The major advantages of nuclear imaging modalities are sensitivity, quantity, and low tissue penetration [6]. SPECT requires radiopharmaceuticals labeled with gamma-emitted radionuclides with 100-250 keV energy. These gamma rays pass through the collimator, and two-dimensional images are produced by the detector [7].

Radiolabeled NPs show several advantages in molecular imaging: 1. increased sensitivity; 2. ability to conjugate different targeting agents (due to their large surface area); 3. Providing multimodality imaging; 4. combination of diagnosis and therapy, which can be called "Image Guide Systems"; 5. combination of different therapy agents [8]. Radiolabeled NPs can also be used for biodistribution and pharmacokinetic studies by measuring the radioactivity in tissues [9, 10]. Radiolabeled NPs show higher tissue accumulation in this field, providing a better signal-to-noise ratio than other methods [11].

Technetium-99m (Tc-99m) is one of the most common radionuclides used in imaging, biodistribution and drug monitoring in clinics and preclinics. It has 6.02 hours half-life, and 140.5 keV gamma energy. Tc-99m is a cost-effective radionuclide; its easy availability, and photon energy make it a common radionuclide [12]. Moreover, Tc-99m can be easily and stably labeled with direct methods or with chelating agents [11].

In this study, PLGA NPs were radiolabeled with Tc-99m at different conditions to obtain optimum radiolabeling conditions. Before the radiolabeling process, prepared NPs were analyzed by dynamic light scattering (DLS) to find particle size, zeta potential and polydispersity index (PDI) values. Afterward, NPs were radiolabeled with different amounts of reducing agent at different pH, incubation temperature, and

How to cite this article: Sarcan ET, Battal H, Silindir Gunay M, Erdogan S. Development of Tc-99m radiolabeled PLGA nanoparticles: Preparation, characterization and evaluation of radiolabeling parameters. J Res Pharm. 2025; 29(4): 1753-1769.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1734690

incubation times and analyzed for 6 hours to compare these parameters' effects on stability. After finding the optimum formulation, stability tests were performed in saline at room temperature and 37 °C for two half-life times of Tc-99m. Tc-99m radiolabeled PLGA NPs were prepared successfully, and results proved that they could be designed as a more specific therapy, diagnosis or theragnostic agent.

2. RESULTS and DISCUSSION

2.1. Characterization of NPs

Preparation methods play a crucial role in NP properties and directly affect their particle size, surface properties, drug loading capacity, drug release profiles, etc. [13]. The nanoprecipitation method, also known as the solvent displacement method, provides several advantages. NPs are spontaneously and rapidly formed without agglomeration [9].

PLGA NPs are generally formed between 100-400 nm, and the ideal size of PLGA NPs is <300 nm with around 20 mV zeta potential [2]. The characterization results of the NP solution are summarized in Table 1, and this shows a homogeneous size distribution (Figure S1). The results obtained from these NPs were parallel with the literature [2], and NPs can be considered ideal.

DLS analysis was performed to understand the NPs' morphology, size and shape. SEM images confirmed that the NPs have a spherical shape and homogeneous size distribution (Figure 1).

Table 1. Characterization of nanoparticles (n:3).

Nanoparticle Properties	
Size (nm)	190.1±2.36
PDI	0.1056±0.02
Zeta Potential (mV)	8.35±0.43

