The labeling of ^{99m}Tc-PSMA-HBED-CC for prostate cancer imaging

Miss Benchamat Phromphao

จุฬาลงกรณ์มหาวิทยาลัย
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การติดฉลากพีเอสเอ็มเอ เอชบีอีดีซีซี กับเทคนีเชียม-99 เอ็ม เพื่อใช้สำหรับถ่ายภาพวินิจฉัยโรคมะเร็งต่อมลูกหมาก

น.ส.เบญจมาศ พรหมเผ่า

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จากรายงานสถิติอุบัติการณ์โรคมะเร็งทั่วโลกเมื่อปี ค.ศ. 2018 พบมะเร็งต่อมลูกหมากมากเป็นอันดับที่ 2 และเป็นสาเหตุการเสียชีวิตสูงในอันดับที่ 5 (1) โดยทั่วไปการวินิจฉัยโรคมะเร็งต่อมลูกหมากทำได้หลายวิธี แต่วิธีที่มักจะนำมาใช้ในปัจจุบันได้แก่ การตรวจระดับพีเอสเอซึ่งมีความไวและความจำเพาะต่ำ และการตรวจชิ้นเนื้อซึ่งถือเป็นวิธีการที่ล่วงล้ำเข้าไปในร่างกายผู้ป่วยและทำให้เกิดบาดแผล ดังนั้นวิธีการวินิจฉัยโรคด้วยการถ่ายภาพในระดับโมเลกุลจึงได้เข้ามามีบทบาทสำคัญในการวินิจฉัยมะเร็งต่อมลูกหมากมากยิ่งขึ้น โดยอาศัยการจับของสารที่มีมวลโมเลกุลต่ำกับตัวรับพีเอสเอ็มเอที่อยู่บนเซลล์มะเร็งต่อมลูกหมาก สารดังกล่าวที่ได้รับการพัฒนาขึ้นมีหลายชนิด เช่น พีเอสเอ็มเอ-เอชบีอีดีซีซี, พีเอสเอ็มเอ-ไอแอนด์ที ซึ่งสามารถนำมาติดฉลากกับแกลเลียม-68 เพื่อใช้สำหรับวินิจฉัยโรคมะเร็งต่อมลูกหมากด้วยเครื่องเพทสแกน ถึงแม้ว่าการวินิจฉัยมะเร็งต่อมลูกหมากด้วยเครื่องเพทสแกนจะให้ภาพที่มีความคมชัดและให้รายละเอียดที่ดี แต่ก็ยังมีค่าใช้จ่ายในการตรวจและค่าสารเภสัชรังสีที่ค่อนข้างสูง ในขณะที่การตรวจวินิจฉัยมะเร็งต่อมลูกหมากด้วยเครื่องสเปคนั้นมีค่าใช้จ่ายที่ต่ำกว่า และสารเภสัชรังสีที่ใช้มีค่าครึ่งชีวิตที่ยาวกว่า ดังนั้นโอกาสการเข้าถึงการตรวจวินิจฉัยด้วยเครื่องเพทสแกนและจำนวนเครื่องเพทสแกนในโรงพยาบาลต่างๆ ที่มีไม่มากจึงเป็นข้อจำกัดสำหรับผู้ป่วยส่วนใหญ่ คณะผู้วิจัยจึงได้ทำการศึกษาและรายงานการติดฉลากสารเภสัชรังสีชนิดใหม่ที่มีชื่อว่า เทคนีเชียม99เอ็ม-พีเอสเอ็มเอ-เอชบีอีดีซีซี เพื่อใช้เป็นทางเลือกสำหรับการถ่ายภาพวินิจฉัยโรคมะเร็งต่อมลูกหมาก งานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาวิธีการติดฉลากเทคนีเชียม99เอ็ม-พีเอสเอ็มเอ-เอชบีอีดีซีซี ภายในหน่วยงาน เพื่อใช้สำหรับวินิจฉัยโรคมะเร็งต่อมลูกหมากด้วยเครื่องสเปค โดยวิธีการติดฉลากเทคนีเชียม99เอ็ม-พีเอสเอ็มเอ-เอชบีอีดีซีซี เริ่มจากการนำเทคนีเชียม-99เอ็ม ที่มีความแรงรังสี 370 เมกะเบคเคอเรล ผสมกับสารละลายพีเอสเอ็มเอ-เอชบีอีดีซีซี ปริมาณ 10 ไมโครกรัม และ 4% สแตนนัสคลอไรด์ไดไฮเดรต ในขวดปราศจากเชื้อขนาด 10 มิลลิลิตร นำไปให้ความร้อนที่อุณภูมิ 100 องศาเซลเซียส เป็นเวลา 15 นาที แล้ววางทิ้งไว้ให้เย็นลงจนถึงอุณหภูมิห้อง จากนั้นทำการทดสอบความบริสุทธิ์ของสารเภสัชรังสีด้วยวิธีโครมาโตกราฟี และตรวจหาค่าความเป็นกรดด่าง ผลการวิจัยพบว่าวิธีการติดฉลากสารเภสัชรังสีเทคนีเชียม99เอ็ม-พีเอสเอ็มเอ-เอชบีอีดีซีซี ที่คณะผู้วิจัยพัฒนาขึ้นมานี้สามารถเตรียมสารเภสัชรังสีได้ผลผลิตทางเคมีรังสี 71.49% และมีความบริสุทธิ์ 98.29% ซึ่งได้คุณภาพดีและเพียงพอสำหรับการวินิจฉัยโรคมะเร็งต่อมลูกหมากตามคำแนะนำของทบวงการพลังงานปรมาณูระหว่างประเทศ

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Benchamat Phromphao : The labeling of ^{99m}Tc-PSMA-HBED-CC for prostate cancer imaging. Advisor: Asst. Prof. YOTHIN RAKVONGTHAI, Ph.D. Co-advisor: Assoc. Prof. SHUICHI SHIRATORI, Ph.D.

Prostate cancer (PCa) is the second most common cancer and the fifth leading cause of death worldwide in 2018 (1). Initially, PCa diagnosis is based on Prostate Specific Antigen (PSA) blood test, which is low sensitivity and low specificity, and sonography guided needle biopsy, which is invasive. Therefore, molecular imaging recently enrolls as an important technique in PCa diagnosis using some small molecules which are well developed to bind to overexpressed Prostate Specific Membrane Antigen (PSMA). The small molecules, for example PSMA-HBED-CC, PSMA I&T, can be chelated with Ga-68 for diagnostic purpose using positron emission tomography (PET) scan. Even though PET scan provides good contrast and spatial resolution, the related cost is high. Meanwhile, single photon emission computed tomography (SPECT) imaging offers more affordable with longer half-life of radiotracer. Therefore, it is difficult for most patients to access to PET scan in limited PET facilities. Herein, we report a new tracer using Tc-99m labeled with PSMA-HBED-CC aim to be alternative option for PCa diagnosis. The purpose of this study was to develop in-house preparation of ^{99m}Tc-PSMA-HBED-CC for prostate cancer imaging using SPECT system. ^{99m}Tc-pertechnetate 370 MBq was added to mixture solution of PSMA-HBED-CC 10 μg and 4% $SnCl_2 \cdot 2H_2O$ in 10 mL sterile vial, then heated to 100 ^{o}C 15 minutes and incubated while cool down to room temperature. Labeling parameters were optimized to obtain the maximum radiochemical yield of ^{99m}Tc-PSMA-HBED-CC. The completeness chelation was determined by instant thin layer chromatography (iTLC) and pH of ^{99m}Tc-PSMA-HBED-CC was measured. ^{99m}Tc-PSMA-HBED-CC was successfully chelated using ^{99m}Tc-pertechnetate solution in high radiochemical yield (71.49%) and radiochemical purity (98.29%) which is sufficient to administer to patient for SPECT imaging of PCa diagnosis according to International Atomic Energy Agency (IAEA) recommendation.

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LIST OF ABBREVIATIONS

CT	Computed Tomography
DTPA	Diethylene Triamine Penta-Acetate
F-18	Fluorine-18
Ga-68	Gallium-68
g	Gram
HCl	Hydrochloric Acid
In-111	Indium-111
Lu-177	Lutetium-177
MBq	Megabecquerel
mCi	Millicurie
mL	Milliliter
MRI	Magnetic Resonance Imaging
MW	Molecular Weight
PCa	Prostate Cancer
PET	Positron Emission Tomography
PSA	Prostate Specific Antigen
PSMA	Prostate Specific Membrane Antigen
SPECT	Single Photon Emission Computed Tomography
Tc-99m	Technetium-99m
$^{99m}TcO_4^-$	Technetium-99m pertechnetate
µCi	Microcurie
µg	Microgram
TLC	Thin Layer Chromatography

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CHAPTER 1
INTRODUCTION

1.1 Background and rationale

Prostate cancer (PCa) was the second most common cancer and the fifth leading cause of death worldwide in 2018 (1). Early and accurate detection is necessary, which has a benefit for staging, treatment management and prognosis. Elevated plasmatic levels of prostate specific antigen (PSA) and sonography guide needle biopsy are the most frequently used for diagnostic method in PCa (2), (3), (4), (5), (6), (7). PSA level as a biomarker for PCa has low sensitivity and low specificity because of possible false positive. Meanwhile, sonography guide needle biopsy is the only technique to find out certain cancer stage but biopsy is an invasive method and cancer might be possibly undetected. In case PSA test and biopsy results can not confirm pathologic PCa, CT and/or MRI scan will be acquired. However, CT imaging provides poor soft tissue contrast and high radiation. On the other hand, MRI requires long acquisition time and not always reliably identify local recurrence lymph node involvement or visceral metastasis. Therefore, molecular imaging allowing specific detection is highly preferable which is potentially employed to accurately diagnose PCa.

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The molecular imaging of PCa became a mighty tool under the concept of specific binding of radiopharmaceuticals to the surface receptor on prostate cancer cell, called Prostate Specific Membrane Antigen (PSMA). PSMA is a glycoprotein type II transmembrane, which composes of 750 amino acids; 19 amino acids in intracellular portion, 24 amino acids in transmembrane portion and 707 amino acids in extracellular portion. PSMA is expressed primarily in prostate epithelial and found in normal cell slightly such as salivary gland, proximal intestine and kidneys. PSMA overexpression has found in almost all type of prostate cancers, metastatic and hormone-refractory carcinomas. These characteristics render PSMA a promising target for imaging and therapy of PCa (8).

PSMA has a short intracellular domain containing a binding site for 7E11 antibody (9), (10). Two decades ago, this binding site was a target for PCa imaging but the antibody has long pharmacokinetics, therefore long half-life radionuclide is necessary to chelate to 7E11. Moreover, it was difficult to penetrate into PCa cell. Radiolabeled 7E11 antibody became not suitable tracer for PCa diagnosis. Recent research publications revealed large extracellular domain contains another binding site for J591 antibody. However, J591 antibody also requires long pharmacokinetics that is not appropriate as a tracer for PCa imaging as well. However, this extracellular binding site guided an idea to develop the small molecule for imaging, as PSMA inhibitor.

Nowadays, PSMA inhibitor divided into three families [FIG.1.1], phosphate-based, thiol-based and urea-based. Urea-based inhibitors showed highest affinity to PSMA among them. Urea-based inhibitors are a small molecule developed from the binding part of J591 antibody. Currently, several commercially available PSMA inhibitors are used in PCa imaging. For example, Glu-NH-CO-NH-Lsy (Ahx)-HBED-CC (PSMA-11® or PSMA-HBED-CC), suitable for Ga-68 labeling. PSMA inhibitor Vipivotide tetraxetan (PSMA-617®) (11) and PSMA I&T® (imaging and therapeutic) contain DOTA chelator that are suitable for Ga-68 and Lu-177 labeling. Recently, ^{18}F-PSMA is also available in clinical use. Both ^{68}Ga-PSMA and ^{18}F-PSMA are diagnostic radiopharmaceuticals which required the positron emission tomography (PET) facility. In spite of superior imaging quality, PET tracer is less widely available and more costly to implement in many aspects, while SPECT tracer is routinely use with much more affordable.

