Development of Freeze-Dried DOTMP Kits for Labeling with ^{68}Ga

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Abstract - Lyophilized DOTMP kits were prepared using DOTMP, ammonium acetate, and ascorbic acid. The ^{68}Ga-DOTMP was prepared by incubating the kit dissolved in 0.5 ml of concentrated ^{68}Ga using NaCl method and 0.5 ml of DDW, at 100°C for 7 min. The labeling yield was evaluated by two solvent systems of TLC. 1 MBq of concentrated ^{68}Ga was labeled with 0.8 μg of DOTMP by high radiolabeling yield (>98%), which was determined by two TLC methods. The composition of the prepared freeze-dried vial is 400 μg of DOTMP, 19.27 mg of ammonium acetate and 17.62 mg of ascorbic acid. ~555 MBq of ^{68}Ga-DOTMP was prepared with excellent radiochemical purity (>98%) and it was stable for 4 hr at room temperature. In conclusion, Freeze-dried DOTMP kits for the convenient preparation of ^{68}Ga-DOTMP have been developed. Availability of this kit is expected to stimulate the widespread use of ^{68}Ga-DOTMP in the fields of nuclear medicine.

Key words : Freeze-dried DOTMP kit, PET, Gallium-68 (^{68}Ga), Bone metastasis

INTRODUCTION

Bone is a favorable site of metastasis and is invaded common primary tumors such as prostate, breast, and lung. Due to the progressive pain and mortality of the bone metastasis, effort has been focused on the detection of bone metastasis in the field of nuclear medicine (Mitterhauser *et al*. 2007; Mirzaei *et al*. 2015). Traditional detection method was bone scanning using ^{99m}Tc-radiolabeled polyphosphonates, and there are some commonly used polyphosphonates such as methylene diphosphonate (MDP), dicarboxypropane diphosphonate (DPD), hydroxymethylene diphosphonate (HDP), and ethylene diamine tetramethylene phosphonate (EDTMP) (Mitterhauser *et al*. 2007). Recently, the use of PET imaging using ^{18}F-fluoride has been increased because of the higher sensitivity and spatial resolution compared with SPECT imaging using ^{99m}Tc-polyphosphonates (Cook and Fogelman 2001). ^{18}F-fluoride is however, prone to have a risk of high false-positive findings in minimal degenerative changes and represent blood flow rather than bone remodeling (Billinghurst 1982; Langsteger *et al*. 2006). Thus, other ligand-based bone seekers have been investigated for PET imaging (Simon *et al*. 2012; Mirzaei *et al*. 2015).

^{68}Ga is produced from a cost-effective generator and its half-life is 67.6 min. In addition, Because ^{68}Ga provides sufficient radioactivity for high quality PET images, and therefore examination time and radiation toxicity to the patient can be minimized. Notably, ^{68}Ga might allow for the theranostic development because most therapeutic radionuclides such as ^{177}Lu is also metals (Velikyan 2013).

In designing suitable imaging agents for bone metastasis, multidentate polyaminophosphonate are regarded as the most promising candidates as carrier ligands owing to their high bone affinity, selective localization in skeletal lesions and ability to form metal chelates with high in-vivo stability (Chakraborty *et al*. 2008). Since EDTMP showed high bone affinity (Das *et al*. 2002), the macrocyclic analog of EDTMP,

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1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylene phosphonic acid (DOTMP) was labeled with ^{153}Sm, ^{166}Ho and ^{177}Lu, and used to treat bone metastasis (Liu and Edwards 2001; Chakraborty *et al*. 2008, Jaime *et al*. 2012).

Lanthanides including ^{166}Ho, ^{177}Lu and ^{68}Ga form more stable and inert complexes with macrocyclic chelators than their acyclic analogs. Because the dissociation of the radiometal from the chelate could result in the accumulation of radioactivity in nontarget organs, thermodynamic stability as well as kinetic inertness of the radio-peptide are very significant (Archana *et al*. 2014). It is pertinent to note that ^{166}Ho-DOTMP has shown excellent pharmacokinetic properties as well as clinical efficacy in the treatment of patients suffering from multiple myeloma (Breitz *et al*. 2006). Thus, DOTMP that is the macrocyclic analog of EDTMP is useful for the diagnosis and treatment of bone metastasis.