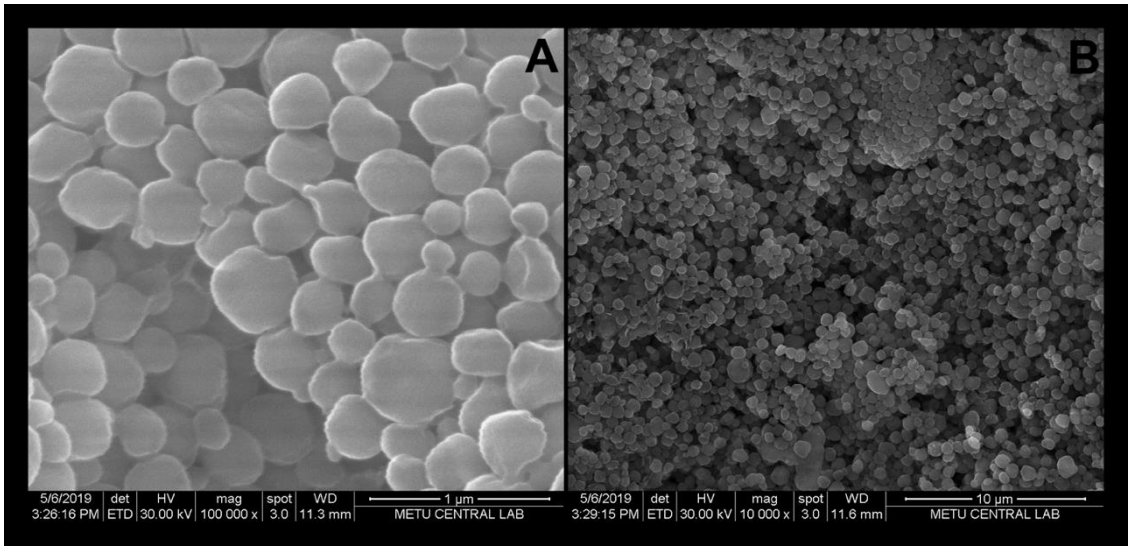

Figure 1. SEM images of PLGA NPs.

2.2. Radiolabeling Studies

2.2.1. Determination of the labelling efficiency of NPs

NPs were radiolabeled with Tc-99m and Tc-99m radiolabeled NPs were evaluated and compared their radiochemical purity (RCP) (%) values in each group. Also, formulations RCP (%) values were investigated for 6 hours (after incubation time) to provide a better understanding of the effects of radiolabeling parameters. After all, Np2 formulation was found an optimum formulation.

Effect of pH

Since radiopharmaceuticals are intravenously administered formulations, the ideal pH value is 7.4. However, pH 5 and pH 9 were investigated in this study to find optimum formulation conditions for Tc-99m

radiolabeled PLGA NPs (Np2, Np4, Np5). At the beginning, their RCP (%) values were found as 99.37±0.33; 76.58±0.50; 84.20±0.47, respectively ($p < 0.05$) (Figure 2A). Results showed that the optimum pH value is 7 and optimum formulation is Np2. Halder et al. [14] also mentioned that pH directly affects the RCP (%) value, and they found that RCP (%) values were changed from 85.9 % to 95.9% from pH 4 to pH 6.5.

Effect of Incubation Temperature

The incubation temperature of Tc-99m radiolabeling for drug delivery systems, especially NPs, was mentioned at room temperature (25 °C) in the literature [9]. In this study, incubation time was another investigated condition for PLGA NPs radiolabeling. 10, 25 and 50 °C temperatures were investigated (Np2, Np8, Np9) and RCP (%) values of them were 99.37±0.33; 77.20±0.25; 78.68±0.26 (Figure 2B). Results showed that the optimum incubation temperature was 25 °C. At lower and higher temperatures, RCP (%) values drop to approximately 77-78%.

Effect of Incubation Time

The incubation time of Tc-99m radiolabeled NPs were investigated to determine the time duration (5, 30 and 60 mins) for higher yield (Np6, Np2, Np7). Results showed Np6, the formulation with 5 mins incubation, had the lowest RCP (%) compared to the other formulation with different incubation times. RCP (%) values, right after incubation times end, RCP (%) of Np6, Np2 and Np7 formulations were found as 74.84±1.03; 99.37±0.33; 85.38±0.52, respectively. After that, RCP (%) values of formulations were also followed up to 6 hours (a half-life of Tc-99m) to provide a better understanding of the effect of incubation time (Figure 2C). Np2 was found stable after 6 hours, and RCP (%) was 98.11±0.08 ($p > 0.05$).

