PURIFICATION AND CONCENTRATION OF GALLIUM-68 VIA ANION EXCHANGE METHOD FROM A SNO_2 -BASED COLUMN GERMANIUM-68/ GALLIUM-68 GENERATOR

Muhammad Fakhrurazi Ahmad Fadzil[1], Mohd Khairul Najah[2], Ng Yen[1], Siti Najila Mohd Janib[1], Noor Hasnah M. Khairullah[1]

[1]Medical Technology Division, Malaysian Nuclear Agency, Bangi 43000 Kajang, Selangor.

[2]*Nuclear Medicine Department, National Cancer Institute, 62250 Putrajaya.*

ABSTRACT

PET/CT Scan with ^{68}Ga-labelled analogs is of increasing interest in Nuclear Medicine and currently is being performed all over the world. However, such labeling procedure requires high purity and concentrated solutions of ^{68}Ga. Here we report the purification and concentration of the eluate of SnO_2-Based $^{68}Ge/^{68}Ga$ generators via the anion exchange method. Three different anion columns were selected to purify and concentrate the^{68}Ga eluates which are Chromafix, Oasis WAX and AG 1-X8 columns. The different anion columns were compared and evaluated in terms of their capability in adsorption and desorption of ^{68}Ga from the generator. While the optimum molarity of Hydrocholric Acid (HCl) for highest ^{68}Ga retention was also determined starting from the ranges of 4M to 7M of HCl. The results showed that the percentage of ^{68}Ga retention or adsorption started to be plateau at molarity of 5.5M for all three anion cartridges. One-way ANOVA analysis proved that there is no significant difference between 5.5M with 6.0M, 6.5M and 7.0M which means that the retention of gallium-68 is equal at those molarities. At 5.5M, Chromafix and Oasis WAX cartridges showed the highest retention of gallium-68 which is 98.30%. The lowest ^{68}Ga retention was gained by AG 1-X8 column which is 97.07%. While for desorption of ^{68}Ga, the highest percentage was obtained by using Oasis WAX cartridges which is 70.49% followed by Chromafix which is 70.36%. The lowest desorption of gallium-68 was obtained by using AG 1-X8 column which is only 58.56%. Therefore, from this study, the most suitable cartridge and HCl molarity that should be applied in purification and concentration of Gallium-68 eluate from a SnO_2 Based $^{68}Ge/^{68}Ga$ generator is Oasis® WAX column with a HCl molarity of 5.5M respectively.

ABSTRAK

PET / CT Scan dengan analog 68Ga-labelled adalah faedah yang meningkat dalam Ubat Nuclear dan kini sedang diusahakan seluruh dunia. Bagaimanapun, prosedur melabelkan sedemikian memerlukan kesucian yang tinggi dan larutan pekat 68Ga. Di sini kami melaporkan penulenan dan tumpuan eluat 68Ge -Based SnO2 / generator-generator 68Ga melalui kaedah pertukaran anion. Tiga tiang-tiang anion yang berbeza dipilih menyucikan dan menumpukan the68Ga eluates yang mana ialah Chromafix, Oasis WAX and AG tiang-tiang 1-X8. Tiang-tiang anion yang berbeza telah dipertandingkan dan dinilaikan dalam soal kemampuan mereka dalam penjerapan dan desorption of 68Ga dari penjana. Manakala kemolaran optimum Hydrocholric Acid untuk pengekalan 68Ga tertinggi juga ditentukan mula dari banjaran 4M kepada 7M of HCl. Keputusan menunjukkan bahawa peratusan pengekalan 68Ga atau penjerapan dimulakan untuk menjadi dataran tinggi di kemolaran 5.5M untuk semua tiga sarung peluru anion. Analisis ANOVA yang sehala membuktikan bahawa tiada perbezaan penting antara 5.5M dengan 6.0M, 6.5M and 7.0M yang bermaksud pengekalan galium 68 menyamai

di yang molarities. Di 5.5M, kartrij-kartrij Chromafix and Oasis WAX menunjukkan pengekalan tertinggi galium 68 yang mana 98.30%. Pengekalan 68Ga terendah telah diperolehi oleh AG lajur 1-X8 yang mana 97.07%. Manakala untuk desorption of 68Ga, peratusan tertinggi telah diperolehi dengan menggunakan kartrij-kartrij Oasis WAX yang mana 70.49% diikuti oleh Chromafix yang mana 70.36%. Penyaherapan terendah galium 68 telah diperolehi dengan menggunakan AG lajur 1-X8 yang mana hanya 58.56%. Lantarannya, daripada kajian ini, kartrij paling sesuai dan kemolaran HCl yang seharusnya digunakan dalam penulenan dan tumpuan 68 eluat, Gallium dari SnO2 Based 68Ge / penjana 68Ga ialah lajur Oasis® WAX dengan kemolaran HCl 5.5M masing-masing.

Keywords: Anion Exchange, Gallium-68, PET/CT Scan, Germanium-68, Generator

INTRODUCTION

Background

Nuclear medicine is a specialty of medicine and medical imaging that uses radionuclides (radiopharmaceuticals) in a very small amount and relies on the process of radioactive decay in the diagnosis and treatment of disease. It is the safe use of radioactive materials in the diagnosis and treatment of various diseases. In imaging, the radiopharmaceuticals are detected by special types of cameras like gamma camera that work with computers that have special software in order to provide very precise pictures about the area of the body being imaged. For a treatment purpose, the radiopharmaceuticals go directly to the organ being treated. The amount of radiation in a nuclear typical treatment is kept within safe limits.

Certain compounds (pharmaceuticals) which by their nature, concentrate in different organs of their human body, are chemically labelled with specific radioactive materials (radioisotopes). These radiopharmaceuticals, once administered, concentrate within the organ or organ system and the distribution is determined by specialised equipment.

Nuclear medicine was started with the invention of the cyclotron by Ernest Orlando Lawrence (1901-1958) and the widespread clinical use of nuclear medicine began in the early 1950s. In Malaysia, nuclear medicine unit was formed in 1964 as a subunit under the Department of Radiotherapy, Kuala Lumpur Hospital.

In nuclear medicine procedures, elemental radiopharmaceuticals are combined with other elements to form chemical compounds, or else combined with existing pharmaceutical compounds, to form radiopharmaceuticals. These radiopharmaceuticals, once administered to the patient, can localize to specific organs or cellular receptors. This property of radiopharmaceuticals allows nuclear medicine the ability to image the extent of a disease-process in the body according to the cellular function and physiology of particular organs.

Positron Emission Tomography (PET)

Positron emission tomography (PET) is an imaging method in nuclear medicine. It combines the potential to quantify the tracer uptake within lesions with a relatively high resolution. High resolution means that the picture of a specific organ or area in the human body can be displayed in a high quality. In addition, biomolecules are frequently labeled with 'biological' positron-emitting isotopes such as ^{11}C, ^{13}O, ^{15}N which render any molecule chemically unchanged compared to the original molecule.

