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Development of a Ready-to-Use PSMA-11 Kit Formulation and Biological Evaluation of Binding Affinity

Maria Renata Valente Brandão Freire[1*], Natália Marchesan Bexiga[2], Marco Antonio Stephano[2], João Ezequiel Oliveira[3], Carlos Roberto Jorge Soares[3], Ana Cláudia Camargo Miranda[4], Anna Carolina de Alcântara Barbosa[4], Marycel Figols de Barboza[4], Laura Fernanda Garcia[3], Luiz Felipe S. Teixeira[3], Maria Helena Marumo Bellini[3], Natanael Gomes da Silva[1], Luiza Mascarenhas Balieiro[1], Joel Mendes[1], Elaine Bortoleti de Araújo[1]

[1]Centro de Radiofarmácia, Instituto de Pesquisas Energéticas e Nucleares (IPEN), Cidade Universitária, São Paulo, Brasil
[2]Departamento de Tecnologia Farmacêutica, Faculdade de Ciências Farmacêuticas (USP), Cidade Universitária, São Paulo, Brasil
[3]Centro de Biotecnologia, Instituto de Pesquisas Energéticas e Nucleares (IPEN), Cidade Universitária, São Paulo, Brasil
[4]Departamento de Medicina Nuclear do Hospital Israelita Albert Einstein, São Paulo/SP, Brasil
Email: *renatabrd@gmail.com

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Abstract

Introduction: ^{68}Ga-PSMA-11 is considered the gold standard in detection of micro and oligometastases in advanced prostate cancer, being used for therapeutic planning, as well as, potentially, for evaluating response to treatment. The development of ready-to-use lyophilized kit of PSMA-11 adds quality and safety to the routine use of this radiopharmaceutical and represents a pharmacotechnical challenge as it must preserve the integrity and specificity of the ligand. **Methods:** PSMA-11 kit formulation was proposed, considering radiolabeling parameters and the preservation of the peptide during the lyophilization process, using mannitol as an excipient. Critical temperature characterization studies were carried out using DSC equipment and the freeze-drying process was developed. The direct radiolabeling conditions were evaluated and standardized using ^{68}Ge/^{68}Ga generator eluate from two different manufacturers (ITG and Eckert & Ziegler). The radiochemical purity was evaluated by TLC and HPLC. Biological evaluation was carried out with lyophilized PSMA-11 to demonstrate the integrity of the peptide and preservation of biological activity after the lyophilization process. **Results:** Based on critical temperature characterization studies, the freeze-drying cycle was designed to reach a freezing temperature of around −40˚C and primary drying at 2˚C. Using 20 mg of mannitol, an intact and elegant lyophilized cake was obtained. PSMA-11 lyophilized kit was directly labeled with ^{68}Ga eluate from ^{68}Ge/^{68}Ga GMP generators (ITG and Eckert & Ziegler) resulting

in % RP > 95% at pH 4.0 to 4.5. The results obtained from *in vitro* and *in vivo* biological competition studies confirmed the preservation of PSMA-11 affinity for the receptor after lyophilization. **Conclusion:** A lyophilized formulation (Kit) of PSMA-11 was successfully obtained, which preserved the integrity and biological activity of the peptide and guaranteed radiolabeling efficiency.

Keywords

^{68}Ga-PSMA-11, Prostate-Specific Membrane Antigen (PSMA), Ready-to-Use Kit, GMP-Grade Generators, Metastatic Prostate Cancer, PET Imaging

1. Introduction

According to a study published by The Lancet's scientific commission, the aging population is expected to drive the incidence of prostate cancer worldwide in the coming decades, and the trend is that the rate of disease growth will be higher in low- and middle-income countries [1]. Currently, in the detection of metastatic prostate cancer, molecules targeting the transmembrane receptor type II, called "PSMA" due to the receptor they bind to, prove to be a superior alternative to other conventional imaging modalities and other PET imaging agents like ^{18}F-FDG or ^{18}F-Choline [2] [3]. High levels of PSMA expression are found in metastatic and hormone-resistant prostate cancers, indicating that PSMA expression is correlated with disease progression, making PSMA an ideal target for both diagnosis and treatment of this condition [4].

In 2020, ^{68}Ga-PSMA-11 was approved in the United States by the Food and Drug Administration (FDA) for the PET imaging of PSMA-positive prostate cancer whose specificity and efficiency allow for appropriate therapeutic planning and monitoring of treatment effectiveness [5] [6]. The PSMA-11 molecule consists of the hydrophilic pharmacophoric group Glu-NH-CO-NH-Lys combined with the efficient acyclic Ga (III) chelator HBED-CC [7]. The acyclic radiometal chelator HBED-CC represents a highly effective complexing agent for ^{68}Ga, particularly for radio-labeling at room temperature, a fact that makes it highly convenient for preparation in the form of a ready-to-use lyophilized kit [8].