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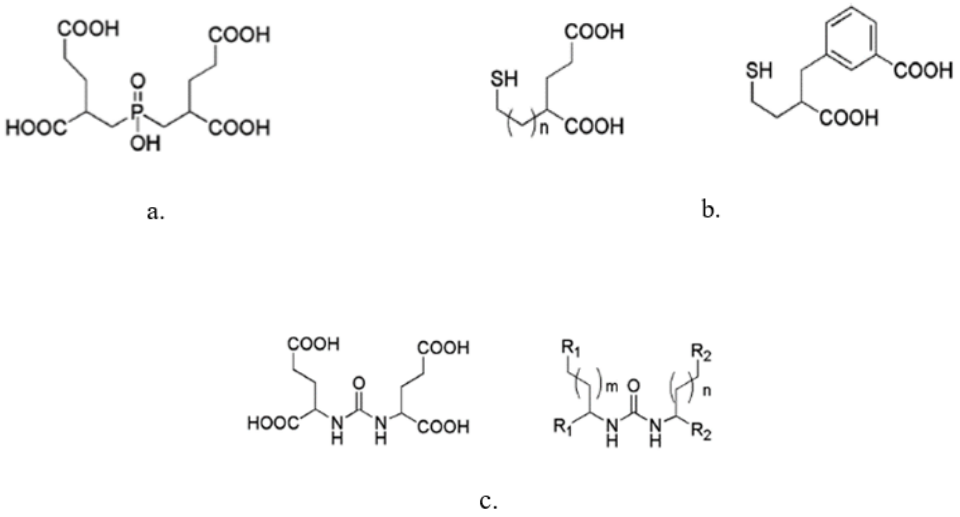

Figure 1.1 PSMA inhibitor families; (a.) Phosphate-based family, (b.) Thiol-based family, (c.) Urea-based family

Recently, radiosynthesis of ^{99m}Tc-PSMA had been reported (12), (13), (14), (15), (16), (17). There are some drawbacks such as complicate preparation, low radiochemical yield, low radiochemical purity and time-consuming process. These drawbacks could be improved in methodology to provide more efficient and practical labeling. Our rationale of ^{99m}Tc-PSMA labeling was derived from similarity of chemical structure between PSMA-HBED-CC and diethylene triamine pentaacetate (DTPA) [FIG.1.2]. With this characteristic of PSMA-HBED-CC and DTPA, it is possible that PSMA and DTPA can bind with Tc-99m. The chemical structure of DTPA that highlighted in blue line, shows the dentates involved in chelating to Tc-99m. The groups of dentates composes of 3 N and 3 OH groups. While the chemical structure of PSMA-HBED-CC that highlighted in red, shows 2 N and 4 OH groups of dentate for Tc-99m chelation. These two highlight structures can be almost superimposed. Moreover, the proposed coordinating complex of ^{99m}Tc-DTPA and ^{99m}Tc-PSMA-HBED-CC are shown in figure 1.3, which support our rationale to develop ^{99m}Tc-PSMA-HBED-CC as a new promising SPECT radiopharmaceutical for PCa diagnosis in cost-effective manner.

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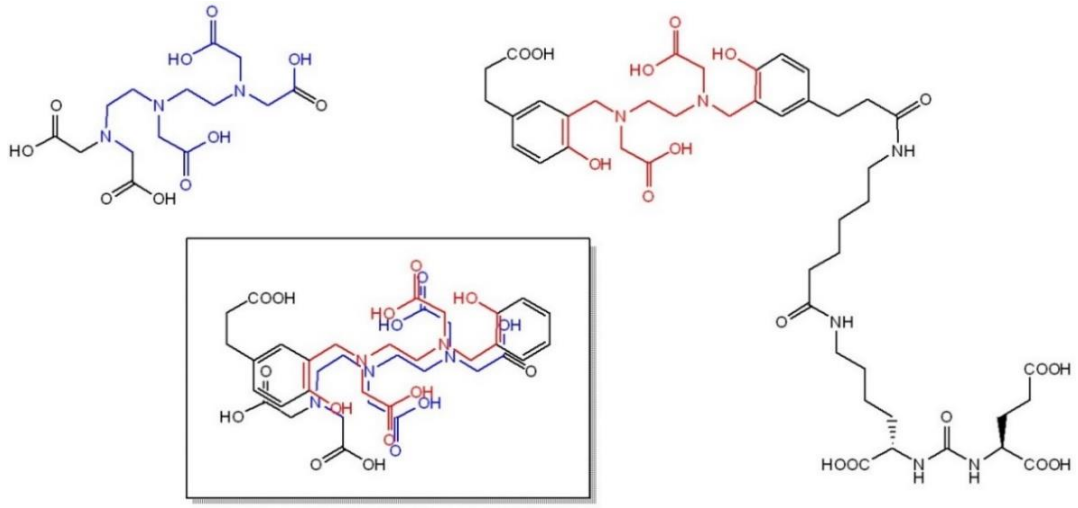

Figure 1.2 Chemical structure of DTPA and PSMA-HBED-CC with superimposed structure

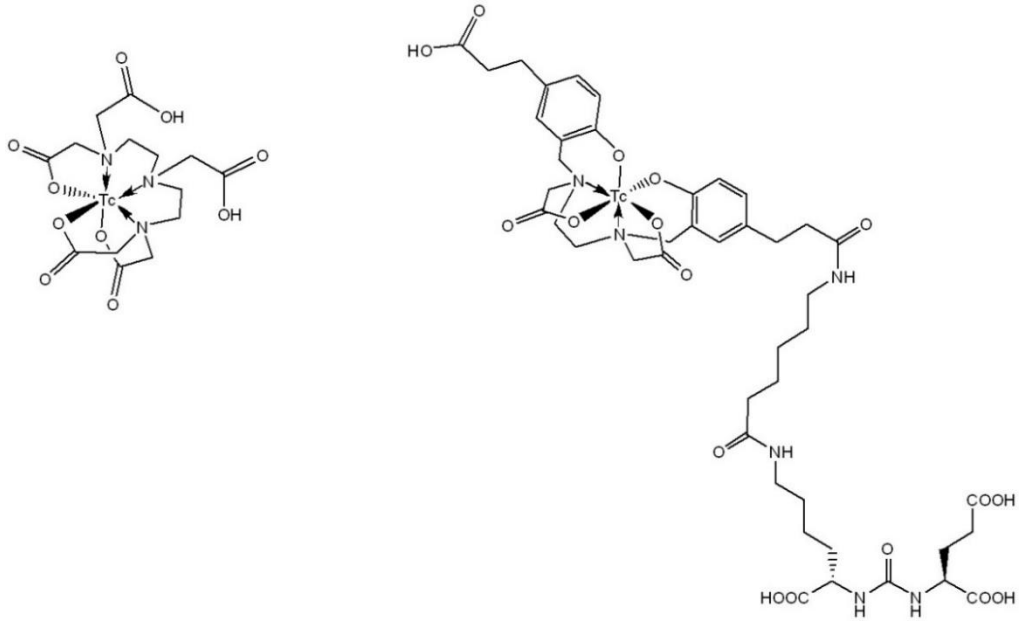

Figure 1.3 ^{99m}Tc-DTPA and ^{99m}Tc-PSMA-HBED-CC complex

1.2 Research objective

To develop in-house preparation of ^{99m}Tc-PSMA-HBED-CC for prostate cancer imaging using SPECT system.

CHAPTER 2
REVIEW OF RELATED LITERATURE

2.1 Theory

2.1.1 Prostate cancer

2.1.1.1 The prostate gland

Male reproductive system composes of penis, prostate gland, seminal vesicles and testicles. The prostate gland, so called prostate, is a walnut-size gland located below the bladder and in front of rectum. The prostate surrounds the urethra, which is a tube that carries urine from the bladder and out of the body. Above the prostate and behind the bladder are two seminal vesicles that make a fluid as a part of semen. Semen is made up of sperm from the testicles and fluid from prostate (18).

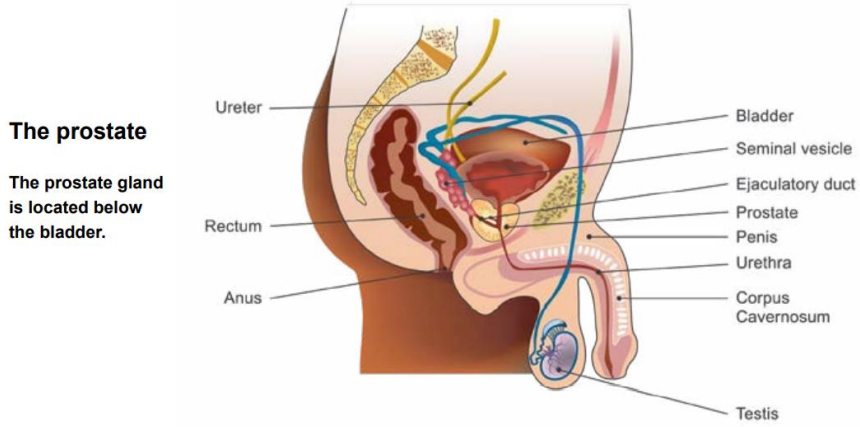

Figure 2.1 Male reproductive system and the prostate gland

2.1.1.2 Prostate cancer

Cancer is a disease in which cells grow out of control. Prostate cancer is cancer of prostate gland that uncontrolled cells in prostate were abnormally developed. Almost all prostate cancers are adenocarcinomas, which are cancer in the cells secreting fluids or other substances. Cancer can grow or spread to form tumors in other parts of the body. Cancer that has spread is called metastasis.

Localized prostate cancer contained entirely within the prostate, meanwhile cancer that has spread from the prostate gland to nearby lymph nodes, is called regional prostate cancer and cancer that has spread beyond the prostate or regional lymph nodes is called distant metastasis and may be referred to as metastatic prostate cancer (18). Prostate cancer can metastasize through blood into the bones, lymph nodes, liver, lung and other organs.

2.1.1.3 Risk factors in the prostate cancer

Risk factor is anything that increases a person's chance of prostate cancer development. All men are at risk for prostate cancer with 1 out of 9 men, especially African-American men who are more likely to get prostate cancer at a younger age (7), (18). Cancer in African-American men tend to be more aggressive and more advanced. However, once diagnosed, African-Americans have similar treatment results as other men with the same cancer stage. Meanwhile, incidence rates are lower in Asian. Rates are on the rise in countries where PSA testing became more widely use later, including Thailand, Japan and many other countries in other region [1]. Moreover, age is important factor, which is the most common risk factor for developing prostate cancer. The older men have found the higher chance of getting prostate cancer. In addition, men with family history of cancer have a greater chance of getting prostate cancer (18). Currently, there are known effects of dietary factors that have been associated with prostate cancer such as high alcohol intake (7). In addition, a relation between intake of dietary and fried foods enhanced risk of prostate cancer may exist (7), (19).

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2.1.1.4 Prostate cancer diagnosis

Prostate cancers are detected on the basis of elevated plasmatic levels of prostate specific antigen or PSA. However, not only prostate cancer but also enlargement of prostate gland has been found the elevated PSA level. Therefore, a tissue biopsy is the standard diagnosis to confirm cancer's presence. European Associated of Urology (EAU) Guidelines on prostate cancer and National Comprehensive Cancer Network (NCCN) (18), (20) has recommended diagnosis stratification by PSA test, digital rectal exam (DRE), sonography guide needle biopsy, computed tomography and magnetic resonance imaging. Summary as following:

2.1.1.4.1 Patient history and physical exam

It's well known that patient's age, family history and ethnic background are associated with an increased prostate cancer incidence. History of family records become useful information for example direct and/or indirect family members who are diagnosed as any types of cancer patient. In addition, physical examination is also important for signs of disease such as difficulty of urination, often cystitis, enlargement of prostate gland.

2.1.1.4.2 Prostate Specific Antigen (PSA) level

Blood test is the basis method for elevated plasmatic levels of prostate specific antigen (PSA). PSA as a serum biomarker has revolutionized prostate cancer diagnosis, check for signs of disease and chance of prostate cancer including use for follow up, check organ function and treatment results. PSA level is measurement of a specific protein made by the fluid-making cells that line the small glands inside the prostate in the number of nanogram of PSA per milliliter (ng/mL) of the blood. However, high PSA level can be a sign of prostate cancer, benign prostatic hypertrophy (BPH), prostatitis or just enlargement of prostate gland. So, PSA level has low sensitivity and low specificity. Moreover, the rising of PSA levels are also at increased risk of PCa metastasis. In addition, PSA levels are rising after prostate cancer treatment with surgery or radiation therapy, it is called "PSA recurrence". This means that the cancer is returned or treatment did not succeed for reduce the amount of cancer (7), (18).