The most commonly used PET-radionuclide is ^{18}F and is being produced in a cyclotron. Another strategy to produce positron-emitting radionuclides is via a generator such as ^{68}Ge/^{68}Ga, ^{82}Sr/^{82}Rb and the ^{62}Zn/^{62}Cu generators. One of the advantages of generators is they allow clinical studies without an on-site cyclotron or if cyclotron beam time may not be available.

Chemistry of Gallium

Gallium is the third element of Group 13 of the Periodic Table. The +3 oxidation state of this metal is the most stable in aqueous solution. Ga^{2+} would exist in the presence of high concentration of Cl^- ions but it is thermodynamically unstable to further oxidation to Ga^{3+}. In aqueous solution, the free hydrated Ga^{3+} ion is stable only under acidic conditions. In the pH range of 3-7, it can hydrolyse to insoluble trihydroxide if its concentration exceeds nanomolar level. However, this precipitation can be avoided in the presence of stabilizing agents. At physiological pH, the solubility of gallium is high due to the almost exclusive formation of $[Ga(OH)_4]^-$ ions.

In order to be suitable as a radiopharmaceutical, a Ga^{3+} chelate has to be thermodynamically stable towards hydrolysis at physiological pH or to be kinetically stable during the period of clinical use. Another requirement is the Ga^{3+} chelate also does not undergo exchange with the abundant blood serum protein transferrin.

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

The ^{68}Ge/^{68}Ga generator provides an excellent source of positron-emitting ^{68}Ga. Radionuclide generators have the advantage of providing radionuclides on demand. This is potentially an inexpensive and convenient alternative to the on-site cyclotron production of short-lived radionuclides. Radionuclide generators usually contain a long-lived parent radionuclide which decays to a short-lived daughter. There needs to be a sufficient chemical difference to allow efficient chemical separation of the stationary parent from the soluble daughter. The development of the ^{68}Ge/^{68}Ga generator has been reviewed in several articles (Lambrecht & Sajjad 1988; Mirzadeh & Lambrecht 1996). The long half-life ($t_{1/2}$ =270.8 days) of the parent ^{68}Ge combined with the half-life of ^{68}Ga ($t_{1/2}$=68 min), suitable for radiopharmaceutical synthesis, makes this pair ideal for a generator strategy.

The preferred production route of ^{68}Ge is via the (p, 2n) reaction on gallium targets (Ga_2O_3). This reaction provides a significant cross section but experimental yields amount to 0.74 MBq/μ A/h only; therefore high current accelerators are needed for sufficient batch yields (Rösch and Knapp 2003).

An important aspect for wide use of ^{68}Ga in clinical PET is its chemical form and concentration after elution from the generator. In addition, there is concern about ^{68}Ge-breakthrough and contamination of the generator column material. Nowadays, TiO_2-based generator has become commercially available.

Due to the long half-life of ^{68}Ge, a good separation system of mother and daughter to avoid breakthrough is mandatory. Two different strategies have been employed to afford this separation of ^{68}Ge (IV) from ^{68}Ga (III).

Metal ions in eluate

The presence of metal ions, especially Zn in the eluate is frequently a concern for labelling DOTA-peptides with radiometals as ^{68}Ga, since the incorporation of radiometals in DOTA-peptides is negatively influenced (Breeman et al., 2005, 2003). Zn ions will always be present in the eluate of ^{68}Ge/^{68}Ga generators, due to formation of ^{68}Zn as decay product of ^{68}Ga.

The amount of formed ^{68}Zn is dependent on the time between prior elution. All other metals are low (<1 ppm), including Sn as also reported by Loc'h et al. (1980). Although the concentration of these metals (Zn, Sn, Fe and Ti) is low, their concentration can be much higher than the concentration of ^{68}Ga.

Ion Exchange Column in ^{68}Ga Eluate Purification

The most popular method for the purification of charged molecules is ion exchange chromatography which also was known as ion exchange column. In anion exchange column, negatively charged molecules are attracted to a positively charged solid support. Conversely, in cation exchange chromatography, positively charged molecules are attracted to a negatively charged solid support. Anion exchange columns include AG 1-X8 resins, Chromafix and Oasis Wax cartridges.

In order to optimize of all charged molecules, generally, the mobile phase is a low to medium conductivity solution. The adsorption of the molecules to the solid support is driven by the ionic interaction between the oppositely charged ionic groups in the sample molecule and in the functional ligand on the support. . The strength of the interaction is determined by the number and location of the charges on the molecule and on the functional group.

By increasing the salt concentration (generally by using a linear salt gradient) the molecules with the weakest ionic interactions start to elute from the column first. Molecules that have a stronger ionic interaction require a higher salt concentration and elute later in the gradient. The binding capacities of ion exchange resins are generally quite high. This is of major importance in process scale chromatography.

Although the commercially available 68Ge/ 68Ga generator system provides an excellent source of 68Ga but not optimized for the routine synthesis of 68Ga-labelled radiopharmaceuticals. The eluates have low specific volume of 68Ga, high acid concentration and different trace elements. Hence, pre-concentration and purification of the initial generator eluate should be done.

BACKGROUND

The ^{68}Ge/^{68}Ga radionuclide generator provides an excellent source of positron emitting ^{68}Ga for the routine synthesis and application of ^{68}Ga-labelled peptides using positron emission tomography (PET). Currently, the method of ^{68}Ga purification from SnO_2-based ^{68}Ge/^{68}Ga generator is via fractionation method. There are several problem imposed by adopting the fractionation method in the radiolabelling processes.

One of them is low percentage of recovery of Ga-68. Furthermore, the current eluation system of the Ga-68 produces low specific volume of ^{68}Ga eluate within the 2 ml fraction of 0.6M HCl. One of the factors that contribute to this matter is the 'dead volume' remains in the outlet tubing to the reactor. The eluates have low specific volume of ^{68}Ga, high acid concentration, a breakthrough of ^{68}Ge of 10^{-2}% increasing with time or usage frequency and impurities such as stable Zn(II) generated by the decay of ^{68}Ga and Fe(III) as a general impurity (Decristoforo et al., 2007; Erik et al., 2010; Mattia et al., 2008;). Hence, pre-concentration and purification of the initial generator eluate is mandatory.

Secondly, the obtained volume of Ga-68 also is not consistent. This problem is also due to the 'dead volume' remains within the outlet tubing between the generator and the reactor. This condition will cause the adjustment of pH in the reactor become unpredictable and finally may lead to inconsistent labelling yield.