Effect of the Reducing Agent ($SnCl_2$)

NPs were radiolabeled with Tc-99m by using $SnCl_2$ as a reducing agent. $SnCl_2$ directly affects the ratio of free and hydrolyzed Tc-99m amount. Radioactive colloids are formed with high $SnCl_2$ amounts although low amount of $SnCl_2$ causes lower radiolabeling efficiency [12]. Different volumes of $SnCl_2$ were used in 3 formulations (Np1, Np2, Np3) as 10, 50, and 250 µl at (2mg/ml) concentration (Table 2). After 30 minutes incubation, formulations were analyzed, and Np2 was the optimum formulation among them. Both 3 formulations RCP (%) values were found 98.64±0.02, 99.37±0.33, and 69.24±0.90 ($p < 0.05$) after incubation time, respectively. This result showed that 10 µl (Np1) and 50 µl (Np2) were appropriate for high labeling; however, the Np1 formulation RCP (%) value decreased very quickly following 6 hours (Figure 2D). So, the study showed that 50 µl $SnCl_2$ was the optimal amount for radiolabeling PLGA NPs.

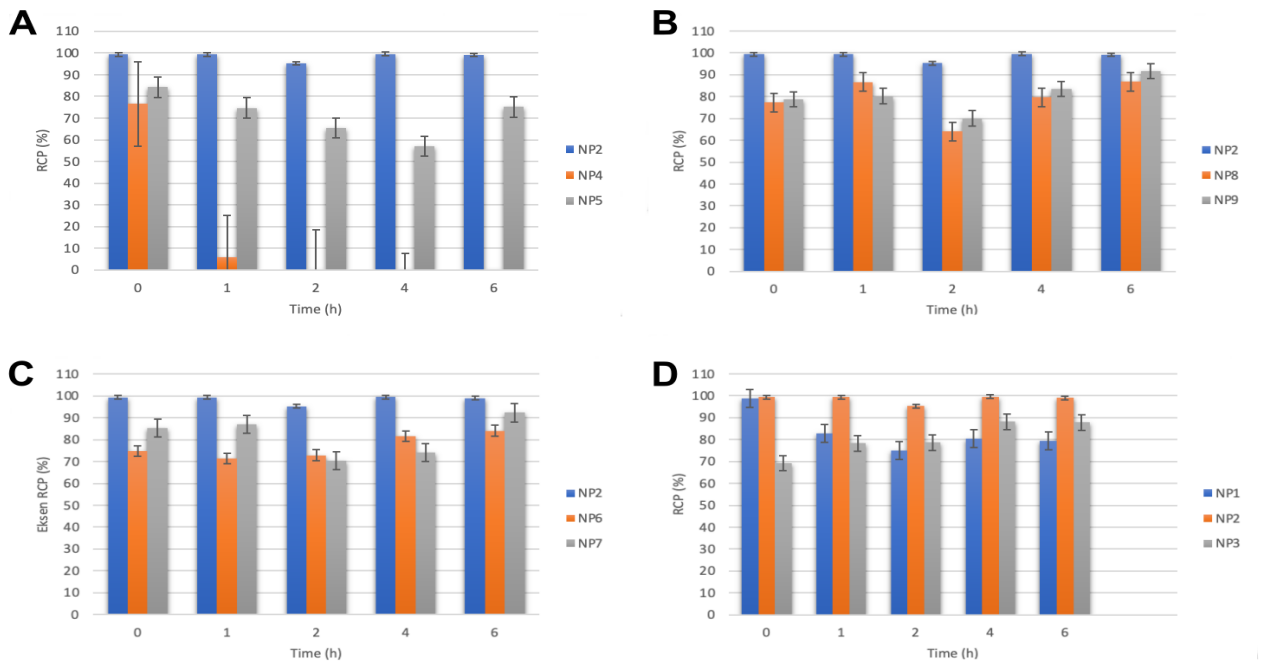

Figure 2. Radiochemical purity of Tc-99m radiolabeled PLGA NP formulations at (A) different pH (7, 5, 9); (B) different temperatures (25°C, 10°C, 50°C); (C) different incubation times (30, 10, 60 min); (D) different volume of $SnCl_2$ (50, 10, 250 µl).