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2.1.1.4.3 Digital rectal exam (DRE)

Most prostate cancer are located in peripheral zone and may be detected by digital rectal exam (DRE). DRE is used to screen for prostate cancer, the cancer stage evaluation, and assess how patient cancer is responding to treatment. Doctor will insert a lubricated, gloved finger into patient rectum to feel the prostate for abnormalities. Not all parts of the prostate can be felt during this exam.

However, DRE alone in the primary care setting had a sensitivity and specificity below 60% (7), possibly due to inexperience. It can not be recommended to exclude prostate cancer. An abnormal DRE is associated with an increased risk of a higher grade and is an indication for biopsy.

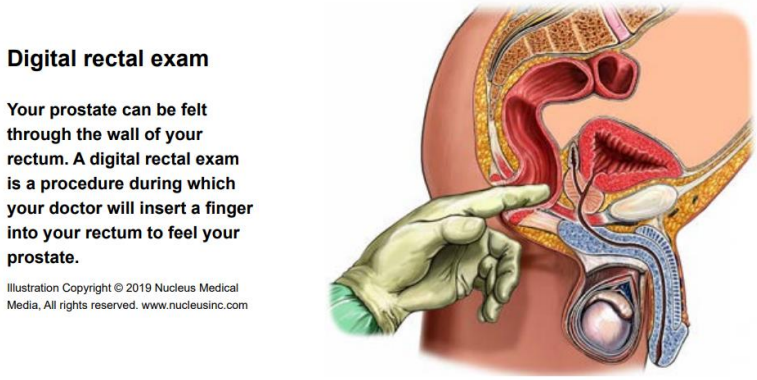

Figure 2.2 Digital rectal exam

2.1.1.4.4 Prostate guide needle biopsy

The prostate biopsy is based on PSA level and/or suspicious DRE and/or imaging. In preliminary limitation of PSA elevation, PSA elevation alone should not prompt immediate biopsy. Therefore, ultrasound guided biopsy is now the standard of care (7). Prostate guided biopsy is performed by either the transrectal or transperineal approach.

A biopsy is performed by removing samples of fluid or tissue under a microscope to confirm prostate cancer. A hollow needle is used to remove one or more samples. Core samples will be taken from different parts of prostate. Biopsy samples will be sent to a pathologist, who will test the biopsy and write a report called a pathology report. The pathologist may perform other tests to see if the cancer cells have specific genes or proteins.

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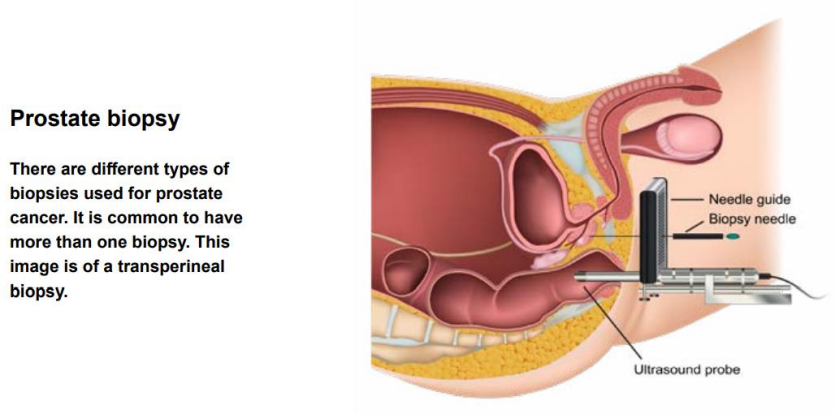

Figure 2.3 Sonography guide needle biopsy

2.1.1.4.5 Conventional imaging modalities

Imaging modalities for cancer diagnosis are Computed Tomography (CT) scan, Magnetic Resonance Imaging (MRI) and radionuclide imaging. CT uses x-rays and computer technology to take pictures of the inside of the body. It takes many x-rays of the same body part from different angles. All images are combined to make one detailed picture. CT has poor soft tissue contrast and high radiation dose. Therefore, CT scan especially CT of abdomen and/or pelvis may be one of the tests used to look for cancer that has spread to other areas (metastasized). CT scans are good at seeing lymph nodes and the area around the prostate. Before CT scan, patient may be given contrast media. It is not dyes but substances that help certain areas in the body stand out (7), (18).

Magnetic resonance imaging (MRI) scan uses radio waves and powerful magnetic field to take pictures inside the body. MRI does not use x-rays. Like a CT scan, a contrast media may be used to make the pictures clearer. An MRI might provide more detail about the cancer within the prostate while it is more sensitive detection of lymphnode and cancer metastasis to nearby lymph nodes or the bones in pelvis. However, MRI takes long acquisition time and not always identify local recurrence lymph node involvement or visceral metastasis because the size of non-metastatic lymphnodes varies widely and may overlap the size of lymphnode metastasis. A multiparametric MRI (mpMRI), a special type of MRI scan, remains the

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most useful method for local staging when combined with clinical data. In an mpMRI, multiple scans are performed without contrast followed by another MRI with contrast. The patient might have more than one mpMRI during the course of treatment. It might be done to learn more about the prostate cancer or to look for bleeding after a biopsy. An mpMRI might help detect certain types of tumors. It also might help determine risk group for active surveillance (7), (18).

2.1.2 Single Photon Emission Computed Tomography (SPECT)

Single Photon Emission Computed Tomography (SPECT) comprises a conventional scintillation camera mounted on a special gantry and connected to an appropriate computer system. This type of system enables a series of images acquired around a patient to be reconstructed to give a set of transaxial images, similar to those obtained by x-ray, CT, which constitute a 3D image of that part of the patient being scanned. While SPECT imaging is extensively used in nuclear medicine imaging, special attention is needed with respect to quality control. SPECT systems will not produce adequate results unless great care is taken with the performance and set up of both the scintillation camera and all the other component parts of the system.

The basic principle of a SPECT system depends on the rotating camera concept in a series of collected planar images. While the camera is rotated through either 180^{o} or 360^{o} around the patient. These planar images are called projection images and are used to create transaxial slice images by filtered back projection of the data into the transaxial plane. Figure 2.4 is a diagram of such a system with various axes and, in particular, with the axis of rotation indicated and identified. Each row of pixels across the projection image gives a projection line, a profile of counts for a common Y value in that image. The counts in these projection lines may be back projected at the appropriate angle across the transaxial plane, which would result in a first order approximation of the data that gave rise to the set of projection images (21).

SPECT imaging is widely used for cancer detection in nuclear medicine because it has a good sensitivity. In addition, cost of SPECT and radiotracer is lower than PET modality. Therefore, SPECT is more widely available than PET in many

regions. For prostate cancer diagnosis, SPECT is mostly used for detected cancer metastasis especially bone metastasis evaluation. At present, SPECT imaging is used after PCa treatment because PSMA's therapeutic radiopharmaceuticals labeled with beta emitting isotope such as Lu-177 (7).

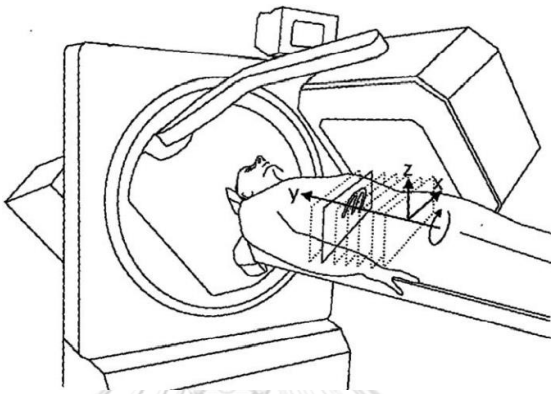

Figure 2.4 SPECT system showing the axis of rotation

2.1.3 PSMA and metal-based PSMA radioligands

2.1.3.1 Prostate specific membrane antigen (PSMA)

Prostate specific membrane antigen (PSMA) is a type II transmembrane protein expressed in all forms of prostate tissue, including carcinoma. The PSMA gene is located on the short arm of chromosome 11 in a region that is not commonly deleted in prostate cancer (10). In addition, PSMA has known enzymatic activities and acts as a glutamate-preferring carboxypeptidase. Importantly, PSMA expression seems to be inversely related to androgen levels. Scientists discover that PSMA activity in PCa cell lines increased as cells became more androgen independent. Furthermore, PSMA is a key player in prostate carcinogenesis and disease progression, glutamatergic neurotransmission, and folate absorption. These various functions and the tissue distribution of the protein result in different inhibitor designations. The NAALDase activity of PSMA has been extensively investigated for the development of PSMA-specific based ligands with the potential to be used for prostate cancer diagnosis and/or therapy (8).

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2.1.3.2 PSMA-based radioligands

As part of the ongoing efforts of the scientific community to develop new anti-PSMA-specific ligands. The dual nature of PSMA, not only as a receptor protein, but also as an enzyme, has paved the way for the establishment of several approaches for its targeting via radiolabeled molecules. Firstly, based on the macromolecular protein structure of PSMA, specific monoclonal antibodies and smaller molecules called aptamers have been developed. These molecules bind tightly, selectively and specifically to PSMA. Secondly, the enzymatic activity of PSMA served as the trigger for the synthesis and the further evaluation of a variety of anti-PSMA inhibitors of low molecular weight, with the potential to be used as nuclear imaging probes (8). PSMA was originally developed with a type of prostate cancer cell line known as LNCaP cells, the monoclonal antibody 7E11 was the first anti-PSMA antibody. It recognizes and binds a PSMA intracellular or cytoplasmic epitope which is accessible on necrotic tumors only (8), (10). Therefore, this fails to gain wide acceptance in the nuclear medicine field for PCa detection. Hence, there is the need for monoclonal antibodies development which bind to the extracellular site of PSMA such as the humanized monoclonal antibody J591. These antibodies exhibit high and specific binding to cell-adherent PSMA and it has been evaluated preclinically by SPECT, PET as well as radioimmunotherapy. Despite the improved targeting of J591 and the subsequent generation of anti-PSMA monoclonal antibodies, the major impediment to the use of antibodies for diagnosis continues to be the slow clearance from the non-target tissues. This is mitigated by introducing a time interval of several days between radiotracer administration and imaging. An alternative approach is to optimize the pharmacokinetics of the monoclonal antibodies by the generation of antibody fragments, such as single domain antibodies (called nanobodies), PSMA inhibitors, etc.

Nanobodies, the comparatively low molecular mass of nanobodies leads both to good permeability in tissues and a short plasma half-life. Meanwhile, PSMA inhibitors with lower molecular mass have been devoted to generate further molecules with inhibitory action towards PSMA. The main strategy for the discovery of those inhibitors was to find zinc-binding groups which are linked to a glutamate moiety.

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Three functionalities with affinity for zinc, including phosphorus-based (phosphonate, phosphates, phosphoramidates), thiols-based and ureas-based have been identified. In the radiopharmaceutical development, phosphorus-based and urea-based inhibitors have been synthesized and modified to labeled with radiometals (8). Especially, urea-based inhibitors showed high affinity to PSMA (9). Urea-based PSMA is a small molecule developed from the binding part of J591 antibody.

2.1.3.3 Urea-based PSMA inhibitors

Currently, a variety of urea-based PSMA inhibitors have been developed and labeled with different SPECT, PET and therapeutic radiometals using several chelators and showed great promise not only preclinically but also in the clinical assessment. Urea-based inhibitors have a high specificity for PSMA, fast and efficient internalization in LNCaP cells (9). The urea-based PSMA precursors development requires the attachment of a relatively bulky chelator to the peptidomimetic structure of the PSMA inhibitors labeled with radiometals. The PSMA extracellular surface with the active site of the enzyme, rendered difficult the entry of the radiometal-bearing chelator into the enzymatic binding site. Therefore, the insertion of a spacer between the urea-based motif and the chelator is essential. Although the binding affinity can be considered as the most crucial parameter which affects the tumor uptake, the overall pharmacokinetic performance of a radiotracer is determined by many other factors which certainly need to be taken into consideration during the development of potential radiopharmaceuticals. In particular, parameters such as the nature of the spacer, lipophilicity, charge, plasma protein binding and molecular weight also influence the pharmacokinetic performance of a radiotracer. Indeed, the presence of the spacer not only greatly influences the affinity of the derived radiotracers towards PSMA but also the pharmacokinetic properties of the chelator-conjugated PSMA inhibitors (8).