Thirdly, high acidity of Ga-68 eluate (0.6M in 2ml fraction) from the generator will affect the radio labelling activity. The rather large volume (up to 10 ml for complete elution) and high proton concentration of the generator eluate require pre-concentrationof the activity for labeling nanomolar amounts of peptides. For all these reasons, purification and concentration of the ^{68}Ga-eluate must be performed before labelling step (Mattia et al., 2008).

Currently, the method that are being practiced for the SnO_2 Based Generator is by fractionation method in order to purify and concentrate the Ga-68 eluate. However, this kind of method does not totally eliminate the metallic impurities instead of only decrease the impurities (Mattia et al., 2008). Purification method of the generator-produced ^{68}Ga and requires an eluate fractionation (Breeman et al., 2005). Although this approach does not eliminate but only decreases the metallic impurities, it has been successfully employed for ^{68}Ga-DOTATOC synthesis (Breeman et al., 2005; Decristoforo et al., 2007).

One of the methods to purify Ga(III) is by using a cation-exchange column which eluted with HCl/acetone mixtures was reported (Strelow et al., 1971). ^{68}Ga in 0.1 M HCl can be quantitatively absorbed on acation-exchange resin and further purified from Ge, Ti, Fe, Zn and Al impurities by adjusting the HCl concentration and HCl/ acetone ratio of the eluent (Zhernosekov et al., 2007). Finally, ^{68}Ga can be released from the resin in a chemical form suitable for labelling by increasing the acetone content. The overall content of acetone in the purified Ga-68 fraction is small and non-toxic. However, this method could not be done in the research project because the ^{68}Ge/ ^{68}Ga generator that has been used in the experiments was using 0.6M HCl to eluate the Ga-68 from the generator where the eluate is in Ga^{3+} ions form. Ga^{3+} ions have higher tendency to form the negatively charged gallium tetrachloro complex $[GaCl4]^-$ complex that can be adsorbed on a strong anion exchange resin (Meyer et al., 2004) instead of cation exchange column.

Purification and concentration procedures of Ga-68 eluate also can be done via solvent extraction method where an organic solvent has been used like methyl ethyl ketone (MEK). According to T.H. Bokhari et al. 2008, this method showed that at 1:2 ratio of eluate and MEK, almost 100% Ga-68 activity is extracted in MEK. The extraction is finished within 2 to 4 minutes. The mixing and shaking steps had been done for 4 minutes. Then, the organic solvent (MEK) was evaporated. The purpose of doing so is to concentrate the Ga-68 eluate. The activity of ^{68}Ga recovered was noted after dissolving it in buffer solution. Finally, the whole procedure of ^{68}Ga concentration was finished in 10 min. Although the yield or outcome of the solvent extraction method is good but there are a lot of modifications must be done before running the procedures and it is a time-consuming method. Therefore, this method has not been recommended for the research project.

Recently, another method to purify and concentrate Ga-68 eluate is by increasing the HCl concentration to ~6 M and passing the mixture through an anion exchange column where the anionic $[GaCl_6]^{3-}$and $[GaCl_4]^-$complexes are strongly absorbed, while ^{68}Ge(IV), Al(III), Ti(IV) and In(III) are practically passed through the column. ^{68}Ga is then eluted with <200 µl of pure water [Breeman et al., 2005; Decristoforo et al., 2007; Velikyan et al., 2004).

Furthermore, in this project, anion-exchange column will be used as our method in order to achieve objectives of the project. Ga^{3+} ions form the negatively charged gallium tetrachloro complex $[GaCl_4]^-$ at concentration of HCl is more than 5.0 M. Therefore, this feature can be used advantageously to concentrate the eluted Ga-68, since the $[GaCl_4]^-$complex can be adsorbed quantitatively on a strong anion exchange resin according to Meyer et al. (2004).Ga-68 activity in 6 ml 5 M HCl was adsorbed (>98%) on all anion columns.

Desorption of Ga-68 activity from total Ga-68 activity on anion columns ranged between 50% and 83%. The highest desorption was obtained with the 30mg weak anion Oasis WAX column where 83% was desorbed in 1mL Milli-Q. In order to reduce acidity of the eluate of anion column, H^+ was replaced by Na^+ whilst keeping $[Cl^-]$ constant at 5 M. Furthermore, no statistically significant changes in Ga-68 desorption were observed while decreasing at constant $[Cl^-]$ (Erik de Blois et al. 2010).

Thus, in this study, anion-exchange resin has been chosen rather than fractionation because it can be used to reduce ionic impurities, to increase the concentration of generator eluate and to reduce acidity (Meyer et al., 2004; Velikyan et al., 2004; Zhernosekov et al., 2007). Although fractionation results in a ready to use eluate containing approximately 80% of the elutable Ga-68 activity but here we must be noticed that the major limitations for direct use of Ga-68 for radiolabelling of peptide (DOTA-TATE) are the large volume of generator eluate, high $[H^+]$, Ge-68 breakthrough and also potential metal ion impurities (Erik de Blois et al. 2010).

Furthermore, a study by Velikyan et al. (2004) describes most suitable volume reductions for the ^{68}Ga eluate in which they purified and concentrated the radionuclide by anion exchange resins. By this means the volume of the final aqueous elute could be further decreased to 50-200 µl thus obtaining a high volume activity of 5 to 6 MBq/ µl using a ^{68}Ge/^{68}Ga generator of ~1250MBq initial ^{68}Ga activity (Gebhardt et al., 2010).

Another reason that supports the method of choice is although in fractionation method, contents of ^{68}Ge and metallic impurities are lowered principally but not chemically removed (Zhernosekov et al. 2007).

The paper describes the method to purify and concentrate Ga-68 eluate from a SnO_2-based $^{68}Ge/^{68}Ga$ generator by using anion-exchange purification method; compare the retention and desorption of Ga-68 eluate by using three different types of anion cartridges; identify the optimum hydrochloric acid molarity which can gives the highest retention of ^{68}Ga in three different types of cartridges; determine the most suitable anion-exchange column that can be implemented into the SnO_2-based $^{68}Ge/^{68}Ga$ generator

MATERIALS AND METHODS

Study Design

This research mainly involved laboratory works. Starting from synthesizing the Ga-68 until the last steps would be the desorption step. Proper protection and safety while handling the radioactive compound would be taking under full consideration and pre-caution. Particular attention would be paid at reducing the personnel radiation exposure during the manual operation steps.

$^{68}Ge/^{68}Ga$ generator

A 30 mCi, $^{68}Ge/^{68}Ga$ generator used was SnO_2 Based Column generator manufactured by iThemba LABS (Somerset West, South Africa) was used in this study. The column was loaded with the parent ^{68}Ge (half-life 271 days). At this time, the generator capability of eluting Ga-68 is 16 mCi.