Gallium-68 labeled radiopharmaceuticals such as ^{68}Ga-PSMA-11 can be also prepared by automated synthesis modules, in a robust and reproducible way, while reducing operator exposure to radiation. However, synthesis modules require high installation costs for hot labs to meet radiological protection requirements and good manufacturing practices (GMP) [9]. The development of lyophilized kits of receptor-specific peptides in individual vials for direct labeling with ^{68}Ga is an economically viable alternative to automated module-based production [10]. However, there are few PSMA-11 kits approved by regulatory agencies commercially available in the world. The Illumet™ kit developed by the

company ANMI (Belgium) consists of 2 vials, one containing the lyophilized peptide, and a second sterile vial containing sodium acetate [11]. The IsoPRO-Trace-11® kit developed by Isotopia Molecular Imaging company (Israel) consists of a single vial containing the lyophilized peptide [12]. A commercially approved kit recommends the use of a pH adjustment solution, which indicates that the employed concentration of sodium acetate has not been optimized or formulated as a buffer to allow the use of HCl solutions with different concentrations. Another issue is that research groups do not usually publish detailed information on the development of the ready-to-use PSMA-11 kit formulation and the lyophilization cycle. Thus, the development of ready-to-use lyophilized PSMA-11 formulations remains an ongoing area of research for the expansion of PET technology in screening patients with metastatic prostate cancer.

The preparation of ^{68}Ga-PSMA-11 via lyophilized kit has a strong dependence on a high-quality generator (GMP grade) [13]. The commercial generators Eckert & Ziegler GalliaPharm® and ITG Isotope Technologies Garching are both GMP-grade. The GalliaPharm® generator has a type II drug master file with the FDA, and ITG features a unique characteristic in the market regarding the reduction of ^{68}Ge breakthrough with usage [14]. The characteristics of eluates from different commercial generators vary, particularly in relation to the concentration of the eluting acid solution, a fact that directly interferes with the pH of radiolabeling and must be strongly considered in the development of a ready-to-use kit for labeling PSMA-11 with ^{68}Ga. Lyophilized kits for radiolabeling with ^{68}Ga should contain, in addition to the lyophilized precursor, components such as a suitable buffer and stabilizing agents to ensure radiochemical purity and stability of the desired product [15]. It is also important to emphasize that for any lyophilized formulation, the minimum number of components necessary to confer stability to the peptide and the cake structure should be used. A bulking agent is desired to provide volume and form an elegant cake with good mechanical properties [16]. Additionally, a bulking agent is important in cases where very small amounts of the active ingredient are used, to prevent mass loss during the freeze-drying process [17] [18].

To design an ideal lyophilization process, it is necessary to understand the critical properties of the formulation and how to apply this information to the cycle. The critical properties of the formulation include the glass transition temperature ($T_g^{'}$) in the frozen state and the eutectic temperature (T_{eu}). Typically, thermal analysis techniques such as differential scanning calorimetry (DSC) are employed to evaluate the thermal stability of the protein and to improve lyophilization conditions [19]. Keeping the formulation below the $T_g^{'}$ during freezing and lyophilization can preserve the structure and activity of the product [20]. The goal of this study was to develop a ready-to-use kit for the preparation of ^{68}Ga-PSMA-11 for commercialization in Brazil. As mentioned, a domestic PSMA-11 kit represents an economically viable alternative to automated production for nuclear medicine centers, and it will enable the expansion of PET-based prostate lesion detection applications in Brazil. The robust PSMA-11

kit described here was optimized for parameters like buffer content, pH conditions, temperature, and bulking agent content. It was demonstrated to effectively prepare the radiopharmaceutical using Ga-68 from two different commercial generators: Eckert & Ziegler (Germany), and Isotope Technologies, ITG (Germany). Kit-formulated ^{68}Ga-PSMA-11 was evaluated in biological assays to demonstrate the integrity and stability of the PSMA-11 peptide after the freeze-drying process.

2. Materials and Methods

2.1. Chemicals

The Precursor peptides for labeling with ^{68}Ga (PSMA-11) and ^{177}Lu (PSMA-I&T) were purchased from ABX (Advanced Biochemical Compounds, Germany) and CMR (Center of Molecular Research, Russia), respectively. A 37% solution of Suprapur® hydrochloric acid was purchased from Merck, Germany, with purity of 99.9%. Sodium acetate was purchased from Merck, Germany. All other reagents and solvents were of analytical grade and were purchased from Sigma-Aldrich, USA. All materials and media for cell culture, including RPMI (Roswell Park Memorial Institute medium), were purchased from Gibco (USA). Isoflurane was purchased from Cristália Laboratory, Brazil, and Matrigel® from Corning®, USA.