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2.1.4 The technetium-99m

2.1.4.1 Chemistry of technetium-99m

Technetium, the 43rd element in the periodic table, is a transition metal. There are 51 isotopes of technetium ranging from Tc-85 to Tc-120. The two most

studied and used are Tc-99 (half-life 211,000 y) and Tc-99m (half-life 6.01 h). The half-life of Tc-99m is nearly ideal for nuclear medicine because it is long enough to facilitate the preparation, transportation and administration of radiopharmaceuticals for patients imaging. Meanwhile, Tc-99m's half-life is short enough to allay concerns surrounding radiation exposure and disposal. In addition, Tc-99m emits gamma rays with an energy of 140 keV, which is sufficient to allow for clinical tomographic whole-body imaging at any depth without imparting a burdensome radiation dose. Therefore, this radiometal has widespread availability in clinical diagnostic nuclear medicine (22).

Tc-99m is produced by the decay of the parent Mo-99. The generator system is composed of alumina column on which the Mo-99 is absorbed in the chemical form of molybdate $^{99}MoO_4^{2-}$. Eluting with normal saline leads to the ^{99m}Tc-pertechnetate formation ($^{99m}TcO_4^-$), which is less tightly bound to the alumina column because of its single negative charge compared to the double negative charge of Mo. $Na^{99m}TcO_4$ can be eluted from the column with a saline solution tank. Then, the $Na^{99m}TcO_4$ is ready for radiopharmaceuticals preparation (23).

Generally, Tc-complexes have been reported with oxidation states -1 to +7. Tc-99m complexation is controlled by several factors including pH, the type of reducing agents and the nature of the coordinating ligands. The formation of multiple structural isomers is also common, which must be taken into account on designing ^{99m}Tc-based radiopharmaceuticals. Characterizing ^{99m}Tc-complex can be a challenge because the doses used for nuclear imaging typically contain only a very small molar amount of metal complex (22). Deep knowledge of inorganic chemistry allows for developing convenient approaches to introduce stable Tc-99m into a bioactive molecule with the aim of not affecting its bioactivity. A number of inorganic technetium functional groups, are called "cores" or "metal fragments". For example, the Tc-oxo ($[Tc \equiv O]^{3+}$), Tc-nitrido $[Tc \equiv N]^{2+}$, Tc-HYNIC [Tc=N-nH-C_5H_5N] and Tc-*tris*-carbonyl(*fac*-[$Tc(CO_3)]^+$) [FIG.2.5] (23), (24). These groups are chemical motifs comprising a characteristic arrangement of atoms bounded to the metallic center and determining the formation of a variety of coordination complexes and molecular geometries.

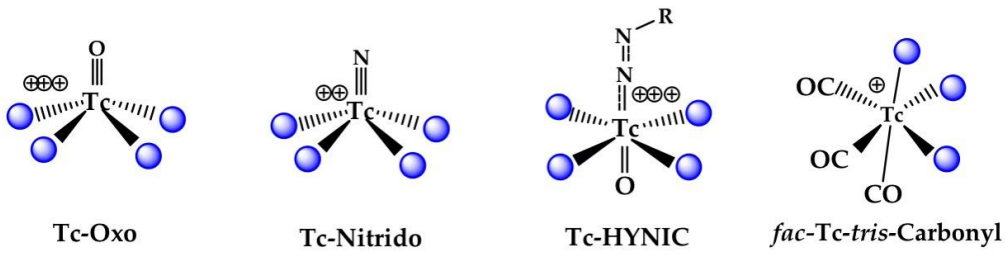

Figure 2.5 Technetium-ligand complexation

2.1.4.2 Quality control methods of Tc-99m pharmaceuticals

The determination of the radiochemical purity in nuclear medicine commonly uses thin layer chromatography (TLC). The principle of this method is a mobile phase (solvent) moves along a layer of adsorbents (stationary phase) due to capillary forces. Depending solubility on the distribution between the stationary and mobile phase, the radioactive sample spotted onto the adsorbent will migrate with different velocities and thus, impurities are separated. The main impurities in Tc-99m pharmaceutical are free pertechnetate ($^{99m}TcO_4^-$) and reduce hydrolyzed technetium (colloidal Tc-99m). These impurities may be tested by simple TLC procedures.

The migration properties of free pertechnetate are influenced by the choice of different mobile and stationary phases. Silica gel, instant TLC (iTLC) material, is usually used as stationary phase migration of free pertechnetate depends on the solubility of this anion in the solvent. In a polar solvent such as saline, 80% methanol, acetone or 2-butanone (methyl ethyl ketone, or MEK) pertechnetate migrates with the solvent front (R_f = 0.6-1.0). When a nonpolar, lipophilic solvent (e.g., ethylacetate, chloroform) is used and the sample is dried (no water content), free pertechnetate remains at the origin. Colloids do not migrate in most TLC systems since insoluble material will stay at the origin. Changing the mobile or the stationary phase will not affect the migration properties of colloidal Tc-99m (25).

TLC method is frequently used and is recommend in many official procedures. It might be used for simple separation techniques. The strip is cut into two segments and measured in the ionization chamber. This procedure is a quick analysis before the radiopharmaceutical is used in patients. However, limitation is low

resolution of measurements. An amount of impurities overestimation and if not cut properly, the results may be wrong. This problem can be avoided by cutting the strip into more segments, which are measured separately (25).

2.2 Review of related literature

There are few reports of the ^{99m}Tc-PSMA labeling. One interesting publication among them is ^{99m}Tc-PSMA MIP-1404 (trofolastat chloride) by Julia Reinfelder et al (12). In their study, they labeled PSMA MIP-1404 (Progenics Pharmaceuticals, Inc, Tarrytown, NY) with ^{99m}Tc for PCa diagnosis. The aim of their study was to improve experience and clinical use ^{99m}Tc-PSMA MIP-1404 in patients with biochemical relapse of PCa. In this study, 60 male recurrence PCa, age range 49-85 years old were recruited. The recruited criteria were histopathology confirm PCa and complete primary therapy, prostectomy with or without lymphnode dissection radiotherapy, or androgen deprivation therapy. In addition, PSA level increased greater than 0.2 ng/mL after radical prostatectomy and greater than 2 ng/mL after radiation therapy or androgen deprivation therapy.

MIP-1404 was synthesized and labeled with $^{99m}Tc(CO)_3^+$ to form the desired ^{99m}Tc metal complexes ($^{99m}Tc(CO)_3$-MIP-1404) [FIG.2.6] and commercially available IsoLink kits (Covidien) in a volume of 1 mL to form the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ intermediate, which was subsequently neutralized with 0.2 mL of 1N HCl and reacted with 0.1 mg of compound (MIP-1404) at a concentration of 10^{-4} M in an equal volume mixture of acetonitrile and water in a sealed vial. The sealed vial was heated at 100 ^{O}C for 30 min. After cooling and evaporation of the solvent, the tert-butyl ester protecting groups were removed by treatment with 2 mL of 50% trifluoroacetic acid in dichloromethane for 45 min at room temperature. The final ^{99m}Tc-labeled complexes were dried under a stream of nitrogen and reconstituted in 0.9% sodium chloride solution (pH 5), resulting in no-carrier-added products (14).

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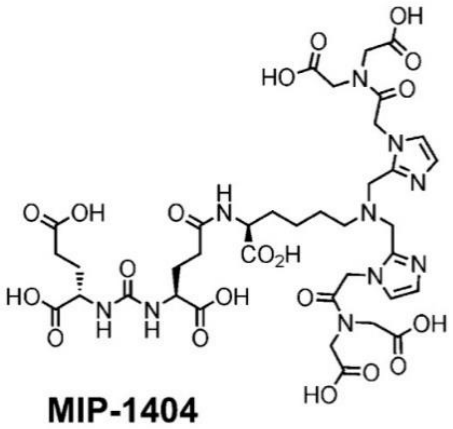

Figure 2.6 Chemical structure of PSMA MIP-1404

PSMA MIP-1404 was labeled with Tc-99m resulted in high radiochemical purity in 97%±1 with 70% radiochemical yield. Each patient was injected ^{99m}Tc-PSMA MIP-1404 612 to 842.5 MBq based on body weight calculation. In all subject, a Symbia T2 SPECT/CT system, Siemens Healthcare was used. All scans were reviewed and interpreted by 2 nuclear medicine physicians. Blinded to patient related medical data and the PSA value. All lesion with visually determined focal uptake greater than background activity in the gluteal muscle was considered MIP-1404 positive. Therefore, interpreted results are assessed as suggestive of PCa recurrence or metastasis [FIG.2.7].

42 from 60 patients have found positive lesions with detection rate 70% at 95% confidence interval. At PSA level greater than 2 ng/mL, detection rate was 91.4% and 40% detection rate at lower PSA values (P value < 0.001)

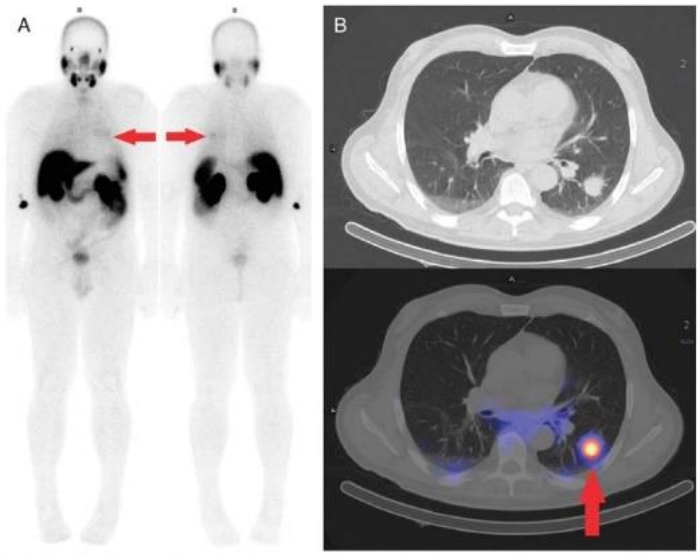

Figure 2.7 Planar whole-body scintigraphy (A) and transaxial SPECT/CT fusion (B)

In conclusions, ^{99m}Tc-PSMA MIP-1404 is capable to detect locally recurrent or metastasis. SPECT tracer to the array of diagnostic tools for PCa will provide valuable assistance to patients where PET facility is not a viable option.

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CHAPTER 3
RESEARCH METHODOLOGY

3.1 Research design

This study is an experimental laboratory research.

3.2 Research design model

Research design model for experimental laboratory has followed the pattern of basic science experimental model in chemistry. The flowchart [FIG.3.1] depicts the preparation of reducing agent using $SnCl_2 \cdot 2H_2O$ as the first step. This reducing agent is freshly prepared to reduce the most stable oxidation state of Tc-99m (+7) to the appropriate lower oxidation state, which is active enough to coordinate with ligand. Subsequently, chelation of starting material with Tc-99m forms a complex of ^{99m}Tc-PSMA-HBED-CC. After chelation, the crude product undergoes purification. In the third step, the purified product is submitted to quality control process in 3 checkpoints; measurement of pH, radiochemical yield and radiochemical purity. All experimental data from each experiment is collected. Finally, statistical analysis is performed to conclude the endpoint of research.