Anion exchange columns

There are three different types of anion exchange columns were investigated in the study that are an in-jouse made cartridge using AG 1-X8 Resin (250 mg, 200-400 dry mesh, 45-106 µm wet bead, BioRad, USA), Oasis® WAX (30mg, Waters, USA) and Chromafix® PS-HCO_3 column (45 mg, Machery-Nagel, Duren, Germany).

Dose calibrator

In the experiments, researcher used a calibrated Atomlab™ 300 dose calibrator to measure the activity of ^{68}Ga at specific time. The time was taken for each reading in order to do the decay correct for the activity of the Ga-68.

Deionized water

Deionized water was prepared by the Barnstead, Easypure II, RF ultrapure water system (USA). Basically, the deionized was used for the dilution of HCl.

Consumables

All the consumables that had been used such as tubing, connectors, syringes and needles are of one time-used in order to prevent any cross contamination.

$^{68}Ge/^{68}Ga$ generator elution procedures

The generator was eluted with 10 ml of 0.6 M HCl. The first 1 ml fraction of 0.6 M HCl was eluted into the waste vial followed by eluting next 2 ml of the 0.6M HCl into the collecting vial of ^{68}Ga eluate. After that, another 7 ml of the 0.6M HCl was eluted into the waste vial. For the safety purpose, the elution vial of ^{68}Ga was placed into lead pot.

Anion Chromatography

Three different types of anion exchange columns were used for the adsorption of Ga-68 process. They were AG 1-X8 resin, Oasis WAX and Chromafix® PS-HCO_3 columns. Before starting the adsorption process, all three anion exchange columns were pre-conditioned with 2 ml 5M HCl and 10 ml of air to remove excess HCl within the column. Then various molarities of Ga-68 eluate in HCl which ranged from 4.0 M to 7.0 will be investigated to find the optimum HCl molarity which gave the higest ^{68}Ga retention. Subsequently, ^{68}Ga will be desorbed

from the column with 1 mL of water for injection (WFI). Desorption was expressed as % ^{68}Ga desorption of total ^{68}Ga applied to the columns. After each elution step, the anion column was flushed with air.

Molarity of HCl

Various molarities of HCl which ranged from 4.0 M to 7.0 M HCl had been tested. The molarities of HCl were 4.0 M, 4.5 M, 5.0 M, 5.5 M, 6.0 M, 6.5 M and 7.0 M. Two mL of the ^{68}Ga eluate was added to 4 mL 5.70 M HCl and finally total volume would be 6 mL with final [HCl] was 4.0 M. In order to get 6 mL of ^{68}Ga solution with final [HCl] was 4.5 M, the ^{68}Ga eluate was added to 4 mL 6.45 M HCl. Then, ^{68}Ga eluate was added to 4 mL 7.20 M HCl and the final solution would be 6 mL with final [HCl] became 5.0 M. In order to produce 6 mL ^{68}Ga solution with final [HCl] was 5.5 M, the two mL eluate which was eluted from generator was added to 4 mL 7.95 M HCl. After that, the next two mL eluate was added to 4 mL 8.70 M HCl in order to obtain ^{68}Ga in 6 mL 6 M HCl. Then, two mL ^{68}Ga eluate was added to 4 mL 9.45M HCl and final solution would be 6 mL with 6.5 M HCl. Finally, two mL ^{68}Ga eluate was added to 4 mL 10.20 M HCl in order to get the final ^{68}Ga solution would become 6 mL with 7.0 M HCl.

Percentage of Gallium-68 retention

Gallium-68 activity in each different molarity as mentioned in the previous section was eluted through the anion exchange columns. There were four readings were taken throughout the experiments. The parameters were syringe activity of ^{68}Ga before the elution, syringe activity of ^{68}Ga after the elution, ^{68}Ga activity in the column after the elution and the ^{68}Ga activity in the waste vials. Those parameters were measured by using Atomlab™ 300 dose calibrator. In this study, time for each measured parameter was taken. This is due to the decay process of the ^{68}Ga activity. Therefore, by taking the time, the researcher was able to do the decay correct in order to obtain an accurate activity of ^{68}Ga.

The percentage of ^{68}Ga retention formula as shown below:

$$\frac{\text{Activity of } ^{68}\text{Ga in anion column after elution}}{\text{Syringe activity of } ^{68}\text{Ga before the elution - Syringe activity of } ^{68}\text{Ga after the elution}} \times 100\%$$

The researcher compared the percentage of ^{68}Ga retention profiles between the three different anion cartridges by taking the reading of %^{68}Ga retention at the best molarity of HCl for retention process.

Percentage of Gallium-68 desorption

After the elution of ^{68}Ga activity in various molarities of HCl as mentioned above, the adsorbed ^{68}Ga in anion exchange column was desorbed with one (1) mL of water for injection. The parameters that had been measured are ^{68}Ga activity in anion exchange column before desorption process, ^{68}Ga activity in elution vial and ^{68}Ga activity in anion exchange column after desorption process. . The parameters were measured by using Atomlab™ 300 dose calibrator. Time was taken for every measured parameter.

The percentage of ^{68}Ga desorption formula as follow:

$$\frac{^{68}\text{Ga activity in elution vial}}{^{68}\text{Ga activity in anion exchange column before desorption}} \times 100\%$$

In this procedure, the researcher tried to identify whether the molarity of HCl may affect desorption of ^{68}Ga or not.

Statistic analysis

Statistical analysis was performed using IBM SPSS Statistics Version 20 software. In this study, data was analyzed by using one-way ANOVA test. Statistical significance was defined at $p<0.05$.

RESULTS

All of data were collected from twenty one samples for each anion columns. In this research, three different types of columns were investigated. Therefore, the total of samples was sixty three which consists of three samples for each molarity of hydrochloric acid. The results and data that had been collected were percentage of ^{68}Ga adsorption and percentage of ^{68}Ga desorption. Generally, the data obtained were different for diverse HCl molarities. In this study, all the activity of gallium-68 was in millicurie unit (mCi).

Adsorption (Retention) of Ga-68 in different HCl molarities

^{68}Ga activity in fraction of 6 mL at various [HCl] was measured by using dose calibrator and time was taken in order to make decay correction of gallium-68 activity.

AG 1-X8 resins column

In the AG 1-X8 resins cartridge, a range of HCl molarities was run to determine which molarity that can give the highest retention of gallium-68. The ^{68}Ga adsorption in the column was expressed as percentage of ^{68}Ga retention. For identification of the best molarity which would give the highest ^{68}Ga retention, test that had been used was one-way ANOVA analysis due to more than two groups (seven groups) involved in the experiments. There were seven groups in the experiment which is seven different molarities were tested.