To introduce the theranostic approach, the development of molecular based imaging technology is needed. However, to our knowledge, PET imaging study of DOTMP using ^{68}Ga has not reported yet. In addition, the preparation of ^{68}Ga-labeled imaging agent requires delicate and rapid handling because of its short half-life and possible radiolysis (Garnuszek *et al*. 2003). To solve these problems, development of a straightforward freeze-dried kit formulation strategy is required to promote its widespread use (Archana *et al*. 2014).

Here, we report the methodologies of formulation of the freeze-dried DOTMP kit for labeling with ^{68}Ga.

MATERIALS AND METHODS

1. Materials

All chemicals were of analytical grade and thus used without further purification (Anaspec, California, USA). For all experiments, a ^{68}Ga was produced from a ^{68}Ge/^{68}Ga generator (ITG, Germany). The radioactivity was determined with a Wallac 1470 automated gamma counter (PerkinElmer Life Science, Massachusetts, USA) and an ionizing chamber (Atomlab 200, Bio-dex, New York, USA). The radiolabeling yield and radiochemical purity (RCP) were determined using a Tracemaster 20 automated TLC-linear analyzer (Berthold, Germany).

2. Radiolabeling of DOTMP with ^{68}Ga

The ^{68}Ge produced by ^{68}Ge/^{68}Ga generator was eluted with a total 4 ml of 0.05 M HCl according to the manufacturer's guidance., and concentrated using a NaCl-based ^{68}Ga eluate concentration method as described by Mueller *et al*. (Mueller *et al*. 2012). Briefly, the ^{68}Ga generator eluate was collected by a SCX cation exchange cartridge and eluted from the cartridge with 0.5 ml of a 5 M NaCl solution containing a 12.5 μl of 5.5 M HCl. This eluate (~555 MBq) was slowly added to a 500 μl of reaction buffer containing DOTMP (OXCHEM, CA, USA), pH 4.5 ammonium acetate buffer (final 0.25 M) and ascorbic acid (final 0.1 M). The mixture was reacted at 100°C for 7 min for the radiolabeling. Various concentration of DOTMP (10~400 μg) was labeled with 111 MBq of ^{68}Ga to evaluate the mass of DOTMP required for a high radiolabeling yield (>98%), and the results were converted by 1 MBq of ^{68}Ga.

3. Quality control of ^{68}Ga-DOTMP

The radiolabeling yield was determined by TLC method, using 0.5 M sodium citrate buffer (pH 4.5) as the eluting solvent. In brief, 2~4 μl of the reaction mixture was spotted on the silica-gel (SG) TLC paper. The strip was developed using 0.5 M sodium citrate buffer (pH 4.5), dried, and analyzed using TLC-linear analyzer. To confirm its radiochemical purity, TLC using cellulose TLC plates developed with 2 : 1 mixture of B1 : B2 (B1: 9 mL Millipore water; 0.6 mL HCl (conc. 37%); 88 mL acetone/B2: 2,4-pentadione) was evaluated (Fellner *et al*. 2012).

4. Formulation of freeze-dried DOTMP kits

Freeze-dried DOTMP kits (ten numbers in each batch) were prepared following the protocol mentioned below. 4 mg of DOTMP was dissolved in 2.5 ml of 1 M ammonium acetate buffer (pH 4.5), and 2.5 ml of 0.4 M Ascorbic acid was added under aseptic conditions. The resultant solution was thoroughly mixed and subsequently passed through Millipore® (0.2 μm) filter paper. Then, it was aliquoted into ten sterile glass vials, each vial containing 0.5 ml of the solution. All these preparative steps were carried out under aseptic conditions. The vials were incubated for a period of 24 h at −20°C. Finally, the vials were freeze-dried in a lyophilizer and stored at −20°C.