2.2. ^{68}Ge/^{68}Ga Generator

The $^{68}GaCl_3$ eluate was obtained from GMP-grade generators provided by two different manufacturers: Isotopen Technologies Garching (ITG) and Eckert & Ziegler (both based in Germany).

The ^{68}Ge/^{68}Ga generator from ITG was eluted with 4 mL of sterile 0.05 M hydrochloric acid solution and the ^{68}Ge/^{68}Ga generator from Eckert & Ziegler was eluted with 7 mL of sterile 0.1 M hydrochloric acid solution. The hydrochloric acid solution was prepared using 37% ultrapure HCl (99.99%, Merck, Germany) and water for injection as sterile solution. The eluate from both generators was analyzed and met the quality criteria specified by the manufacturers.

$^{177}LuCl_3$ was obtained from INM (Russia) with a specific activity of around 1332 GBq/mg.

3. Experimental

3.1. Kit Formulation

Pre-formulation experiments were conducted to select sodium acetate buffer concentration, pH, and volume to achieve the desired pH range (4.0 - 5.0) for the optimal complexation of ^{68}Ga after the addition of eluates from two ^{68}Ge/^{68}Ga generators (GMP grade, ITG and Eckert & Ziegler) [21] [22] [23]. The maintenance of the desired pH range (4.0 - 5.0) of the buffer after lyophilization was evaluated following lyophilization of the vials by adding an acid-base indicator (Congo Red). The amount of mannitol to be used in the formulation was

also evaluated in the freeze-drying process. Labeling experiments were conducted using the intended formulation with the aim of optimizing the reaction pH, assessing the radiolabeling temperature, and investigating the influence of adding two different concentrations of mannitol to the formulation.

3.2. Thermal Analysis and Lyophilization Cycle Design

The thermal analysis of the formulation containing mannitol and the PSMA-11 peptide was conducted using a differential scanning calorimeter (DSC 4000 Perkin Elmer, USA). The primary objective of this analysis was to investigate the thermal properties of the formulation, including phase transitions and thermal behavior during cooling and heating. Initially, a 30 µL sample of the formulation was carefully pipetted into a hermetically sealed aluminum container. The analyses were conducted using a cooling program, starting from room temperature to −60˚C at a rate of 5˚C/minute, followed by heating to 20˚C at a rate of 10˚C /minute, with the insertion of hold times to stabilize the temperature at specific points.

The results obtained from the thermal analysis were used to guide the lyophilization process designed to ensure the stability and integrity of the final product. The lyophilization test cycle took place in a benchtop lyophilizer model Advantage Pro with Intellitronics controller from SP Scientific, Pennsylvania (USA).

3.3. PSMA-11 Kit Radiolabeling with ^{68}Ga

The PSMA-11 kit was radiolabeled by adding 1 mL of sodium acetate buffer (1.5 M, pH 5.0) and 4 mL of Ga-68 eluted in 0.05 M HCl from the ITG generator (~223.8 MBq) or 3.5 mL of $^{68}GaCl_3$ eluted in 0.1M HCl from Eckert & Ziegler generator, at room temperature for 10 minutes.

3.4. PSMA-I&T Radiolabeling with ^{177}Lu

The radiopharmaceutical ^{177}Lu-PSMA-I&T for in vivo studies was prepared by incubating 20 µg (13.3 nmols) of PSMA-I&T with 429.2 MBq of $^{177}LuCl_3$ at 95˚C and 450 rpm for 30 minutes in a 0.52 M sodium ascorbate buffer (200 µL, pH 4.7).

3.5. Quality Control

Two chromatographic systems determined the radiochemical purity of PSMA-11 kit with Ga-68. High Pressure Liquid Chromatography (radio-HPLC) (Shimadzu, Japan) analysis was conducted using a C-18 column (Waters model Symmetry® C18, 5 µm, 250 mm × 4.6 mm), eluted at a flow rate of 0.6 mL/min by (A) water 0.1% TFA and (B) acetonitrile 0.1% TFA, in the following gradient mode: 0 - 0.5 min 5% B; 0.5 - 10.0 min 40% B; 10.0 - 16.0 min 5% B. Thin Layer Chromatography (TLC-SG) was performed on silica gel 60 plate (Merck, Germany) and ammonium acetate 77 g/L: methanol (1:1) RF, ^{68}Ga-colloidal = 0.0 - 0.1; RF, ^{68}Ga-peptide = 0.7 - 1.0. The TLC-SG strips were quantified with a radio

TLC-scanner (AR-2000 Eckert & Ziegler Radiopharma Inc., USA) [24].