Percentage of Ga-68 adsorption results for AG 1-X8 resin cartridges are shown in Figure 1. Based on the line graph, the percentage of Ga-68 adsorption (retention) for AG 1-X8 columns begin to be plateau at HCl molarity of 5.5.

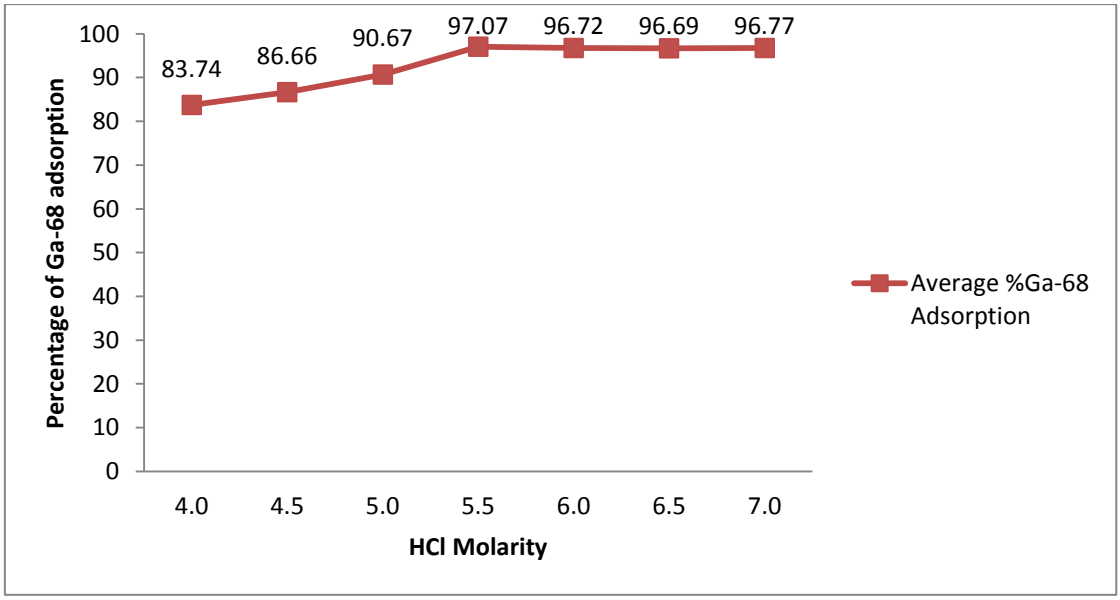

Figure 1: Graph of percentage of Ga-68 adsorption on AG 1-X8 column with different HCl molarities.

By using one-way ANOVA, the analysis of data is shown in Table 1. The significance level for this study is 0.05 ($p<0.05$). It means that the mean difference is significant at the 0.05 level. Therefore, percentage of ^{68}Ga retention in AG 1-X8 column at 5.5M HCl is significantly different compared to 4.0 M, 4.5 M and 5.0 M. However, the percentage of ^{68}Ga retention at 5.5M, 6.0 M, 6.5 M and 7.0 M are equal. The equal variances assumed by using Bonferroni.

Chromafix® PS-HCO$_3$ column

In the Chromafix cartridge, a range of HCl molarities was run in order to determine which molarity that can give the highest retention of gallium-68. The ^{68}Ga adsorption in the column was expressed as percentage of ^{68}Ga retention. Data normality was tested by using Kolmogorov-Smirnov test. For identification of the best molarity which would give the highest ^{68}Ga retention, test that had been used was one-way ANOVA analysis due to more than two groups (seven groups) involved in the experiments. There were seven groups in the experiment which is seven different molarities were tested.

Percentage of Ga-68 adsorption results for Chromafix® PS-HCO$_3$ columns are shown in Figure 2. Based on the line graph, the % Ga-68 adsorption (retention) for Chromafix column begins to plateau at HCl molarity of 5.5.

Table 1: One-way ANOVA data analysis of percentage of Ga-68 retention in AG 1-X8 anion columns at 5.5M compared to other HCl molarities

(I) Molarity	(J) Molarity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5.5M	4.0M	13.32667*	2.46562	.002	4.2058	22.4476
	4.5M	10.40667*	2.46562	.018	1.2858	19.5276
	5.0M	6.40000	2.46562	.444	-2.7209	15.5209
	6.0M	.34667	2.46562	1.000	-8.7742	9.4676
	6.5M	.38000	2.46562	1.000	-8.7409	9.5009
	7.0M	.29333	2.46562	1.000	-8.8276	9.4142

*. The mean difference is significant at the 0.05 level.

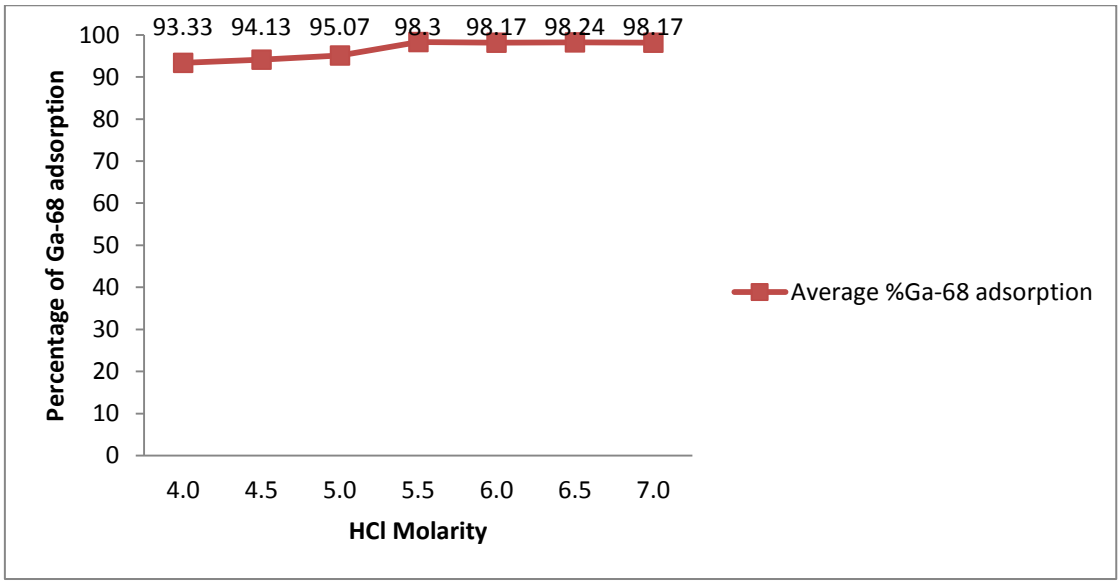

Figure 2: Graph of percentage of Ga-68 adsorption on Chromafix® PS-HCO$_3$ columns with different HCl molarities.