2.3. Stability Test

Stability test of Np2, the optimum formulation, were performed at two different temperatures (25°C and 37°C) to evaluate the Tc-99m release in saline (Figure 3).

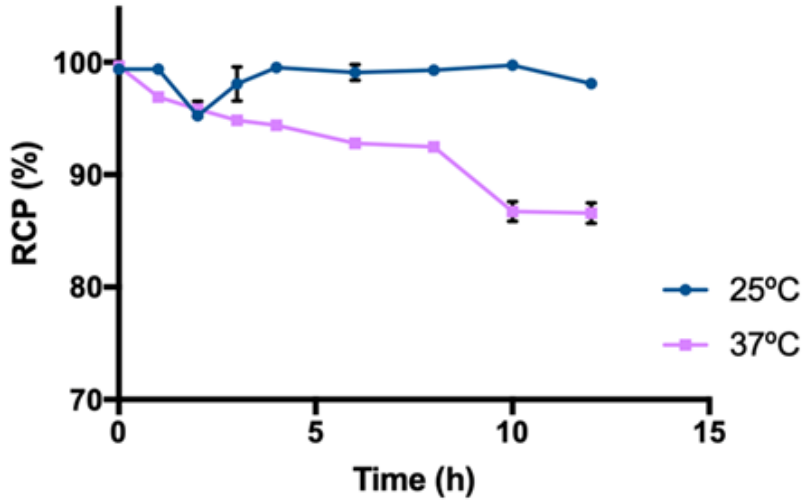

Figure 3. Stability results of Np2 formulation at 25ºC and 37ºC in saline up to 12 hours (n:3).

The RCP (%) value of Np2 was found more than 95% up to 12 hours at 25°C. However, the stability at 37 °C showed stability only for 3 hours. Moreover, the RCP (%) value was reduced to %86.58 after 12 hours of incubation at 37 °C ($p<0.05$). Tc-99m radiolabeled PLGA NPs are found stable in saline at 25°C for 24 hours in many studies in literature [9]. Our results were parallel with the literature [9], and RCP (%) values were more than 95% at the end of 12 hours ($p>0.05$). In another study, Tc-99m radiolabeled PLGA NPs stability was investigated at 37°C in saline [15], and Tc-99m radiolabeled PLGA NPs RCP (%) value was found around 90% in this study, while that value was around 86% in our study.

3. CONCLUSION

PLGA NPs were successfully prepared at around 190 nm in size with 0.1056 PDI. PLGA NPs were radiolabeled with Tc-99m in different conditions to understand the effect of labeling parameters, such as reducing agent amount, pH, incubation time, and temperature, to find the optimum formulations. ^{99m}Tc-PLGA NPs were radiolabeled with high RCP (~99%), and optimum conditions were determined at pH 7, 30 mins incubation time, 25 ºC (RT) and 50 µl $SnCl_2$. Stability tests of optimum formulation (Np2) were performed at 25 ºC and 37 ºC in saline for 12 hours, and it was found stable at 25 ºC for 12 hours (RCP>95%). This study showed the basic principle for radiolabeling PLGA NPs, which are frequently researched, and proved that they can radiolabeled with Tc-99m with high efficiency and high stability. It can be evaluated as the first step to prepare more specific Tc-99m labeled PLGA nanoparticles for targeted treatment or diagnosis. Further information is required to design more specific imaging agents.

4. MATERIALS AND METHODS

4.1. Materials

PLGA polymer (50:50, MW:24.000-38.000), polyvinyl alcohol (PVA) (MW:30.000-70.000 Da) and all other solutions were purchased from Sigma-Aldrich, Inc (Missouri, USA), unlike otherwise mentioned. Nano Zs Zeta sizer (Malvern Instruments, UK) was used for characterization studies of NPs. Tc-99m was obtained from Hacettepe University Hospital, Nuclear Medicine Department.