The HPLC analysis method for determining the radiochemical purity of ^{177}Lu-PSMA-I&T was conducted under the following conditions: Symmetry® C18 column, 5 µm, Waters 250 × 4.6 mm; mobile phase: (A) water/0.1% TFA and (B) Acetonitrile/0.1% TFA; gradient 10% to 45% of B over 20 minutes at a flow rate of 1 mL/minute [25].

3.6. Biological Evaluation

The *in vitro* and *in vivo* competition assays were performed with the radiotracer ^{177}Lu-PSMA-I&T and lyophilized PSMA-11 (employed as a competitor). The cell line used was human androgen-dependent prostate carcinoma (LNCaP-Lymph Node Carcinoma of the Prostate) PSMA-positive (ATCC® CRL-1740™). LNCaP cells were cultured in RPMI 1640 medium (Life Technologies, MD, USA) supplemented with 10% Fetal Bovine Serum (FBS), 300 µg/mL streptomycin and 100 U/mL penicillin. Cultures were maintained at 37˚C under an atmosphere of 5% CO_2/humidified air. One day before the experiments, cells were harvested using Trypsin/EDTA (0.05% and 0.02%), centrifuged and suspended in culture medium, counted and 2×10^5 LNCaP cells were seeded per well in 6-well plates. The plates were maintained at 37˚C under a humidified atmosphere of 5% CO_2/air in an incubator.

In vivo competition study was performed in SCID-BALB/c mice with LNCap cell prostate tumor model. To induce tumor growth, LNCaP cells (5×10^6 cells/100 µL) were suspended in PBS supplemented with 20% Matrigel (Corning® Matrigel®, USA) and inoculated subcutaneously into the dorsal area of SCID mice (4 - 5 weeks old; IPEN Animal Facility). The mice were previously anesthetized with isoflurane.

3.6.1. *In Vitro* Competition Assay

In the competition experiments, the final concentration of the ^{177}Lu-PSMA-I&T radiotracer in each tube was 5 nM/mL, and the concentration of PSMA-11 competitor was 75 nM/mL, in RPMI medium supplemented with 10% FBS [26]. The PSMA-11 was reconstituted from a lyophilized vial obtained with the parameters developed in the present study, aiming to assess the effect of freeze-drying on the ability of the peptide to recognize the PSMA receptor. The experiments were performed in triplicate as two independent experiments. LNCaP cells (2×10^5 per well) were incubated with the radiotracer ^{177}Lu-PSMA-I&T with and without the PSMA-11 competitor at 4˚C for 60 minutes. The incubation was stopped by removing the radiotracer and competitor and washing the wells with 1 mL of PBS. The cells were then lysed using 1 mL of 1M NaOH solution. The lysate was transferred to counting tubes. The quantification of radioactivity in CPM was performed using an automatic gamma counter with NaI (TI) crystals (D5002 Cobra II, Packard Canberra), and the values were converted to fmol of ^{177}Lu-PSMA-I&T. Finally, the data were analyzed using GraphPad Prism software (version 5.00®). Quantitative data were expressed as mean ± standard devi-

ation. Significant difference was assessed using the unpaired *t*-test (two-tailed) with Welch's correction. P values below 0.05 were considered statistically significant.

3.6.2. *In Vivo* Competition Assay

The *in vivo* competition study was conducted by injecting the radiotracer ^{177}Lu-PSMA-I&T into two groups of tumor-bearing mice (n = 5 for each group), one of the groups receiving an excess of PSMA-11 (100 μg/animal), prepared by reconstituting lyophilized PSMA-11 with 0.9% sodium chloride solution, 30 minutes before the administration of the ^{177}Lu-PSMA-I&T. After 60 minutes of the radiopharmaceutical administration, the animals were euthanized, and samples of blood and organs of interest were collected as well as the tumor. The percentages of administered activity per gram of organ or tumor were determined relative to a standard of administered dose using an automatic gamma counter with NaI (TI) crystals (D5002 Cobra II, Packard Canberra). Quantitative data are expressed as mean ± standard deviation. Significant differences were assessed using two-way ANOVA followed by Bonferroni post-test for *in vivo* statistical analysis. P values below 0.05 were considered statistically significant.

3.7. Statistical Analysis

All graphs and statistical analyses were performed using GraphPad Prism software (version 5.00®). Quantitative data are expressed as mean ± standard deviation. The tools used to analyze significant differences were described for each competition assay. P values below 0.05 were considered statistically significant.