By using one-way ANOVA, the analysis of data is shown in Table 2. The significance level for this study is 0.05 ($p<0.05$). It means that the mean difference is significant at the 0.05 level. Therefore, percentage of ^{68}Ga retention in Chromafix® PS-HCO_3 column at 5.5M HCl is significantly different compared to 4.0 M, 4.5 M and 5.0 M. However, the percentage of ^{68}Ga retention at 5.5M, 6.0 M, 6.5 M and 7.0 M are identical. The equal variances assumed by using Benferroni.

Table 2: One-way ANOVA data analysis of percentage of Ga-68 retention in Chromafix® PS-HCO_3 column at 5.5M compared to other HCl molarities

(I) Molarity	(J) Molarity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5.5M	4.0M	4.97333*	.35764	.000	3.6503	6.2963
	4.5M	4.16667*	.35764	.000	2.8437	5.4897
	5.0M	3.23333*	.35764	.000	1.9103	4.5563
	6.0M	.13333	.35764	1.000	-1.1897	1.4563
	6.5M	.06333	.35764	1.000	-1.2597	1.3863
	7.0M	.13333	.35764	1.000	-1.1897	1.4563

*. The mean difference is significant at the 0.05 level

Oasis® WAX column

In the Oasis® WAX cartridge, a range of HCl molarities was investigated in order to determine which molarity may give the highest retention of gallium-68. The ^{68}Ga adsorption in the column was expressed as percentage of ^{68}Ga retention. Data normality was tested by using Kolmogorov-Smirnov test. For identification of the best molarity which would give the highest ^{68}Ga retention, test that had been used was one-way ANOVA analysis due to more than two groups (seven groups) involved in the experiments. There were seven groups in the experiment which is seven different molarities were tested.

Percentage of Ga-68 adsorption results for Oasis® WAX columns are shown in Figure 3. Based on the line graph, the % Ga-68 adsorption (retention) for Oasis® WAX column begins to plateau at HCl molarity of 5.5.

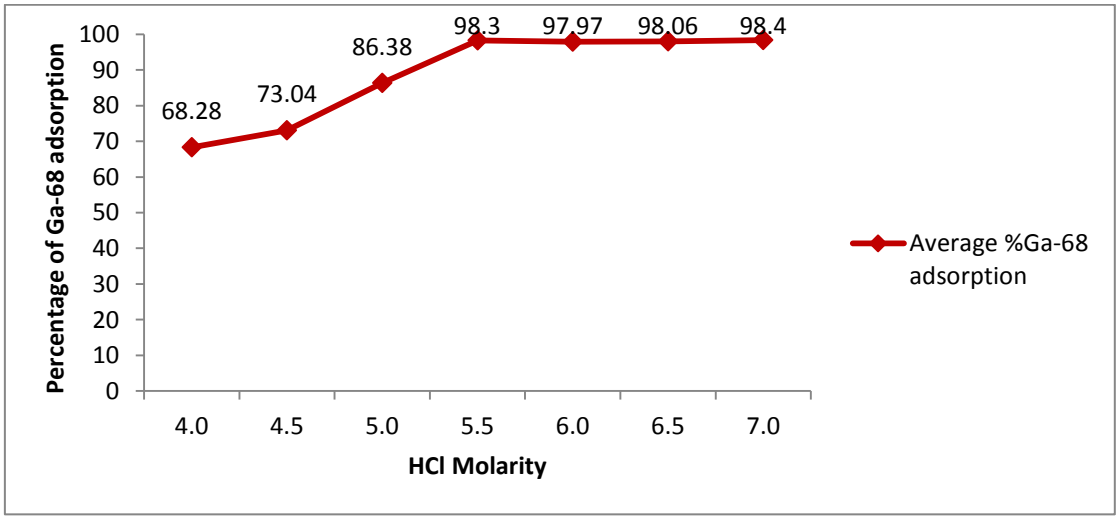

Figure 3: Graph of percentage of Ga-68 adsorption on Oasis® WAX columns with different HCl molarities.

By using one-way ANOVA, the analysis of data is shown in Table 3. The significance level for this study is 0.05 ($p<0.05$) which is the mean difference is significant at the 0.05 level. Therefore, percentage of ^{68}Ga retention in Oasis® WAX column at 5.5M HCl is significantly different compared to 4.0 M, 4.5 M and 5.0 M. However, the percentage of ^{68}Ga retention at 5.5M, 6.0 M, 6.5 M and 7.0 M are identical. The equal variances assumed by using Bonferroni.

Table 3: One-way ANOVA data analysis of percentage of Ga-68 retention in Oasis® WAX column at 5.5M compared to other HCl molarities

(I) Molarity	**(J) Molarity**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
5.5M	4.0M	31.02333*	3.06627	.000	19.6805	42.3662
	4.5M	25.25667*	3.06627	.000	13.9138	36.5995
	5.0M	11.91667*	3.06627	.035	.5738	23.2595
	6.0M	.32667	3.06627	1.000	-11.0162	11.6695
	6.5M	.24333	3.06627	1.000	-11.0995	11.5862
	7.0M	-.09667	3.06627	1.000	-11.4395	11.2462

*. The mean difference is significant at the 0.05 level.

Comparison of % ^{68}Ga retention at 5.5 M HCl between three different cartridges

The descriptive analysis of % Ga-68 retention at 5.5 M HCl for those investigated anion cartridges are shown in Table 4.

Table 4: Descriptive analysis of %Ga-68 retention at 5.5 M HCl

Types of Anion Cartridges	N	Min	Max	Mean	Std. Deviation
AG 1-X8	3	96.40	97.90	97.07	0.764
Chromafix	3	98.10	98.60	98.30	0.264
Oasis	3	97.50	99.10	98.30	0.800

Based on the results analysis, at 5.5 M HCl, the highest %Ga-68 retention is Chromafix and Oasis cartridge which is 98.30% and the lowest retention is in AG 1-X8 which is only 97.07%.

Desorption of Gallium-68

For the desorption procedures, 1 mL of water for injection (WFI) was used in order to eluate the retained ^{68}Ga in anion columns into final vial. The highest average of %^{68}Ga desorption is Oasis WAX which is 70.49% followed by second highest is Chromafix which is 70.36% and the lowest is AG 1-X8 which is 58.56%.

Details of the results are shown in Table 5 and Figure 4.