4.2. Preparation of NPs

PLGA NPs were prepared using the nanoprecipitation method [16]. Briefly, PLGA (10 mg) was dissolved in 2 ml acetone (organic phase) and added drop by drop to the aqueous phase under magnetic stirring to form NPs. In this study, the aqueous phase was prepared with 0.5% PVA in 10 ml water. The

suspension was stirred at 1000 rpm to evaporate the acetone overnight, which resulted in the formation of NPs (Figure 4).

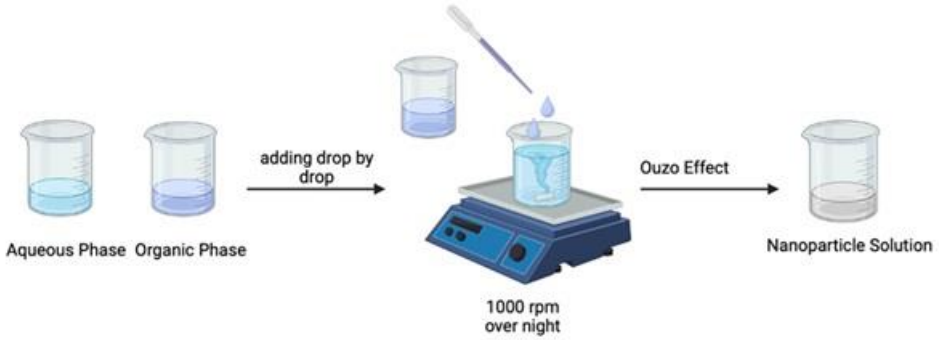

Figure 4. Preparation of PLGA NPs by nanoprecipitation method (Created in Biorender. Sarcan, E. (2025) https://Biorender.com/jfc1fj4).

4.3. Characterization of NPs

Particle size, PDI and zeta potential were measured by DLS with Malvern Nano Zs Zetasizer (Malvern Instruments, UK) at room temperature. NP solution and measured at room temperature (n:3).

The morphology of NPs was examined with scanning electron microscopy (SEM). Certain amounts of dried NP powders were mounted onto the grid and coated with gold, and images were taken at magnifications of x100.000 and x10.000. Analyses were performed by SEM device (QUANTA 400 F Field Emission SEM, Taiwan) at ODTU METU Central Laboratory.

4.4. Radiolabeling Studies

PLGA NPs were radiolabeled with Tc-99m (0.25 mCi) by the direct reduction technique with $SnCl_2$ [9]. This method directly uses a reducing agent (usually an acidic $SnCl_2$ solution). This reducing agent reduces the heptavalent oxidation state to lower oxidation states, creating a metastable species that facilitates the direct binding of radioactive material to the surface of NPs. In the direct reduction technique, no chelators or spacers are required, as in indirect radiolabelling, or they are not trapped inside the nanoparticle by different methods during or after the synthesis of the NPs, as in the encapsulation method [17]. Different formulations were developed by changing the $SnCl_2$ amount, pH, incubation temperature, and incubation time parameters (Table 2).

Briefly, different amounts of $SnCl_2$ (2 mg/ml) were added to 0.5 ml of PLGA NP solutions and the pH of the solution was checked, mixed by adding an acetic acid solution or sodium bicarbonate solution dropwise to match the pH values (pH 5, 7, 9) specified in Table 2. Then, Tc-99m (0.25 mCi) was added to the solution and incubated at different temperatures and different times.

Table 2. Different radiolabeling conditions of NP formulations.

Formulation Code	$SnCl_2$ Amount (µl)	pH	Incubation Temperature (°C)	Incubation Time (min)
Np1	10	7	25	30
Np2	50	7	25	30
Np3	250	7	25	30
Np4	50	5	25	30
Np5	50	9	25	30
Np6	50	7	25	5
Np7	50	7	25	60
Np8	50	7	10	30
Np9	50	7	50	30

4.4.1. Determination of the labelling efficiency of NPs

The labelling efficiency of formulations was determined by instant thin-layer chromatography (ITLC) using by gamma-counter (Berthold-LB 2111). Tc-99m radiolabeled NP suspensions were applied to the ITLC-SG and placed into the mobile phase. Free Tc-99m and hydrolyzed Tc-99m were determined by using acetone and saline mobile phase (Eq.1-Eq.3). RCP % of Tc-99m radiolabeled NPs were determined at different intervals (free Tc-99m and hydrolyzed Tc-99m) to evaluate the effects of various parameters [18].