4. Results

4.1. Kit Formulation

A volume of 1 mL of sodium acetate buffer at a concentration of 1.5 M and pH 5.0 maintained the desired pH between 4 and 5 when adding volumes of 4 mL and 3.5 mL of 0.05 M HCl and 0.1 M HCl, respectively. The peptide should be lyophilized without the presence of the labeling buffer. The high concentration of salts in the labeling buffer resulted in the collapse of the cakes in initial lyophilization cycle tests. Furthermore, buffer freeze-drying tests employing Red of Congo indicator revealed the change in buffer pH during the freeze-drying cycle. In this way, the formulation of the PSMA-11 kit was developed in two vials: vial 1, containing the peptide (20 μg) and the bulking agent mannitol (20 mg), in lyophilized form and vial 2, containing the radiolabeling sodium acetate buffer solution at pH 5.0.

Studies on variation of mannitol mass made it possible to conclude that the use of 20 mg of mannitol instead 5 mg per vial resulted in an elegant and consistent lyophilized cake.

4.2. Thermal Analysis and Lyophilization Cycle Design

The thermal analysis of the formulation containing the PSMA-11 peptide and

mannitol showed a glass transition temperature ($T_g^{'}$) of −42.90˚C and a eutectic temperature (T_{eu}) of 5.10˚C (data shown in "Figure 1").

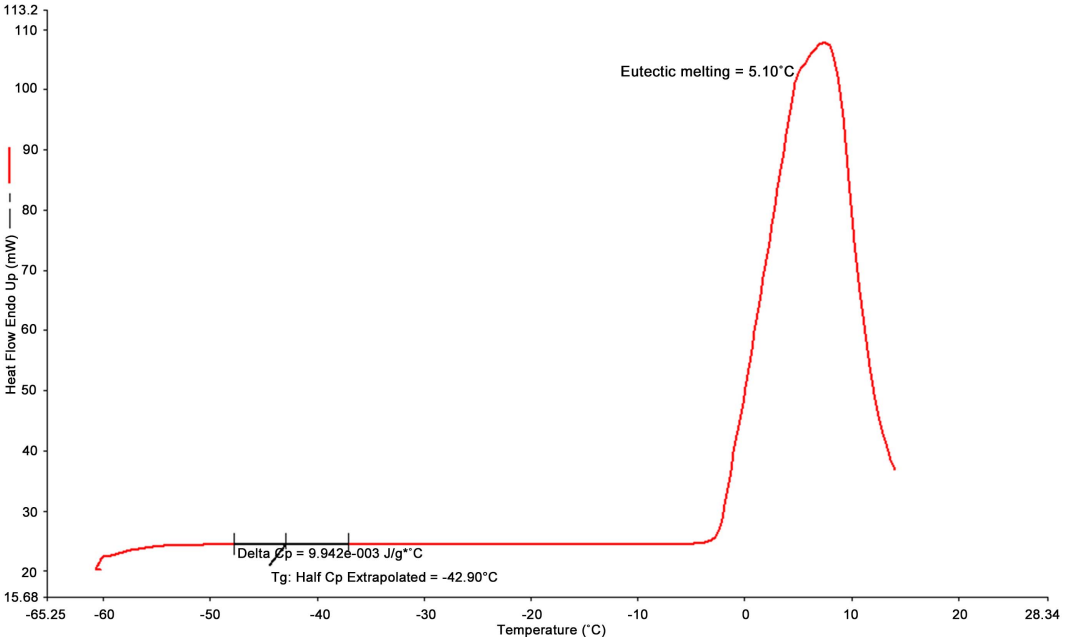

Figure 1. DSC curve obtained for the PSMA-11 with mannitol formulation. The sample was cooled to −60˚C at a rate of 5˚C /minute and then heated to 20˚C at a rate of 10˚C/minute. Critical temperatures can be observed: eutectic melting = 5.10˚C and $T_g^{'}$ = −42.90˚C.

Based on the critical temperature characterization studies, the freezing temperature of the lyophilization cycle was defined and primary drying was conducted at 2˚C. A freezing rate of 0.5˚C/minute was utilized, along with a holding time before the freezing step, to form larger pores and crystals, aiming to facilitate sublimation.

4.3. PSMA-11 Kit Radiolabeling with ^{68}Ga

The direct labeling of the ready-to-use PSMA-11 kit with the $^{68}GaCl_3$ eluted from the ITG generator was evaluated by HPLC immediately after lyophilization and after 40 days of kit storage at −20˚C. The radiolabeling took place at room temperature for 10 minutes and the final pH of the reaction mixture was 4.5. The radiochemical purity was > 95% at HPLC analyses "Figure 2".

The radiolabeling of ready-to-use PSMA-11 kit with gallium-68 obtained from Eckert & Ziegler generator, was evaluated by radio-TLC scanner, after approximately 45 days of storage at −20˚C. All labelings were performed at room temperature for 10 minutes and the final pH of the reaction mixture was 4.0 in this case. The radiochemical purity was > 95% evaluated by TLC "Table 1".