Table 5: Average of %Ga-68 desorption in three different cartridges

Column	Average % ^{68}Ga desorption (%)
AG 1-X8	58.56
Chromafix	70.36
Oasis WAX	70.49

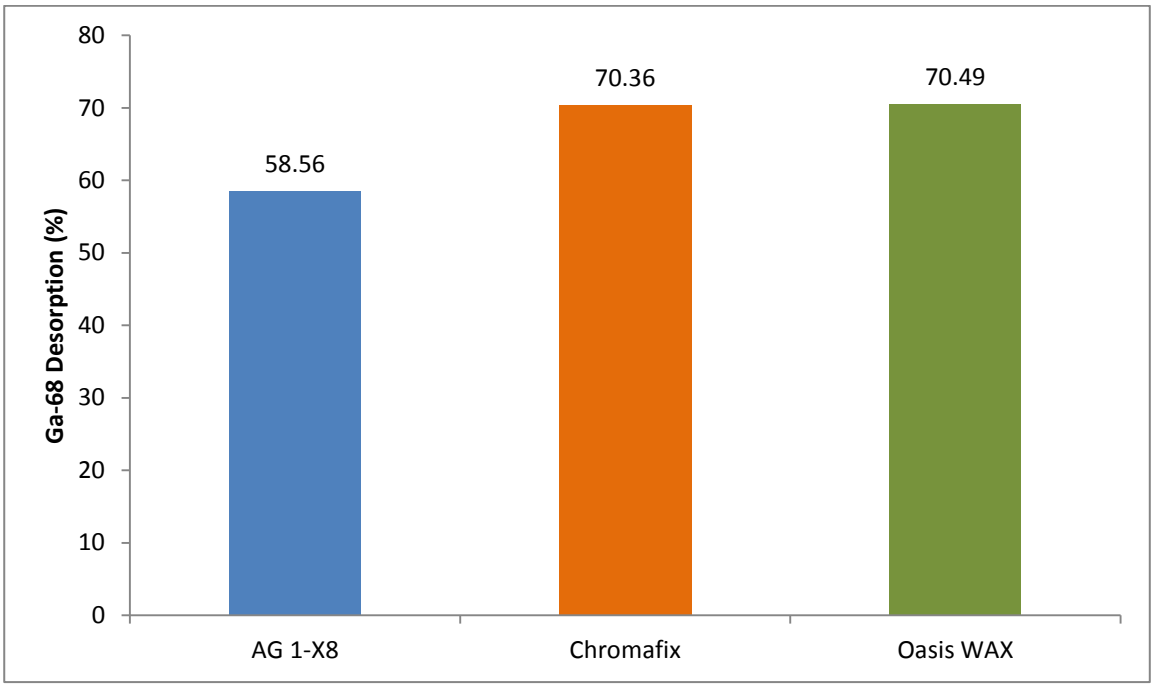

Figure 4: Average %Ga-68 desorption on AG 1-X8 Resin, Chromafix and Oasis WAX columns

DISCUSSION

Characteristics of ^{68}Ge/^{68}Ga generators and eluate

^{68}Ge/^{68}Ga generators are frequently delivered with a given activity of ^{68}Ge on the column, however, the elutable amount of ^{68}Ga activity is not stated and depends, among others on the carrier and eluate. For most commercially available TiO_2-based ^{68}Ge/^{68}Ga generators holds, e.g. 1000MBq $^{68}GeCl_4$ on the column, and 7900MBq ^{68}Ga can be eluted shortly after production, and after 1 half-life of ^{68}Ge the elutable ^{68}Ga activity is reduced to 7350 MBq, thus 770% (Breeman et al., 2005; Decristoforoetal., 2007). iThemba Labs delivers their SnO_2-based generator with a given elutable ^{68}Ga activity, which is >100% in the beginning and ±75% after 1half-life of ^{68}Ge (E. de Blois et al., 2011).

Purification and concentration of Gallium-68 by anion exchange columns

Since the trapping of gallium-68 activity has potential to be increased and improved by using anion exchange column, researcher attempted to compare the ability of three different cartridges which are AG 1-X8 Resin, 500 g, Chloride, 200-400 dry mesh, 45-106 µm wet bead (BioRad, USA) in an in-house made cartridge, Oasis® WAX (30mg, Waters, USA) and Chromafix® PS-HCO_3 column (45 mg), Machery-Nagel, Duren, Germany.

In order to compare the ability of anion exchange cartridge in term of purification, two aspects were focused in this study which are percentage of gallium-68 retention in the columns and also the percentage of gallium-68

desorption on all the investigated cartridges. However, for identification of the percentage of Ga-68 retention, different molarities of hydrochloric acid were used. According to Meyer et al. (2004), at [HCl] >5.0 M, Ga^{3+} ions will form the negatively charged gallium tetrachloro complex $[GaCl_4]^-$ which can be used advantageously to concentrate the eluted gallium , since the $[GaCl_4]^-$ complex can be adsorbed quantitatively on a strong anion exchange column. Therefore, in order to identify the best and most suitable molarities, researcher used a range of different HCl molarities which are 4.0M, 4.5M, 5.0M, 5.5M, 6.0M, 6.5M and 6.0M of HCl.

Identification of the most suitable molarity of HCl for ^{68}Ga retention

In this study, researcher investigated the effect of molarity on the percentage of Ga-68 retention and finally attempted to identify the most suitable HCl molarity for adsorption of Ga-68 purposes.

Based on the result analysis, in all of investigated cartridges, the percentage of ^{68}Ga retention or adsorption started to be plateau at molarity of 5.5M. Based on the One-way ANOVA analysis, there is no significant difference between 5.5M with 6.0M, 6.5M and 7.0M which means that the retention of gallium-68 is equal at those molarities. Therefore, 5.5M is the best molarity for the adsorption of gallium-68 in all three anion exchange cartridges. This shows that at HCl molarity of 5.5M, all Ga^{3+} ions had form the negatively charged gallium tetrachloro complex $[GaCl_4]^-$; thus all of Ga^{3+} ions is quantitatively adsorb on the anion column while other metallic impurities such as Germanium-68, Zinc-68, Stannous and other metallic impurities will be pass through the column. Therefore there is no need to increase HCl molarity more than 5.5M in order to adsorb all of the Gallium ion on the anion column because later the high H^+ ion need to be neutralize by an equal amount of buffer. So it would be sufficient to have an optimum amount of HCl molarity in order to trap the Gallium-68 ion so that it will become safer and easier for later manipulation before it can be applied for clinical used.

Comparison of % ^{68}Ga retention at 5.5 M HCl between three different anion exchange columns

According to the descriptive analysis at 5.5M for all of the three cartridges, Chromafix and Oasis WAX cartridges showed the highest retention of gallium-68 which is 98.30%. The lowest Ga-68 retention was gained by AG 1-X8 column which is 97.07%. There seems to be no difference between both Chromafix and Oasis WAX cartridges in the retention of Gallium-68.

Desorption of ^{68}Ga of total eluate

In desorption process, one milliliter of water for injection was used in order to elute the retained Ga-68 activity in the anion exchange resin. Regarding to the analysis of results, the highest percentage of ^{68}Ga desorption was obtained by using Oasis WAX cartridges which is 70.49% followed by Chromafix which is 70.36%. The lowest desorption of gallium-68 was obtained by using AG 1-X8 column which is only 58.56%.