5. Preparation of ^{68}Ga-DOTMP using freeze-dried DOTMP kits

Freeze-dried DOTMP kits were allowed to attain room

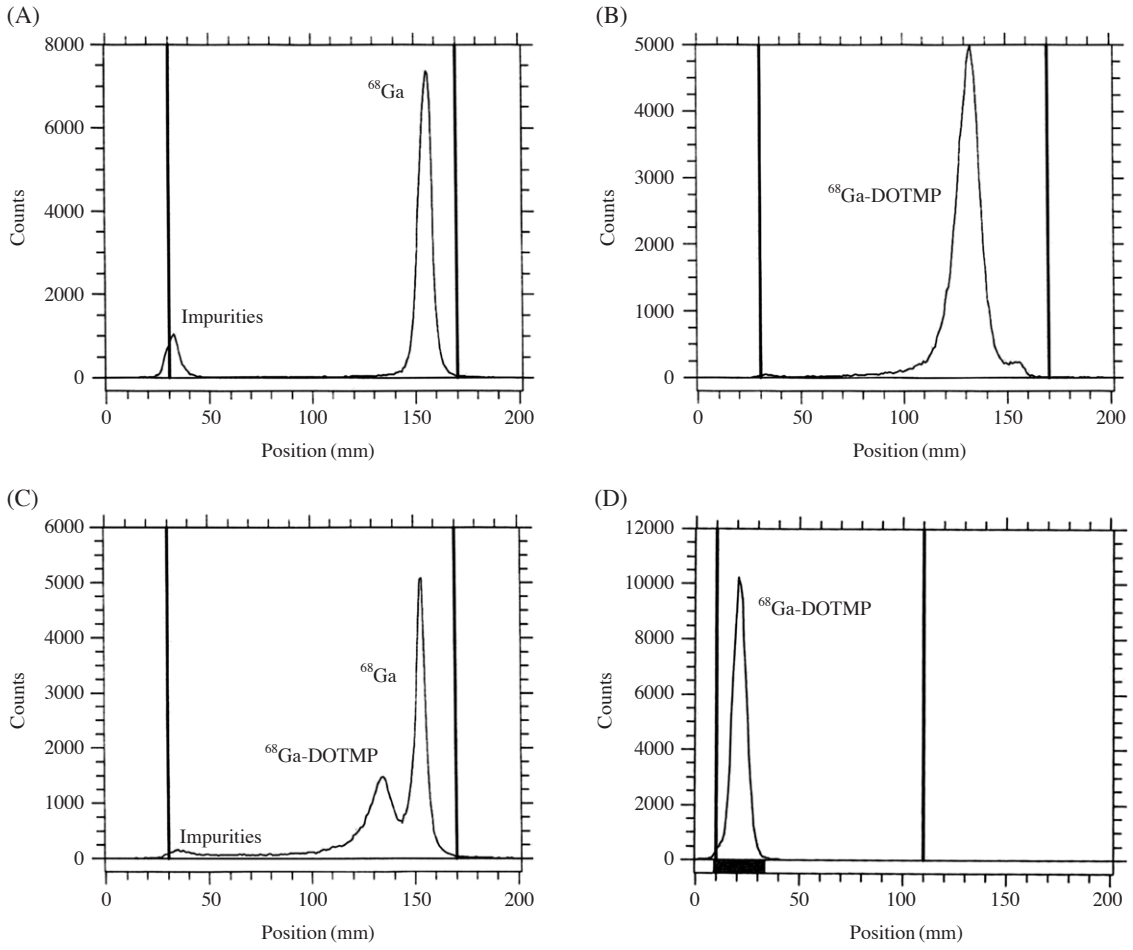

Fig. 1. Typical iTLC profiles of eluted ^{68}Ga (A), ^{68}Ga-labeled DOTMP (B), and mixture of eluted ^{68}Ga and ^{68}Ga-labeled DOTMP (C). Mobile phase was pH 4.5 0.5 M sodium citrate buffer. Impurities (R_f: 0.03 ± 0.01) was removed, and ^{68}Ga (R_f: 0.99 ± 0.01) was radiolabeled with DOTMP that the R_f was 0.82 ± 0.01. The radiochemical purity of ^{68}Ga-DOTMP at origin was confirmed by TLC using cellulose TLC plates (D).

temperature before the radiolabeling. The lyophilized DOTMP powder was dissolved by 0.5 ml of DDW, and 0.5 ml of concentrated ^{68}Ga eluate (~555 MBq) was slowly added to the vial. The vial was heated at 100°C for 7 min for the radiolabeling, and the radiolabeling yield was evaluated by TLC method as described in 2.3. The stability of the ^{68}Ga-DOTMP that was prepared using freeze-dried kit, and was studied by incubating the reacted solution at room temperature. A small drop of the solution was taken at selected times, and analyzed by TLC method as described above.