4.4. PSMA-I&T Radiolabeling with ^{177}Lu

Radiolabeling of PSMA-I&T with lutetium-177 resulted in radiochemical purity > 95% without purification. The SA was 718.9 GBq/mg. The retention time

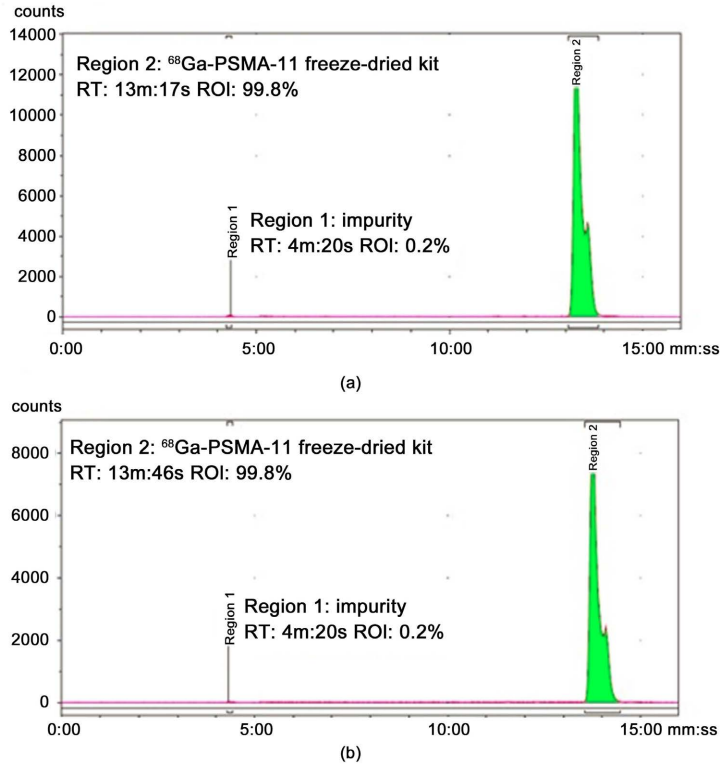

Figure 2. Chromatograms representative of ready-to-use PSMA-11 kit radiolabeled with Ga-68. (a) Analysis conducted immediately after lyophilization. (b) After 40 days of storage at −20˚C. Waters C18 column; mobile phase (a): water (0.1% TFA), (b): acetonitrile (0.1% TFA). Relative areas of ^{68}Ga-PSMA-11 and impurities are reported within the figure as percentages of the total areas (ROI: %).

Table 1. Radiochemical purity of the lyophilized PSMA-11 kits labeling with $^{68}GaCl_3$ solution from the Eckert & Ziegler generator. Analysis by radio-TLC scanner and ammonium acetate/methanol 1:1. (n = 2).

PSMA-11 precursor	Radiochemical purity and final pH		
	Final formulation pH	TLC (%)	Acceptance criteria: TLC* (%)
Lyophilized kit [1]	4.0	99.9 ± 0.1	≥95.0

[1] Lyophilized kit—PSMA-11/ mannitol; *TLC—Thin layer chromatography.

was similar to the cold peptide determined by HPLC with identification of a single peak corresponding to the product of interest.

4.5. Biological Evaluation

4.5.1. *In Vitro* Competition Assay

The integrity and preservation of PSMA-11 affinity post-lyophilization were evaluated in a competitive binding assay with the human prostate carcinoma cell line LNCaP-PSMA + (2×10^5 cells/well), 1-hour incubation at 4˚C with ^{177}Lu-PSMA-I&T (5 nM) as the radioligand and lyophilized, unlabeled PSMA-11

(75 nM) as the competitor. The experiment demonstrated the binding of ^{177}Lu-PSMA-I&T, being 1712.08 ± 63.03 fmol without competitor and 1010.54 ± 243.49 fmol with competitor "Figure 3".

A significant difference was observed between the uptake of ^{177}Lu-PSMA-I&T without the competitor and with the competitor (P = 0.0320, P < 0.05), which confirms the preservation of PSMA-11 affinity for the receptor after one month of lyophilization procedure.