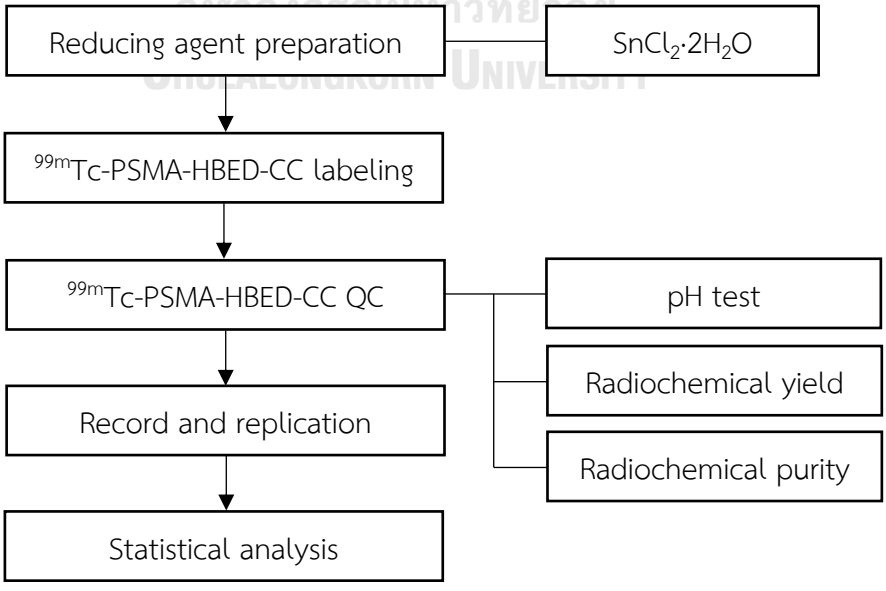

Figure 3.1 Research design model of ^{99m}Tc-PSMA-HBED-CC labeling

3.3 Conceptual framework

There are several parameters that affect labeleing efficacy of Tc-99m [FIG.3.2]. These parameters are concerned into 3 categories in labeling of ^{99m}Tc-PSMA-HBED-CC. The first category of reducing agent preparation is affected by $SnCl_2 \cdot 2H_2O$ itself and temperature in preparation method. The next category is labeling method that is affected by two precursors (PSMA-HBED-CC and $SnCl_2 \cdot 2H_2O$ solution) and temperature of labeling method. The last category of radiopharmaceutical quality control (QC), which is affected by humidity of silica gel paper strip.

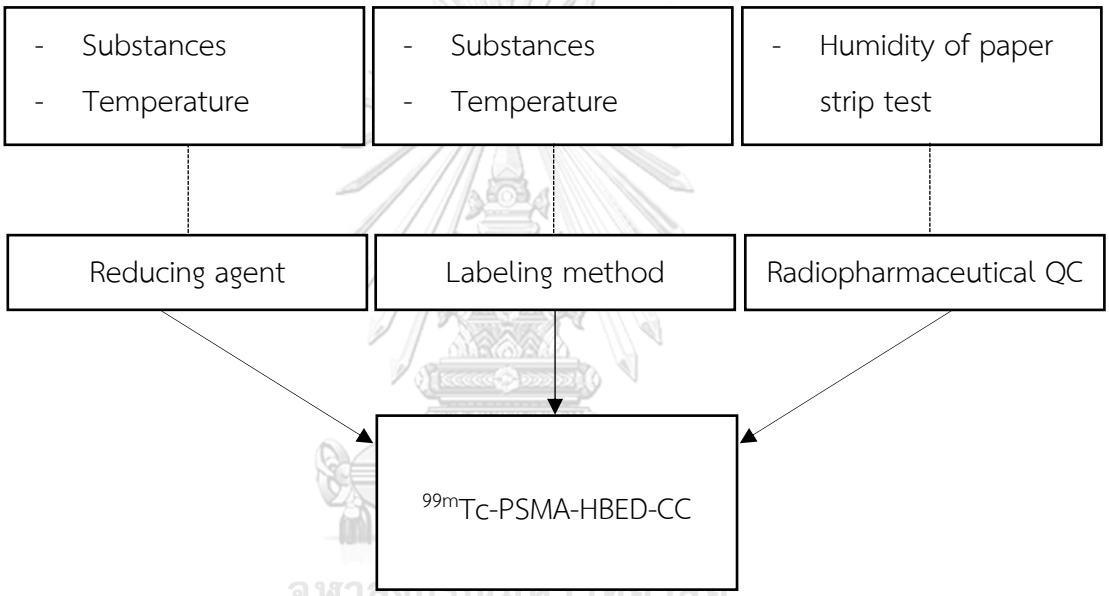

Figure 3.2 Overview of conceptual framework

3.4 Research question

What is the suitable substance quantitation with the labeling method development of ^{99m}Tc-PSMA-HBED-CC for prostate cancer imaging?

3.5 Research objective

To develop in-house preparation of ^{99m}Tc-PSMA-HBED-CC for prostate cancer imaging using SPECT system.

3.6 Key words

Prostate cancer imaging, Technetium-99m, PSMA, ^{99m}Tc-PSMA, PSMA-HBED-CC, SPECT tracer.

3.7 Outcome measurement

According to IAEA and university of Vienna recommendation for quality control in the production of radiopharmaceutical (25), (26), the QC of a certain SPECT radiopharmaceuticals should meet criteria as following;

3.7.1 pH measurement

The pH of the purified radiopharmaceuticals can be measured using pH paper indicators. The advantage of pH paper type is the universal pH range and easy to apply. The pH measurement method is as follows;

- Drop a sample of radiopharmaceuticals on the pH paper.
- Check changing color, which indicates particular pH value (between pH 1 and pH 14). For accurate measurement, a narrow-band pH paper can be used.

3.7.2 Radiochemical yield measurement

The radiochemical yield or percentage of labeling efficiency can be measured by the method as follows;

1. Drop a sample of the radiopharmaceuticals on a Thin Layer Chromatograph (TLC) paper (stationary phase; silica paper strips) and dry the spot with a stream of air [FIG.3.3].

2. Put the silica paper strips into a chamber containing a suitable solvent (mobile phase; acetone for free Tc-99m component and 0.9% saline for hydrolyze-reduce Tc-99m component) which takes a few minutes before put into the dose calibrator chamber.

3. Allow the mobile phase to migrate to the top of the silica paper strips. The mobile phase level must be below the marked spot on paper strips.

4. Cut each TLC strips in the middle. Determine radioactivity of each TLC piece by using a dose calibrator counting.

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Percent labeling is calculated as the ratio of radioactivity counts in each TLC piece compared to total counts on the paper strips in each system (free $^{99m}TcO_4^-$ and hydrolyze reduce (HR) Tc-99m). The radiochemical yield can be obtained using the equation as following;

%Labeling efficiency = 100 - %free $^{99m}TcO_4^-$ - %HR.

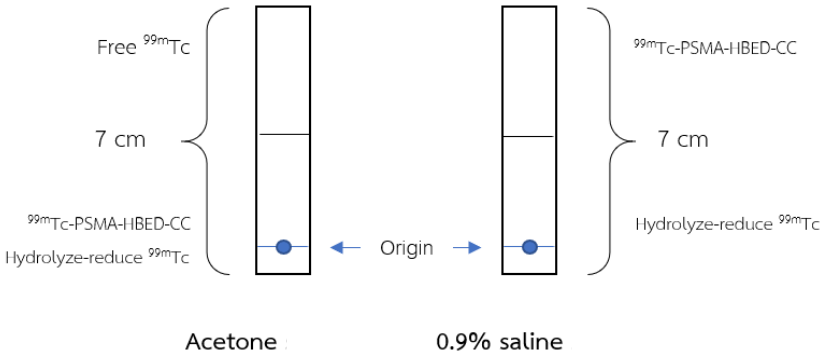

Figure 3.3 TLC manipulation in radiochemical yield measurement

3.7.3 Radiochemical purity measurement

The radiochemical purity (RCP) can be measured after purification using a solid phase extraction (SPE) C-18 cartridge by washing with organic solvent (ethanol : water, 1:1). The method is as follows;

1. Crude product is loaded into C-18 cartridge. ^{99m}Tc-PSMA-HBED-CC is trapped inside cartridge and free Tc-99m is released to waste vial.

2. Using ethanol and water 1 to 1 ratio to wash labeling product to obtain 100% radiochemical purity that is confirmed by TLC.

3. Drop a sample of the radiopharmaceuticals on silica gel paper strip and dry the spot with a stream of air.

4. Insert the silica paper strips into a TLC tank containing a suitable solvent (mobile phase; acetone for free Tc-99m component test and 0.9% saline for hydrolyze-reduce Tc-99m component test) which take a few minutes before put into the chamber.

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5. The radioactivity of each TLC piece is determined by using a dose calibrator counting small pieces in the case of silica paper strips.

Calculate the RCP as the % activity with counts in product divide by total counts on paper strips. The specification requires that the % RCP should be greater than 95% or recommend value in related monograph (26).

$$\% \text{ Free } {}^{99m}TcO_4^- = \frac{\text{Count of free } {}^{99m}TcO_4^-}{\text{Total count}} \times 100$$

$$\% \text{ Hydrolyzed reduced} = \frac{\text{Count of hydrolyze reduce form}}{\text{Total count}} \times 100$$

$$\% \text{ RCP} = 100 - (\%\text{Free } {}^{99m}TcO_4^- + \%\text{Hydrolyze reduce})$$

3.8 Sample size determination

In this study, labeling of ^{99m}Tc-PSMA-HBED-CC using the most optimized labeling condition must be replicated to ensure that consistency of the labeling method is acceptable.

This sample size is required to estimate a population mean with a specified level of confidence and precision. Inputs are the assumed population standard deviation, the desired level of confidence and the desired precision of the estimation. The desired precision of the estimate (sometimes called the allowable or acceptable error in the estimation) is half the width of the desired confidence interval.

$$N = \frac{(Z_{\alpha/2})^2.\sigma^2}{d^2}$$

Where **N** = Replication

$Z_{\alpha/2}$ = 95% Confidence interval

σ = Standard deviation

d = Acceptable error

$$N = \frac{(1.96)^2.(1)^2}{(1)^2} = 4$$

$Z_{\alpha/2}$ = 1.96; the value from the standard normal distribution for the selected confidence level (for a 95% confidence interval, Z = 1.96)

σ = ±1 (reference from literature review (12))

d = ±1 of radiochemical purity, the margin of error to measure

3.9 Materials

3.9.1 Scientific and nuclear medicine instruments

3.9.1.1 Autopipette

3.9.1.2 Glass test tube 6 mL

3.9.1.3 Beaker 80-500 mL

3.9.1.4 Sterile vial 10 mL

3.9.1.5 Needles No.18 and No.21

3.9.1.6 Syringes 3,5 and 10 mL

3.9.1.7 Rubber gloves

3.9.1.8 Scientific digital balance scale (Denver Instrument Company AA-160)

3.9.1.9 Hot plate stirrer (FRONTLAB FLHS-A-Pro)

3.9.1.10 Dry block heater (MTOPO DMB-1)

3.9.1.11 Dose calibrator (CAPINTEC CRC-25R)

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3.9.2 Reducing agent

3.9.2.1 Stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$)

3.9.2.2 Hydrochloric acid (HCl)

3.9.3 ^{99m}Tc-PSMA-HBED-CC labeling precursors

3.9.3.1 ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$)

(Purchased from Global Medical Solution (Thailand) CO., LTD.)

3.9.3.2 PSMA-HBED-CC

(Imported from Germany by Biomedia (Thailand) CO., LTD.)

3.9.4 ^{99m}Tc-PSMA-HBED-CC quality control

3.9.4.1 pH indicator

3.9.4.2 TLC; Silica paper strips (Silica paper, storage at 18-20 °C with regulated humidity in desiccator (27).

3.9.4.3 Acetone and 0.9% saline

3.9.4.4 Solid phase extraction (SPE) C-18 Cartridge

3.9.4.5 Ethanol and sterile water

3.10 Methods

3.10.1 Dose calibrator quality control

Physical inspection, accuracy and precision of dose calibrator CAPINTECH CRC-25R were performed according to quality control of nuclear medicine instruments 1991, IAEA-TECDOC-602 (28). [APPENDIX A]

^{99m}Tc-PSMA-HBED-CC labeling methodology is adopted according to the standard ^{99m}Tc-labeling. However, optimized factors in labeling process are investigated and formulated to reach the requirement criteria for radiopharmaceutical injection as the following;

3.10.2 PSMA-HBED-CC quantitation calculation

Refer to the rationale in chapter 1, PSMA-HBED-CC was selected according to the coordinating mimic to chemical structure of DTPA. Therefore, starting materials in labeling of ^{99m}Tc-PSMA-HBED-CC is calculated from chemical amounts in the same proportion of ^{99m}Tc-DTPA cold kit.