This is in agreement with the study done by Eric et al (2010), where the highest desorption was also in Oasis WAX 30mg column. The desorption of Gallium-68 from the anion column is concentrated in one (1) ml which yielded a high specific activity with less H^+ ion than the fractionation method where the Gallium-68 ion is in 2 ml fraction of 0.6M HCl.

Determination of Most Suitable Anion Column

By considering all of aspects, the best anion exchange column that should be implemented for a SnO_2 Based ^{68}Ge/^{68}Ga generator is Oasis® WAX (30mg, Waters, USA).

Although there are no significance difference in terms of adsorption of Ga-68 ion at 5.5 M HCl on both Oasis WAX and Chromafix column which are 98.30%, the desorption profile of the Oasis WAX column is high compared to other column which are 70.49%. Therefore, from this study, the most suitable cartridge and HCl molarity that should be applied in purification and concentration of Gallium-68 eluate from a SnO_2 Based ^{68}Ge/^{68}Ga generator is Oasis® WAX column with a HCl molarity of 5.5M respectively.

CONCLUSION

This study gave an important info on the methods to be used in the preparation of gallium-68 elution. By identifying the best molarity and anion exchange column for the retention of Ga-68, the activity in the collecting or final vial can be increased effectively. Other than that, it also can help to optimize the uses of each Ga-68 from the ^{68}Ge/ ^{68}Ga generator. Several limitations occurred throughout this study. The first limitation is all the purified and concentrated Gallium-68 from the anion cartridges ion shall be assessed their metal contamination by conducting Atomic Absorption Spectrophotometry so that the reduction of metal contaminants can be seen by adopting the anion purification method. Other than that, the study is limited to the ability of ^{68}Ge/^{68}Ga generator to elute the Ga-68 for every five hours.

ACKNOWLEDGEMENTS

The authors wish to thank Malaysian Nuclear Agency and Nuclear Medicine Department of National Cancer Institute Putrajaya, Ministry of Health for providing facilities for this research.

REFERENCES

B´e MM, Duchemin B, Browne E, Chechev V, Helmer RG, Schonfeld E. Table of Radionuclides: Comments on Evaluations. ISBN 2 7272 0211 3. Paris, France: CEA/DIMIRI/LNHB; 1999.

Breeman, W.A.,DeJong,M.,Visser,T.J.,Erion,J.L.,Krenning,E.P.,2003.Optimising conditions forradiolabellingofDOTA-peptideswith 90Y, 111In and 177Lu athigh specific activities.*Eur.J.Nucl.Med.Mol.Imaging*30,917–920.

Breeman, W.A.,deJong,M.,deBlois,E.,Bernard,B.F.,Konijnenberg,M.,Krenning, E.P., 2005. Radiolabelling DOTA-peptides with 68Ga. *Eur. J. Nucl. Med. Mol. Imaging* 32,478–485.

Decristoforo C, Knopp R, von Guggenberg E, Rupprich M, Dreger T, Hess A, et al. A fully automated synthesis for the preparation of 68Galabelled peptides.*Nucl Med Commun* 2007; 28:870–5.

Erik de Blois, Ho Sze Chan, CkiveNaidoo, et al. Characteristics of SnO_2-Based ^{68}Ge/^{68}Ga Generator and Aspects of Radiolabelling DOTA-Peptides. *Applied Radiation and Isotopes 2010.*

IDB Holland bv. 2011. *Products / Gallium-68 Generator / Product details Gallium-68 generator.* (online)

http://www.idb-holland.com/products/7/15 product details gallium-68 generator.html (10 February 2011).

K. P. Zhernosekov, D. V. Filosofov, R. P. Baum, P. Aschoff, H.-J. Adrian, H. Bihl, A.A. Razbash, M. Jahn, M. Jennewein, F. Rösch, *Processing of generator produced 68Ga for medical application.*

Konstantin P. Zhernosekov, Dimitry V. Filosofov, Richard P. Baum, et al. Processing of Generator-Produced ^{68}Ga for Medical Application. *J Nucl Med* 2007.

Lambrecht R, Sajjad M (1988) Accelerator derived radionuclide generators. Radiochimica Acta 43:171–179.

Loc'h, C., Maziere, B., Comar, D., 1980. A new generator for ionic gallium-68. *J. Nucl. Med.* 21, 171–173.

Mattia Asti, Giovanni De Pietri, Alessandro Fraternali, et al. Validation of ^{68}Ge/^{68}Ga generator processing by chemical purification for routine clinical application of ^{68}Ga-DOTATOC. *Nuclear Medicine and Biology* 2008;35: 721-724.

Marchol M. Ion Exchangers in Analytical chemistry: *Their Properties and Use in Inorganic Chemistry.* Prague, Czech Republic: Academia; 1982.

Meyer, G.J.,Macke, H.,Schuhmacher, J.,Knapp, W.H.,Hofmann,M., 2004. 68Ga-labelled DOTA-derivatised peptideligands.*Eur. J. Nucl. Med. Mol. Imaging* 31, 1097–1104.

Mirzadeh S, Lambrecht R (1996) Radiochemistry of Germanium. *J Radioanal Nucl Chem* 202:7–102.

Modlin IM, Lye KD, Kidd M. A 5-decade analysis of 13,715 carcinoid tumors. *Cancer.* 2003;97;934-959.

N.S Loktionova, A. Belozub, et al. Purification of ^{68}Ga Combining Cation and Anion Exchange Process.

P. Gebhardt, Th. Opfermann, H.P. Saluz. Computer controlled ^{68}Ga milking and concentration system. *Applied Radiation and Isotopes* 2010.

Rösch F, Knapp R (2003) Radionuclide generators. In: Rösch F (ed) *Radiochemistry and Radiopharmaceutical Chemistry in Life Sciences.* Kluwer Academic Publishers, Dordrecht/Boston/London, p 81–118.

Strelow FWE, Victor AH, van Zyl CR, Eloff C. Distribution coefficients and cation exchange behaviour of elements in hydrochloric acid-acetone. *Anal Chem* 1971;43:870–6.

TanveerHussainBokhari, A. Mushtaq, Islam Ullah Khan. Concentration of 68Ga Via Solvent Extraction. *Applied Radiation and Isotopes* 2009;67,100-102.

Velikyan I, Beyer GJ, Langstrom B. Microwave-supported preparation of 68Ga bioconjugates with high specific radioactivity. *BioconjugChem* 2004;15:554–60.

Zhernosekov KP, Filosofov DV, Baum RP, Aschoff P, Bihl H, Razbash A, et al. Processing of generator-produced 68Ga for medical application. *J Nucl Med* 2007;10:1741–8.