RCP (%)= 100 -[(Free Tc-99m)+ (Hydrolyzed Tc-99m)] **(Eq. 1)**

Free Tc-99m (%) = ((Rf (9.0-10.0))/(Total Activity))× 100 **(Eq. 2)**

Hydrolyzed Tc-99m (%) = ((Rf (0.0-0.1))/(Total Activity)) x 100 **(Eq. 3)**

Parameters such as reducing agent ($SnCl_2$), incubation pH, incubation time and incubation temperature were evaluated and compared over 6 hours.

4.5. Stability Tests

The stability of the optimum Tc-99m labelled PLGA NP formulation was determined using ITLC. Stability tests were carried out in 1 ml saline with 10 MBq NP solutions at room temperature (25°C) and 37°C. Samples were taken at regular intervals up to 12 hours and analyzed by ITLC. Papers were counted in gamma-ray spectrometry, and the labeling efficiencies were calculated [15]. Calculations were made as described in 4.4.1.

4.6. Statistical Analysis

The statistical analysis was performed to compare the differences between the data groups by using Student's T test and ANOVA. $P < 0.05$ was evaluated as statistically significant. All analyses were evaluated using Prism 7 software (Graph-pad, San Diego, USA), and graphs were created using Prism 7 software (Graph-pad, San Diego, USA).

Acknowledgements: The authors would like to thank Hacettepe University Hospital, Nuclear Medicine Department for Technetium-99m supply.

Author contributions: Conception – E.T.S., H.B., M.S.G., S.E..; Design – E.T.S.; Supervision – E.T.S., H.B., M.S.G., S.E..; Resources – E.T.S., H.B., M.S.G., S.E..; Materials – E.T.S., H.B..; Data Collection and/or Processing – E.T.S., H.B.; Analysis and/or Interpretation –E.T.S., H.B.; Literature Search – E.T.S., H.B.; Writing – E.T.S, H.B.; Critical Reviews – E.T.S., H.B., M.S.G., S.E.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Varani M, Campagna G, Bentivoglio V, Serafinelli M, Martini ML, Galli F, Signore A. Synthesis and biodistribution of 99mTc-Labeled PLGA nanoparticles by microfluidic technique. Pharmaceutics. 2021;13(11):1769. https://doi.org/10.3390/pharmaceutics13111769

[2] Chiu HI, Samad NA, Fang L, Lim V. Cytotoxicity of targeted PLGA nanoparticles: A systematic review. RSC Adv. 2021; 11(16): 9433. https://doi.org/10.1039/D1RA00074H

[3] Schoubben A, Ricci M, Giovagnoli S. Meeting the unmet: From traditional to cutting-edge techniques for poly lactide and poly lactide-co-glycolide microparticle manufacturing. J Pharm Investig. 2019; 49: 381-404. https://doi.org/10.1007/s40005-019-00446-y

[4] Prabhu R, Patravale V, Joshi MD. Polymeric nanoparticles for targeted treatment in oncology: Current insights. Int J Nanomed. 2015; 10: 1001-1018. https://doi.org/10.2147/IJN.S56932

[5] Zhang X, Yan R, Wei Z, Yang D, Hu Z, Zhang Y, Huang X, Huang H, Wang W. Folate decorated multifunctional biodegradable nanoparticles for gastric carcinoma active targeting theranostics. Int J Nanomed. 2022; 12: 2493-2502. https://doi.org/10.2147/IJN.S348380

[6] Hong H, Zhang Y, Sun J, Cai W. Molecular imaging and therapy of cancer with radiolabelled nanoparticles. Nano Today. 2009; 4(5): 399-413. https://doi.org/10.1016/j.nantod.2009.07.001