Amount of DTPA and $SnCl_2 \cdot 2H_2O$ in a cold kit

DTPA cold kit purchased from TINT indicates DTPA 5 mg and $SnCl_2 \cdot 2H_2O$ 0.6 mg

As MW of DTPA 393.35 g/mol (29) ; therefore DPTA 5 mg = $5x10^{-3}$ g

According to mol = g/MW

mol = ($5x10^{-3}$ g) / (393.35 g/mol)

mol = $0.013x10^{-3}$ mol

As MW of $SnCl_2 \cdot 2H_2O$ = 225.64 g/mol (30) ; therefore 0.6 mg $SnCl_2 \cdot 2H_2O$ = 0.6×10^{-3} g

mol = g/MW

mol = (0.6×10^{-3} g) / (225.64 g/mol)

mol = 0.0027×10^{-3} mol

Amount of PSMA-HBED-CC calculation

As MW of PSMA-HBED-CC = 947 g/mol (31) ; therefore PSMA-HBED-CC 10 µg = 10×10^{-6} g

mol = g/MW

mol = (10×10^{-6} g) / (947 g/mol)

mol = 0.011×10^{-6} mol

From DTPA 0.013×10^{-3} mol requires $SnCl_2 \cdot 2H_2O$ = 0.0027×10^{-3} mol

PSMA-HBED-CC 0.011×10^{-6} mol requires

$SnCl_2 \cdot 2H_2O$ = ((0.011×10^{-6} mol) x (0.0027×10^{-3} mol)) / (0.013×10^{-3} mol)

= 0.0023×10^{-6} mol

From mol = g/MW

(0.0023×10^{-6} mol) = g / (225.64 g/mol)

g = (0.0023×10^{-6} mol) x (225.64 g/mol)

g = 0.52×10^{-6} g

Then, $SnCl_2 \cdot 2H_2O$ for PSMA-HBED-CC labeling with ^{99m}Tc-pertechnetate is 0.52 µg

3.10.3 Reducing agent preparation: 4% $SnCl_2 \cdot 2H_2O$ solution

3.10.3.1 Preparation of 6 N HCl (10 mL)

Distilled water 4.7 mL was added in concentrate HCl 5.3 mL.

3.10.3.2 Preparation of 4% $SnCl_2 \cdot 2H_2O$

$SnCl_2 \cdot 2H_2O$ 0.144 mg was add to concentrate HCl 0.75 mL, heated 100 °C 5 minutes. After cool down to room temperature 6 N HCl 2.25 mL was

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added to be 4% $SnCl_2 \cdot 2H_2O$ solution. This reducing agent should prepare every time when label Tc-99m with PSMA-HBED-CC for concentration stability.

3.10.4 ^{99m}Tc-PSMA-HBED-CC labeling process

4% $SnCl_2 \cdot 2H_2O$ solution is added to PSMA-HBED-CC 10 µg in H_2O 100 µL in 10 mL of sterile vial. After mixing, $^{99m}TcO_4^-$ 370 MBq was added to the sterile vial, heated to 100 ^{o}C 15 minutes. Incubate 10 minutes while cool down to room temperature.

3.10.5 ^{99m}Tc-PSMA-HBED-CC quality control

The radiochemical yield was calculated from the count of radioactivity which was obtained by TLC using silica paper strips in acetone for free $^{99m}TcO_4^-$ and 0.9% saline for hydrolyze reduce form systems. Radiochemical purification was performed by solid phase extraction. Crude product was loaded into C-18 cartridge. ^{99m}Tc-PSMA-HBED-CC was trapped inside C-18 cartridge and free Tc-99m was released to waste vial. Finally, labeling product was eluted from C-18 cartridge using ethanol and water 1:1 ratio (2 mL) to obtain pure ^{99m}Tc-PSMA-HBED-CC.

Even though the stability test is not required in this research objective, it is performed to enhance research content. The stability test of ^{99m}Tc-PSMA-HBED-CC was checked by TLC monitoring to make sure that no free Tc-99m contamination after labeling. It was incubated at room temperature and was checked by TLC every hour in 6 times.

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3.11 Statistical analysis

The descriptive statistics describe the basic features of the data and descriptive statistic parameters in this research. We used Statistical Package for the Social Sciences (SPSS) program for statistical analysis. Mean and standard deviation are the descriptive statistics, which determine the continuous data in this study.

3.12 Limitation

The activity of ^{99m}Tc-pertechnetate was changed according to its decay constant in each labeling experiment. Moreover, $^{99m}TcO_4^-$ remains in the needle or

syringe. Therefore, the final activity of ^{99m}Tc-PSMA-HBED-CC are variable in each experiment.

3.13 Ethical consideration

This study was performed in laboratory using chemical experiment devices. The research proposal was submitted to the ethic committee of Faculty of medicine, Chulalongkorn university [APPENDIX B].

3.13.1 Respect for person

This is basic science research. The experimental study is performed in laboratory therefore no personal data is involved.

3.13.2 Beneficence/Non-maleficence

No Beneficence/Non-maleficence applies to this research.

3.13.3 Justice

No justice applies to this research.

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CHAPTER 4
RESULTS

4.1 Radiochemical yields and radiochemical purity

4.1.1 The optimal labeling condition of ^{99m}Tc-PSMA-HBED-CC

The radionuclide used in this research is Tc-99m, the lightest element. According to the position in periodic table, technetium is located in the seventh group in the middle of metallic transition therefore it exhibits 9 oxidation states from -1 to +7. The most stable form, which obtains via ^{99}Mo/^{99m}Tc generator, is sodium pertechnetate ($Na^{99m}TcO_4$). These chemical properties of technetium resulted in variety of coordination complexes with chelators. Therefore, there are several parameters in chelation including the strength and type of reducing agent, pH and nature of coordinating ligands in the chelator (32). These parameters effect the stereochemical structure of technetium complexes with tetrahedral form (N = 4), tetragonal pyramidal form (N = 5), octahedral form (N = 6), capped octahedral (N = 7) and pentagonal bipyramidal form (N = 8). Due to the rationale of this research, the technetium coordination complex should be the same geometry to DTPA as octahedral form. Thus, the labeling condition mimics the labeling procedure of ^{99m}Tc-DTPA.

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The labeling of PSMA-HBED-CC with $^{99m}TcO_4^-$ was investigated by vary parameters in labeling process [Table 4.1]. PSMA-HBED-CC is fixed at 10 µg that is the half amount to labeling of ^{68}Ga-PSMA-HBED-CC in clinical use. According to the formulation in DTPA cold kit, radioactivity of $^{99m}TcO_4^-$ at 370 MBq and pH of solution at 5 are also fixed. So, the amount of $SnCl_2{\cdot}2H_2O$ as reducing agent and labeling temperature are varied to investigate the radiochemical yields.

In the first experiment, the amount of $SnCl_2{\cdot}2H_2O$, which referred to the calculation in chapter 3, is 0.5 µg. The labeling was performed at room temperature in 15 minutes, resulted in radiochemical yield 0.25% while %free of $^{99m}TcO_4^-$ was 99.58%. Next, only reaction temperature was raised to 100 $^{\circ}$C 15 min, which was a hint from standard procedure of ^{68}Ga-PSMA-HBED-CC labeling. The radiochemical yield became

11.11%. This result guided us to fix the reaction condition as the fourth parameter at 100 °C 15 mins. In the 3rd to 8th experiment, the amount of $SnCl_2 \cdot 2H_2O$ is varied to reach the highest radiochemical yield. Increasing amount of $SnCl_2 \cdot 2H_2O$ by 0.5 µg in each experiment from 3rd to 8th demonstrated the maximum radiochemical yield that can be obtained in experiment 6th is 65.91%. Although the amount of $SnCl_2 \cdot 2H_2O$ over 3.5 µg showed the complete chelation between PSMA-HBED-CC and $^{99m}TcO_4^-$, it remarkably caused the increasing of %hydrolyze form and resulted in decreased radiochemical yield. Figure 4.1 shows the relationship between the amount of $SnCl_2 \cdot 2H_2O$ and radiochemical yield that obviously illustrated the best radiochemical yield in 6th experiment.

Table 4.1 The optimal labeling condition of ^{99m}Tc-PSMA-HBED-CC

Exp.	PSMA (µg)	$SnCl_2 \cdot 2H_2O$ (µg)	$^{99m}TcO_4^-$ (mCi)	Labeling Condition (°C)	pH	Bg	Free $^{99m}TcO_4^-$ (%)	Hydrolyzed (%)	Yield (%)
1	10	0.5	10.02	room temp. 15 mins.	5	0	99.58	0.17	0.25
2	10	0.5	10.32	100 °C, 15 mins.	5	0	88.89	0.00	11.11
3	10	1.0	9.97	100 °C, 15 mins.	5	0	81.82	0.00	18.18
4	10	2.0	10.16	100 °C, 15 mins.	5	1	64.29	10.00	25.71
5	10	2.5	10.61	100 °C, 15 mins.	5	0	20.00	20.00	60.00
6	10	3.0	11.02	100 °C, 15 mins.	5	0	9.09	25.00	65.91
7	10	3.5	11.64	100 °C, 15 mins.	5	0	0.00	41.67	58.33
8	10	4.0	11.15	100 °C, 15 mins.	4	0	0.00	50.00	50.00

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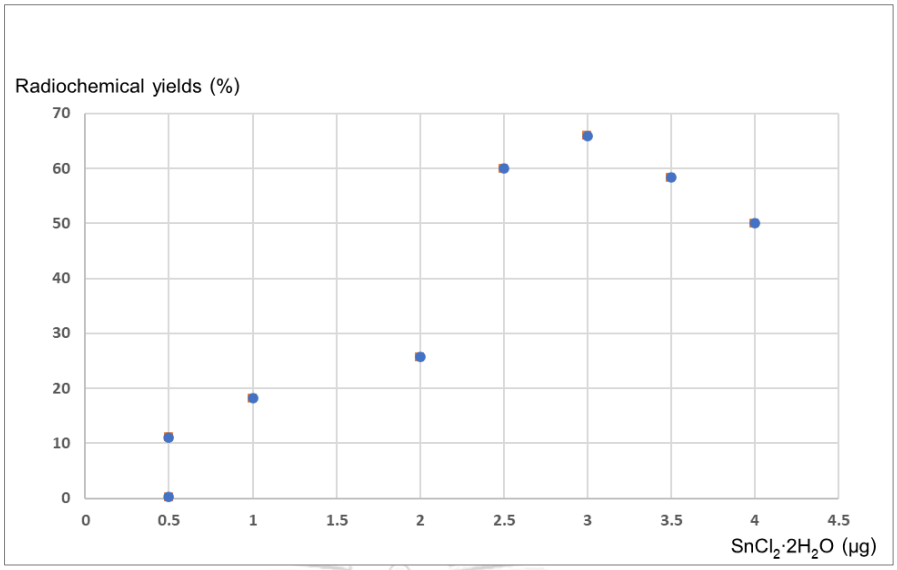

Figure 4.1 Relationship of $SnCl_2 \cdot 2H_2O$ (µg) and radiochemical yield (%)

4.1.2 Replication of the best labeling condition of ^{99m}Tc-PSMA-HBED-CC

Replication of the best labeling condition of ^{99m}Tc-PSMA-HBED-CC was performed to confirm reproducibility for 6 times with statistical reliability. SPSS is used for statistical analysis. The range of radiochemical yield was consistent in 67-75% (71.49±2.42). In 10th experiment, the radiochemical yield was 67.5%. It was dropped from other experiments because PSMA ligand was incompletely drawn out from the vial.