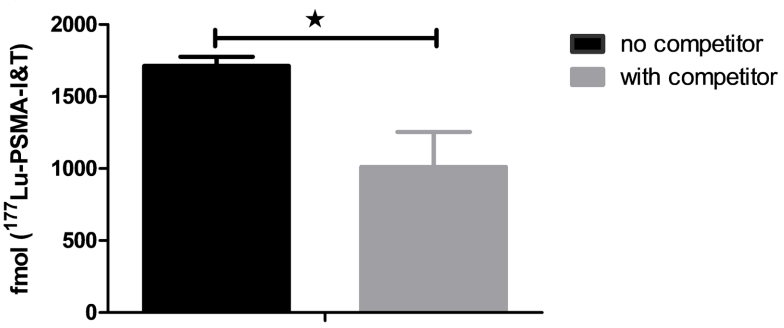

Figure 3. Competition assay in LNCaP-PSMA+ cells, employing ^{177}Lu-PSMA-I&T (5 nM) as the ligand and PSMA-11 (75 nM) as competitor, RPMI 1640 medium + 10% fetal bovine serum, 1-hour incubation at 4˚C. Data are expressed as mean ± standard deviation (n = 3 for each condition).

4.5.2. *In Vivo* Competition Assay

In the *in vivo* competition experiment using ^{177}Lu-PSMA-I&T and lyophilized PSMA-11 as competitor, a reduction in tumor uptake was observed from 2.18 ± 0.27 %ID/g to 0.62 ± 0.17 %ID/g, representing a decrease in the tumor uptake rate by 71.56% "Table 2".

Table 2. Biodistribution of ^{177}Lu-PSMA-I&T with and without administration of 100 μg/animal of reconstituted lyophilized PSMA-11 (competitor), 30 minutes prior to the radiotracer. Both groups of animals were euthanized 60 minutes post-injection. Data expressed as mean and standard deviation (n = 5).

Biological material	IA%/gram of organ or tissue or/mL of blood	
	60 minutes without competitor	60 minutes with competitor
Blood	0.69 ± 0.21	1.19 ± 0.38
Lungs	1.72 ± 0.37	2.12 ± 0.70
Spleen	0.89 ± 1.10	0.39 ± 0.09
Liver	0.87 ± 0.64	0.72 ± 0.20
Kidneys	86.60 ± 2.75	7.37 ± 0.91
Intestines	0.91 ± 0.79	0.90 ± 0.59
Muscle	0.96 ± 0.47	0.65 ± 0.42
Tumor	2.18 ± 0.27	0.62 ± 0.17

% IA/g = percentage of activity injected per gram or per mL of blood.

Additionally, renal uptake was strongly reduced by the presence of the lyophilized PSMA-11 competitor, with the reduction in renal uptake from 97.68 ± 19.24 %ID/g to 7.99 ± 1.59 %ID/g (***P ≤ 0.001) "Figure 4". Conversely, the percentage of the radiopharmaceutical in circulation increased in the blocking group, a fact that also reflects the competition established between the lutetium-177 radiopharmaceutical and the competitor (lyophilized PSMA-11) for PSMA receptors on tumor cells.

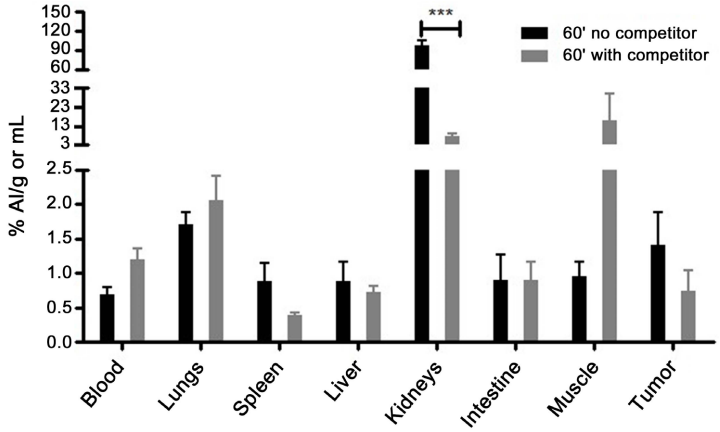

Figure 4. Biodistribution of ^{177}Lu-PSMA-I&T with and without administration of 100 μg/animal of reconstituted lyophilized PSMA-11 (competitor) 30 minutes prior to the radiotracer. Data expressed as mean and standard deviation (n = 5). Asterisks denote significance (***p ≤ 0.001).

5. Discussion

One of the main challenges in developing lyophilized formulations (kits) for labeling with $^{68}GaCl_3$ is related to the fact that generators with GMP-grade purity from different manufacturers diverge in terms of matrix composition, volume, and concentration of hydrochloric acid (HCl) solution used for generator elution. The $^{68}Ge/^{68}Ga$ ITG generator is eluted with a volume of 4 mL of 0.05 M HCl, while Eckert & Ziegler generator is eluted with 7 mL of 0.1 M HCl. In the formulation study, it was demonstrated that 1 mL of 1.5 M sodium acetate buffer (pH 5.0) maintained the desired pH between 4 to 5 for the evaluated eluate volumes from both commercial GMP-grade generators. This buffer volume can also be increased to consider larger volumes of eluate generators in radiolabeling procedures. PSMA-11 peptide should be lyophilized without the presence of the labeling buffer. The results of collapsed cake when the peptide was lyophilized in presence of the buffer were consistent with data found in the literature regarding the impact of lyophilization and extreme freezing on the pH of protein-buffered solutions, which can lead to protein denaturation with the possibility of generating irreversible aggregates [27].