[7] Loudos G, Kagadis GC, Psimadas D. Current status and future perspectives of in vivo small animal imaging using radiolabelled nanoparticles. Eur J Radiol. 2011; 78(2): 287-295. https://doi.org/10.1016/j.ejrad.2010.06.025

[8] Chakravarty R, Goel S, Dash A, Cai W. Radiolabeled inorganic nanoparticles for positron emission tomography imaging of cancer: an overview. Q J Nucl Med Mol Imaging. 2017; 61(2): 181-204. https://doi.org/10.23736/S1824-4785.17.02969-7

[9] Snehalatha M, Venugopal K, Saha RN. Etoposide loaded PLGA and PCL nanoparticles II: Biodistribution and pharmacokinetics after radiolabelling with Tc-99m. Drug Deliv. 2008; 15(5): 277-287. https://doi.org/10.1080/10717540802006500

[10] Arulsudar N, Subramanian N, Mishra P, Sharma RK, Murthy RS. Preparation, characterization and biodistribution of 99mTc-labeled liposome encapsulated cyclosporine. J Drug Target. 2003; 11(3): 187-196. https://doi.org/10.1080/10611860310001615415

[11] Oda CMR, Fernandes RS, de Araujo Lopes SC, de Oliveria MC, Cardoso VN, Santos DM, de Castro Pimenta AM, Malachias A, Paniago R, Townsdend DM, Colletti PM, Rubello Di Alves RJ, de Barros ALB, Leite EA. Synthesis, characterization and radiolabelling of polymeric nano-micelles as a platform for tumor delivering. Biomed Pharmacother. 2017; 89: 268-275. https://doi.org/10.1016/j.biopha.2017.01.144

[12] He Z, Zhang X, Huang J, Wu Y, Huang X, Chen J, Xia J, Jiang, H, Ma J, Wu J. Immune activity and biodistribution of polypeptide K237 and folic acid conjugated amphiphilic PEG-PLGA copolymer nanoparticles radiolabeled with Tc-99m. Oncotarget. 2016; 7(47): 76635-76646. https://doi.org/10.18632/oncotarget.12850

[13] Sarcan ET, Silindir Gunay M, Ozer AY. Theranostic polymeric nanoparticles for NIR imaging and photodynamic therapy. Int J Pharm. 2018; 551(1-2): 329-338. https://doi.org/10.1016/j.ijpharm.2018.09.019

[14] Halder KK, Mandal B, Debneth MC, Bera H, Ghosh LK, Gupta BK. Chloramphenicol-incorporated poly lactide-co-glycolide (PLGA) nanoparticles: Formulation, characterization, technetium-99m labeling and biodistribution studies. J Drug Target. 2008; 16(4): 311-320. https://doi.org/10.1080/10611860801899300

[15] Baishya R, Nayak DK, Kumar D, Sinha S, Gupta A, Ganguly S, Debnath MC. Ursolic acid loaded PLGA nanoparticles: In vitro and in vivo evaluation to explore tumor targeting ability on B16F10 melanoma cell lines. Pharm Res. 2016; 33(11): 2691-2703. https://doi.org/10.1007/s11095-016-1994-1

[16] Fessi H, Puisieux F, Devissaguet JPH, Ammoury N, Benita S. Nanocapsule formation by interfacial polimer deposition following solvent displacement. Int J Pharm. 1989; 55(1): R1-R4. https://doi.org/10.1016/0378-5173(89)90281-0

[17] Varani M, Bentivoglio V, Lauri C, Ranieri D, Signore A. Methods for Radiolabelling Nanoparticles: SPECT Use (Part 1). Biomolecules. 2022; 12(10): 1522. https://doi.org/10.3390/biom12101522

[18] Saha GB. Quality control of radiopharmaceuticals. In: Saha GB (Ed). Fundamentals of Nuclear Pharmacy. Springer, 2018, pp. 163-183.