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Table 4.2 The replication of best labeling condition of ^{99m}Tc-PSMA-HBED-CC

Exp.	PSMA (µg)	$SnCl_2 \cdot 2H_2O$ (µg)	$^{99m}TcO_4^-$ (mCi)	Labeling Condition (°C)	pH	Bg	Free $^{99m}TcO_4^-$ (%)	Hydrolyzed (%)	Yield (%)	Purity (%)
9	10	3.0	11.35	100 °C, 15 mins.	5	0	14.29	12.50	73.21	100.00
10	10	3.0	10.61	100 °C, 15 mins.	5	0	20.00	12.50	67.50	100.00
11	10	3.0	10.13	100 °C, 15 mins.	5	0	9.09	20.00	70.91	100.00
12	10	3.0	10.94	100 °C, 15 mins.	5	0	14.29	11.11	74.60	94.74
13	10	3.0	10.81	100 °C, 15 mins.	5	0	13.33	15.79	70.88	96.00
14	10	3.0	10.48	100 °C, 15 mins.	5	0	18.18	10.00	71.82	100.00

Moreover, ^{99m}Tc-PSMA-HBED-CC labeling replications of the best labeling condition in 9th to 14th experiments were purified with SPE C-18 cartridge and confirmed by TLC, as shown in table 4.2. After purification, radiochemical purities are about 94.74%-100% (98.29±2.65). In 12th and 13th experiments, radiochemical purities decreased to 94.74% and 96% respectively. These decreasing radiochemical yields were occurred because washing speed with normal saline was too fast. By the way, the consistence of washing speed should be well controlled in order to confirm that a final product was transferred from SPE C-18 cartridge.

4.1.3 Stability test of ^{99m}Tc-PSMA-HBED-CC

The stability test of ^{99m}Tc-PSMA-HBED-CC is evaluated by incubated the purified product at room temperature for 6 hours and was checked radiochemical purity by TLC monitoring to make sure that no free Tc-99m contamination after labeling every hour in 6 times. The results are shown in table 4.3.

IAEA recommended that the radiochemical purity must be more than or equal to 95% (26). The results in this study show the radiochemical purity of ^{99m}Tc-PSMA-HBED-CC more than 95% purity within 4 hrs after labeled process and more than 90% at 5th hr and 6th hr.

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Table 4.3 The ^{99m}Tc-PSMA-HBED-CC stability test

Hr.	Bg	Free $^{99m}TcO_4^-$ (%)	Hydrolyzed (%)	Purity (%)
1	0	0.00	0.00	100
2	0	0.00	0.00	100
3	0	0.00	0.00	100
4	0	0.00	0.00	100
5	0	6.25	0.00	93.75
6	0	8.33	0.00	91.67

4.2 Quality control of dose calibrator system

The quality control of dose calibrator was evaluated in terms of physical inspection, accuracy and precision. The results are shown in APPENDIX A.

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CHAPTER 5
DISCUSSION AND CONCLUSION

5.1 Discussion

In December 2020, U.S. Food and Drug Administration approved ^{68}Ga-PSMA-HBED-CC as the first positron emission tomography (PET) imaging of PSMA positive lesions in men with prostate cancer. This strong evidence motivated us to develop ^{99m}Tc-PSMA-HBED-CC as its analogue utilizing in SPECT modality to ameliorate access to PCa diagnosis in widespread.

Tc-99m remains the radionuclide that widely used for SPECT imaging and has been modified to facilitate the efficient labeling with pharmaceutical in nuclear medicine field. Currently, Tc-99m is one of alternative radionuclides for labeling with PSMA for prostate cancer as well.

^{99m}Tc-PSMA labeling had been previously reported (12), (13), (14), (15), (16), (17). There are different PSMA motifs such as PSMA MIP-1404, PSMA MIP-1504, etc. that were formed metal complex such as ^{99m}Tc-HYNIC and $^{99m}Tc(CO)_3^+$. From pioneer research, there were some drawbacks that could be improved in methodology to provide more practical labeling such as complicated preparation methods and time-consuming procedure. To overcome these drawbacks and with nuclear medicine chemist's experiences in ^{68}Ga-PSMA-HBED-CC preparation, PSMA motif with HBED-CC, an open-chain chelator was an appropriate ligand to label with Tc-99m at high temperature. According to the rationale in this research, the HBED-CC as a chelator was investigated to chelate with Tc-99m.

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In the present study, we successfully chelated PSMA-HBED-CC with $^{99m}TcO_4^-$ to form a complex at high temperature because the entropy of reaction at room temperature was not enough to force the chelation forward. This study revealed that general procedure of ^{99m}Tc-PSMA-HBED-CC labeling should be performed at 100 ^{o}C 15 mins with appropriate amount of 3 µg $SnCl_2 \cdot 2H_2O$ to reduce oxidation state of Tc-99m.

In radiochemical purity QC process, TLC silica gel paper strips should be kept away from high humidity because it can lead to high hydrolyzed form. Therefore, silica

paper strips are recommended storage at temperatures 18-20 °C with regulated humidity in desiccator (27). In addition, radionuclidic impurities can affect the percentage of labeling efficiency. It was recommended that, $^{99m}TcO_4^-$ should be validated the amount of Mo-99 break through and aluminum contamination before use (25), (33).

In addition, the stability of ^{99m}Tc-PSMA-HBED-CC was reported in this study. The stability is evaluated by incubation at room temperature for 6 hours and is checked radiochemical purity by TLC to make sure that no free Tc-99m contamination every hour in 6 times. Therefore, recommend to use within 4 hrs after labeled at room temperature storage.

5.2 Conclusion

The labeling of PSMA-HBED-CC with ^{99m}Tc-pertectnetate has been improved for prostate cancer imaging. The general procedure of ^{99m}Tc-PSMA-HBED-CC labeling performed at 100 °C 15 mins with appropriate amount of 3 µg $SnCl_2 \cdot 2H_2O$, 370 MBq of $^{99m}TcO_4^-$ to give high radiochemical yield and high radiochemical purity. The radiochemical purity of ^{99m}Tc-PSMA-HBED-CC is stable at more than 95% within 4 hrs at room temperature after labeling process. Therefore, it is recommended to use within 4 hrs after labeled.

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APPENDICES

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APPENDIX A

Quality control of dose calibrator

Instrument Dose calibrator CRC-25 R

Date 28 March 2021

Test 1. Physical inspection

2. Accuracy and precision

1. Test of physical inspection

Objective To inspect a radionuclide calibrator for general condition.

Method Physical inspection is tested by dose calibrator automatic system

1. System electronic test
2. Clock accuracy test
3. High voltage test
4. Zero adjustment test
5. Background test

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Results

Test	Results
Physical inspection test	Pass
System electronic test	Pass
Clock accuracy test	Pass
High voltage test	156.00 V
Zero adjustment test	- 0.00 mV
Background test	18.14 μCi

2. Test of accuracy and precision

Objective To test the accuracy and precision of radionuclide calibrator in activity measurements in standard geometry at selected gamma ray.

Materials Radioisotope standard source (Co-57, Ba-133, Cs-137)

Radioisotope standard source

Radioisotope	Energy (keV)	Half-life
Co-57	122	271 days
Ba-133	81, 456	10.7 years
Cs-137	662	30 years

Methods

1. Remove the source holder and note the background
2. Insert the source into the source holder
3. Record the activity for accuracy test and repeat step 2 of 10 successive measurements for precision test

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Data analysis

Accuracy test

$$\% = \frac{100(\bar{A}-C)}{C}$$

$\bar{A}$: The measured activity

C : The certified activity of the source corrected for radioactive decay to the day of measurement

$$A_t = A_0 e^{-\lambda t} \ , \ \lambda = \frac{0.693}{T_{1/2}}$$

A_t : Activity at time t

A_0 : Initial activity

λ : Decay constant

t : Time

Precision test

$$\% = \frac{100(A_i - \bar{A})}{\bar{A}}$$

$\bar{A}$: The measured activity

A_i : The individual measured activities

Results

Accuracy test result

Radionuclide	Activity (μCi)		Differences (%)
	Calculated	Measured	
Co-57 **Initial activity** 5950 μCi **Ref. date** 26/01/2021	5090 μCi	5220 μCi	$\frac{100\ (5220 - 5090)}{5090}\% = 2.55\%$
Ba-133 **Initial activity** 264 μCi **Ref. date** 27/11/2017	212 μCi	211.00 μCi	$\frac{100\ (211 - 212)}{212}\% = -0.47\%$

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Radionuclide	Activity (μCi)		Differences (%)
	Calculated	Measured	
Cs-137 **Initial activity** 267 μCi **Ref. date** 01/07/2006	190 μCi	197 μCi	$\frac{100\ (197-190)}{190} = 3.68\%$

Limits of Acceptability **± 10%** ☑ **Accept**

Precision test result

No.	Radionuclide activity (A_i)					
	Co-57 (μCi)		Ba-133 (μCi)		Cs-137 (μCi)	
	Bg = 0	$\% = \frac{100(A_i - \bar{A})}{\bar{A}}$	Bg = 0	$\% = \frac{100(A_i - \bar{A})}{\bar{A}}$	Bg = 0	$\% = \frac{100(A_i - \bar{A})}{\bar{A}}$
1	5220	0.09	211	0.09	197	-0.15
2	5210	-0.09	210	-0.38	197	-0.15
3	5220	0.09	211	0.09	197	-0.15
4	5220	0.09	211	0.09	197	-0.15
5	5210	-0.09	211	0.09	198	0.35
6	5210	-0.09	211	0.09	197	-0.15
7	5220	0.09	211	0.09	198	0.35
8	5220	0.09	211	0.09	198	0.35
9	5210	-0.09	210	-0.38	197	-0.15
10	5210	-0.09	211	0.09	197	-0.15
$\bar{A}$	5215		210.8		197.3	

Limits of Acceptability **± 5%** ☑ **Accept**

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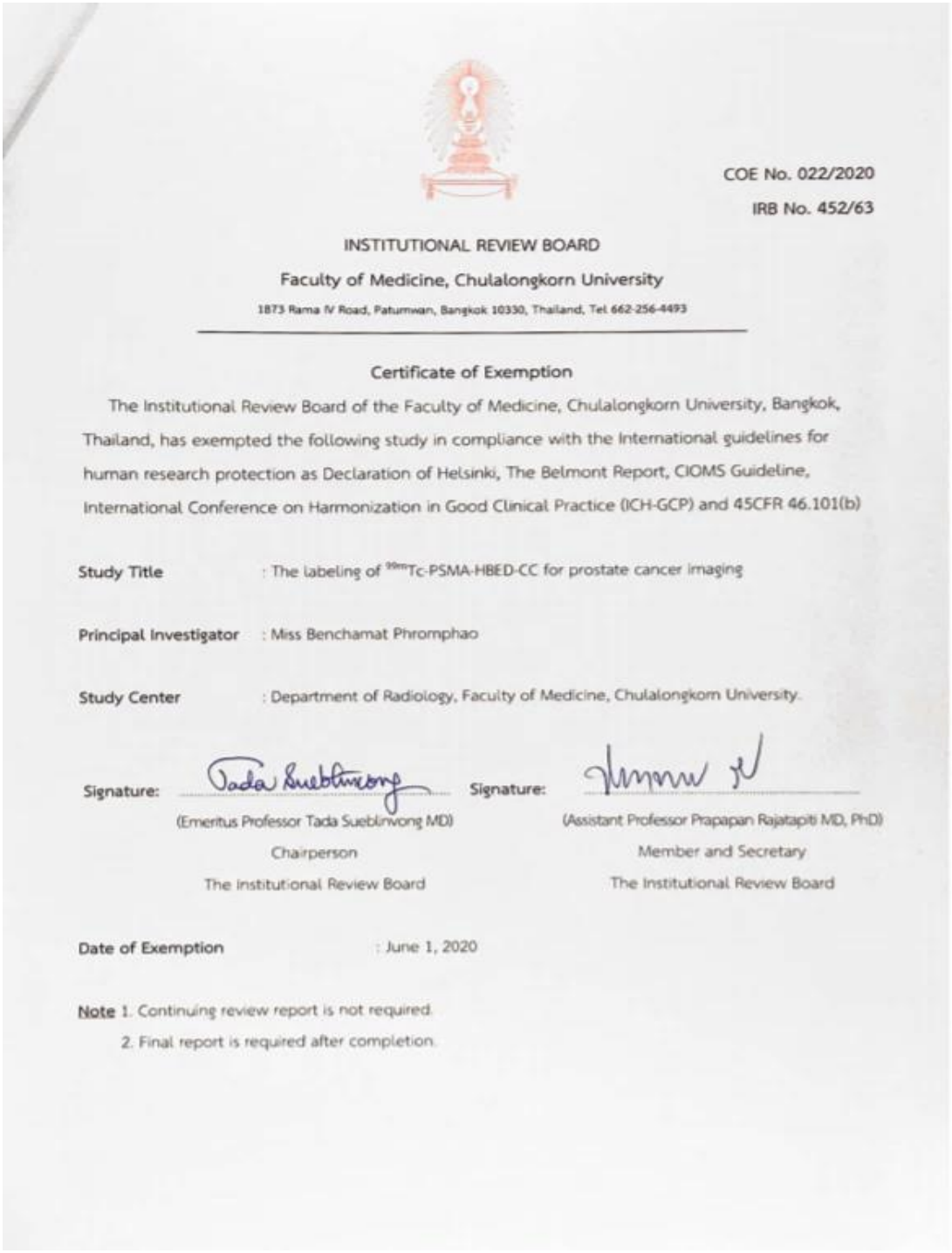
APPENDIX B

The approval of institution review board

The research proposal was exemption on June 1, 2020 by the Institutional Review Board Faculty of Medicine, Chulalongkorn University (IRB No.452/63).