Furthermore, buffer freeze-drying tests employing Red of Congo indicator revealed the change in buffer pH during the freeze-drying cycle. Therefore, in the proposed kit developed in this work, the buffer will be supplied in liquid

form, in an additional vial. Mannitol was proposed in the kit formulation as a volume agent, thereby providing substantial bulk to the formulation. In cases where concentrations of the active ingredient are very low, mannitol prevents the loss of peptide mass during the lyophilization process. Mannitol is also considered a good choice as a tonicity modifier in developing an isotonic formulation [16]. Crystalline volume agents like mannitol yield a visually appealing cake, intact, with uniform consistency and good mechanical properties [17].

Based on critical temperature characterization studies, the lyophilization cycle was designed to achieve a freezing temperature of around −40˚C and primary drying at 2˚C (3˚C below T_{eut}) [20] [28]. It has been demonstrated that ^{68}Ga-PSMA-11 can be obtained from the ready-to-use kit with radiochemical purity exceeding 95%, using the eluate of ^{68}Ga from the ITG or Eckert & Ziegler ^{68}Ge/^{68}Ga GMP generators without previous purification, at room temperature, with the final labeling pH 4.0 to 4.5. These results, enabled by the acyclic radiometal chelator HBED-CC, are in accordance with the concept of a ready-to-use labeling kit. The presence of peaks with "shoulders" in the HPLC profiles for the radiolabeling at room temperature is attributed to the presence of diastereoisomers in the reaction mixture and is consistent with data reported by other researchers [5] [6]. The formation of approximately 50% of the thermodynamically less stable diastereoisomer in the reaction mixture does not reduce specific cellular uptake of PSMA (binding), and thus, does not have any negative influence on the quality of PET images [5].

The presence of mannitol in the labeling medium did not interfere with the binding of Gallium-68 to PSMA-11, as evidenced by the HPLC profiles of the radiolabeling of the lyophilized formulation. Results from *in vitro* and *in vivo* biological competition studies confirmed the preservation of PSMA-11 affinity for the receptor after lyophilization, showing that the freeze-drying process evaluated preserved the integrity and biological properties of PSMA-11 peptide, ensuring radiolabeling efficiency.

6. Conclusion

A lyophilized ready-to-use PSMA-11 kit formulation was successfully obtained, preserving the peptide's integrity and ensuring high radiolabeling yield. The PSMA-11 kit, consisting of two vials (a lyophilized vial containing the peptide in mannitol and a vial with labeling buffer solution), can be directly radiolabeled with ^{68}Ga eluted from two different generators (ITG and Eckert & Ziegler). This represents a fast and easily prepared method for the radiopharmaceutical for clinical application. The use of mannitol allowed the creation of an elegant lyophilized cake, contributing to the preservation of peptide mass and integrity under lyophilization conditions.

Research Involving Animals

The male SCID-BALB/c mice (BALB/c immunodeficient mice resulting from

genetic modification), used in this study, aged four to five weeks, and weighing 18 to 25 grams, were provided by the animal facility at IPEN-CNEN/SP (São Paulo, SP, Brazil). All experiments were previously approved by the Research Ethics Committee of IPEN (Project No. 225/18/CEUA-IPEN/SP) and conducted in accordance with the standards established by the Brazilian Society of Science in Laboratory Animals (SBCAL) and the National Council for Animal Experimentation Control (CONCEA). The animals were housed in the animal facility under the following breeding conditions: room temperature (22˚C ± 2˚C), artificial lighting with a 12-hour light/12-hour dark cycle, and ad libitum access to food and water.

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Conflicts of Interest

The author declares no conflict of interest, financial or otherwise.

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List of Abbreviations

^{68}Ga—Gallium-68
^{68}Ge—Germanium-68
DSC—Differential scanning calorimetry
FDA—Food and drug administration
GBq—Giga-becquerel
GMP—Good manufacturing practice
HCl—Hydrochloric acid
PC—Prostate cancer
PET—Positron emission tomography
PSMA—Prostate-specific membrane antigen
SA—Specific activity
T_{eu}—Eutectic temperature
$T_g^{'}$—Glass transition temperature
TLC—Thin layer chromatography