COE No. 022/2020
IRB No. 452/63

INSTITUTIONAL REVIEW BOARD
Faculty of Medicine, Chulalongkorn University
1873 Rama IV Road, Patumwan, Bangkok 10330, Thailand, Tel 662-256-4493

Certificate of Exemption

The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, has exempted the following study in compliance with the International guidelines for human research protection as Declaration of Helsinki, The Belmont Report, CIOMS Guideline, International Conference on Harmonization in Good Clinical Practice (ICH-GCP) and 45CFR 46.101(b)

Study Title : The labeling of ^{99m}Tc-PSMA-HBED-CC for prostate cancer imaging

Principal Investigator : Miss Benchamat Phromphao

Study Center : Department of Radiology, Faculty of Medicine, Chulalongkorn University.

Signature: Tada Sueblinvong
(Emeritus Professor Tada Sueblinvong MD)
Chairperson
The Institutional Review Board

Signature:
(Assistant Professor Prapapan Rajatapiti MD, PhD)
Member and Secretary
The Institutional Review Board

Date of Exemption : June 1, 2020

Note 1. Continuing review report is not required.
2. Final report is required after completion.

APPENDIX C

Data record form

The optimal labeling condition of ^{99m}Tc-PSMA-HBED-CC

Exp.	PSMA (µg)	$SnCl_2 \cdot 2H_2O$ (µg)	$^{99m}TcO_4^-$ (mCi)	Labeling Condition (°C)	pH	Free $^{99m}TcO_4^-$ (%)	Hydrolyzed (%)	Yield (%)

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The replication of best labeling condition of ^{99m}Tc-PSMA-HBED-CC

Exp.	PSMA (µg)	$SnCl_2 \cdot 2H_2O$ (µg)	$^{99m}TcO_4^-$ (mCi)	Labeling Condition (°C)	pH	Free $^{99m}TcO_4^-$ (%)	Hydrolyzed (%)	Yield (%)	Purity (%)

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APPENDIX D

Laboratory record form

^{99m}Tc-PSMA-HBED-CC labeling

Date .....

Materials and methods

1. $SnCl_2 \cdot 2H_2O$ solution preparation
   1.1 6N HCl preparation
      1.1.1 Conc. HCl 5.3 mL
      1.1.2 Sterile water 4.7 mL
      1.1.3 Mixture and 6N HCl resulting in a solution with 10 mL.
   1.2 4% $SnCl_2 \cdot 2H_2O$ solution preparation
      1.2.1 Boil distilled water 100 mL at 100 ^{O}C on the hot plate.
      1.2.2 Add Conc. HCl 0.75 mL in $SnCl_2 \cdot 2H_2O$ 0.144 g test tube
         $SnCl_2 \cdot 2H_2O$ = ... g
      1.2.3 Boil (1.2.2) in water-bath 5 mins for dissolving.
      1.2.4 After 5 mins add 6N HCl 2.25 mL.
      1.2.5 Nature of the solution = ... pH = ...

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Date .....

2. 4% $SnCl_2 \cdot 2H_2O$ ..... µg, ... mL, pH = ...
3. ^{99m}Tc-PSMA-HBED-CC labeling
   3.1 PSMA-HBED-CC solubility 10 µg with sterile water 100 µL.
   3.2 Add 4% $SnCl_2 \cdot 2H_2O$ to reduce Tc-99m oxidation state in PSMA-HBED-CC, mix and add all to sterile water.
   3.3 Draw $^{99m}TcO_4^-$ 370 MBq (10 mCi) and inject to vial of PSMA-HBED-CC solution without the free air seriously and mixed 10 times, heat at 100 ^{O}C

15 mins, incubate in radioactive fume hood for cooling to room temperature 10-15 mins.

3.4 Perform quality control; measure pH of the product, run TLC for radiochemical yield and radiochemical purity.

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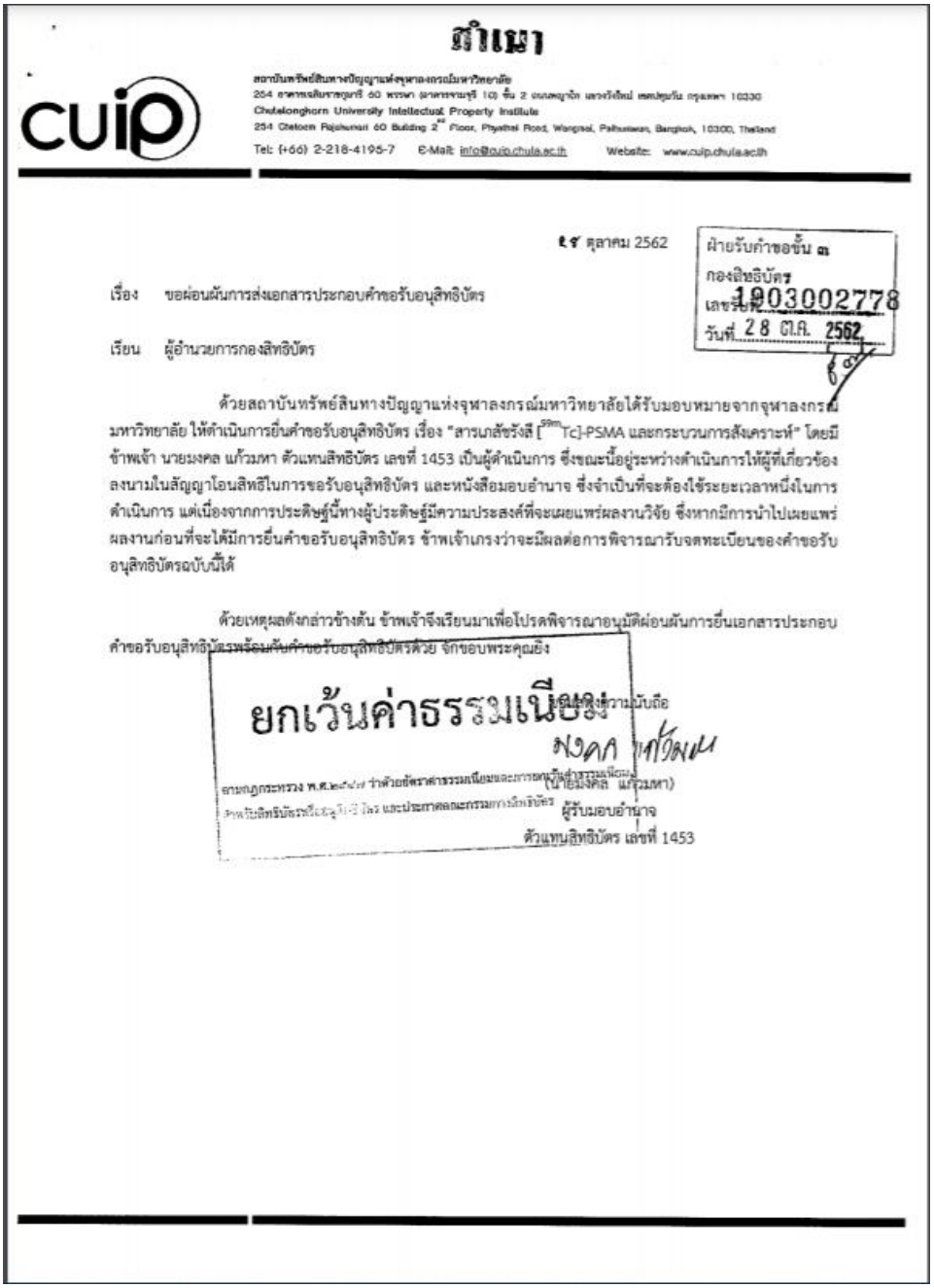
APPENDIX E

Patent of ^{99m}Tc-PSMA-HBED-CC

This original research is novel concept. Therefore, we take this opportunity to register as intellectual property between research inventors and Chulalongkorn university. The patent "^{99m}Tc-PSMA-HBED-CC and its labeling method". Patent no. 1903002778.



สถาบันทรัพย์สินทางปัญญาแห่งจุฬาลงกรณ์มหาวิทยาลัย
254 อาคารเฉลิมพระเกียรติ 60 พรรษา มหาวชิราลงกรณ์ 60 ชั้น 2 ถนนพญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพฯ 10330
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๑๕ ตุลาคม 2562

ฝ่ายรับคำขอชั้น ๓
กองสิทธิบัตร
เลขรับที่ 1903002778
วันที่ 28 ต.ค. 2562

เรื่อง ขอผ่อนผันการส่งเอกสารประกอบคำขอรับอนุสิทธิบัตร

เรียน ผู้อำนวยการกองสิทธิบัตร

ด้วยสถาบันทรัพย์สินทางปัญญาแห่งจุฬาลงกรณ์มหาวิทยาลัยได้รับมอบหมายจากจุฬาลงกรณ์มหาวิทยาลัย ให้ดำเนินการยื่นคำขอรับอนุสิทธิบัตร เรื่อง "สารเภสัชรังสี [^{99m}Tc]-PSMA และกระบวนการสังเคราะห์" โดยมีข้าพเจ้า นายมงคล แก้วมหา ตัวแทนสิทธิบัตร เลขที่ 1453 เป็นผู้ดำเนินการ ซึ่งขณะนี้อยู่ระหว่างดำเนินการให้ผู้ที่เกี่ยวข้องลงนามในสัญญาโอนสิทธิในการขอรับอนุสิทธิบัตร และหนังสือมอบอำนาจ ซึ่งจำเป็นที่จะต้องใช้ระยะเวลาหนึ่งในการดำเนินการ แต่เนื่องจากการประดิษฐ์นี้ทางผู้ประดิษฐ์มีความประสงค์ที่จะเผยแพร่ผลงานวิจัย ซึ่งหากมีการนำไปเผยแพร่ผลงานก่อนที่จะได้มีการยื่นคำขอรับอนุสิทธิบัตร ข้าพเจ้าเกรงว่าจะมีผลต่อการพิจารณารับจดทะเบียนของคำขอรับอนุสิทธิบัตรฉบับนี้ได้

ด้วยเหตุผลดังกล่าวข้างต้น ข้าพเจ้าจึงเรียนมาเพื่อโปรดพิจารณาอนุมัติผ่อนผันการยื่นเอกสารประกอบคำขอรับอนุสิทธิบัตรพร้อมคำขอรับอนุสิทธิบัตรด้วย จักขอบพระคุณยิ่ง

ยกเว้นค่าธรรมเนียม

ตามกฎกระทรวง พ.ศ.๒๕๖๑ ว่าด้วยอัตราค่าธรรมเนียมและการยกเว้นค่าธรรมเนียม
การขอรับสิทธิบัตรหรืออนุสิทธิบัตร และประกาศคณะกรรมการสิทธิบัตร

ขอแสดงความนับถือ
(นายมงคล แก้วมหา)
ผู้รับมอบอำนาจ
ตัวแทนสิทธิบัตร เลขที่ 1453

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PUBLICATION	Phromphao B, Shiratori S. The labeling of 99mTc-PSMA-HBED-CC for prostate cancer imaging. In Proceedings of 20th Asia-Oceania Congress on Medical Physics (AOCMP) - 18th South-East Asia Congress of Medical Physics (SEACOMP) - 12th Annual Meeting of Thai Medical Physicist Society (TMPS), Phuket, Thailand, 2020

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