RESULTS AND DISCUSSION

The multifarious advantages of PET using ^{68}Ga in terms of sensitivity, specificity, accuracy, detection rate, acquisition and examination time stimulate its usage in the field of nuclear medicine (Archana *et al*. 2014). DOTMP is well-known bone seeker, and the present study was aimed to develop ^{68}Ga-labeled DOTMP by using a freeze-dried kit for an easy and simple labeling.

$^{68}Ga^{3+}$ was eluted from the ^{68}Ge/^{68}Ga generator and was concentration using NaCl method within less than five minutes. As shown in Fig. 1 (A), the eluted ^{68}Ga solution contained 5~10% of impurities. Impurities (possibly colloidal ^{68}Ga) were detected at the origin, and ^{68}Ga was dragged to the solvent front by 0.5 M sodium citrate buffer (pH 4.5). NaCl method not only removed impurities, but also concentrated the 4 ml of the eluted ^{68}Ga solution to 0.5 ml.

Impurities such as ^{68}Ga-colloids are hardly labeled with DOTA-chelators. Because unlabeled radioactivity might result in toxicities to the non-targeted organ, purification of

^{68}Ga solution is important. Several purification and concentration methods of ^{68}Ga solution have been reported using cation and anion exchanger. In particular, we applied a rapid and convenient NaCl method to label chelators with ^{68}Ga due to relatively few reagents and a minimal procedural with no subsequent purification steps (Mueller *et al*. 2012). 5∼10% of impurities were detected by TLC analysis, and fast purification and concentration of the solution could be performed by the NaCl method.

The concentrated ^{68}Ga was labeled with DOTMP by heating at temperatures of 100°C for 7 min, and the ^{68}Ga-labeled DOTMP was found in the middle of the plate (R_f = 0.8). Fig. 1 (C) showed the typical TLC profiles of the three components. To confirm the radiochemical purity of ^{68}Ga-DOTMP, another cellulose-TLC was simultaneously performed (Fig. 1 (D)). In this condition, ^{68}Ga-DOTMP was remained at the origin and the unlabeled ^{68}Ga was dragged by the solvent (Fellner *et al*. 2012).

The concentrated ^{68}Ga was labeled with DOTMP by high radiochemical purity (>98%), and further purification was not required. As a result of Fig. 2, 0.8 μg of DOTMP was routinely required per 1 MBq of the concentrated ^{68}Ga solution to obtain the radiochemical purity over 98%.

The presence of high levels of unlabeled peptide might be causes of side effects such as cardiac abnormalities. Thus, a particular labeling condition of metal to ligand is recommended (Tapas *et al*. 2014). In the previous studies about a freeze-dried kit for bone-seeking radiotracers reported that 120∼189 μg of EDTMP was labeled with 1 MBq of ^{68}Ga (Mitterhauser *et al*. 2007; Stefan *et al*. 2008). Compared with the reports, 0.8 μg of DOTMP was successfully labeled with 1 MBq of ^{68}Ga by high radiochemical purity (>98%). This result is comparable to the other DOTA derivatives such as DOTA-TOC which is used for diagnosis of neu-

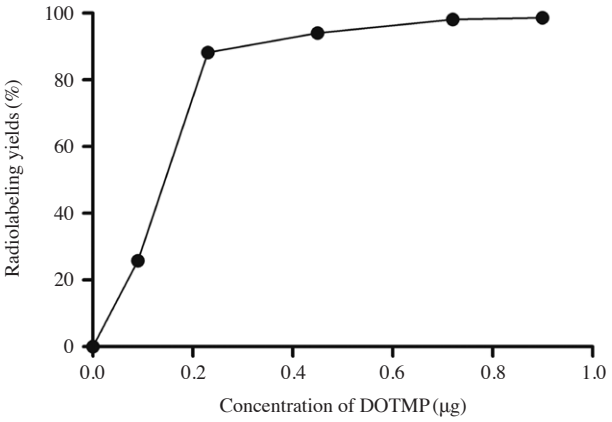

Fig. 2. Radiolabeling yields on the various concentration of DOTMP per 1 MBq of concentrated ^{68}Ga.

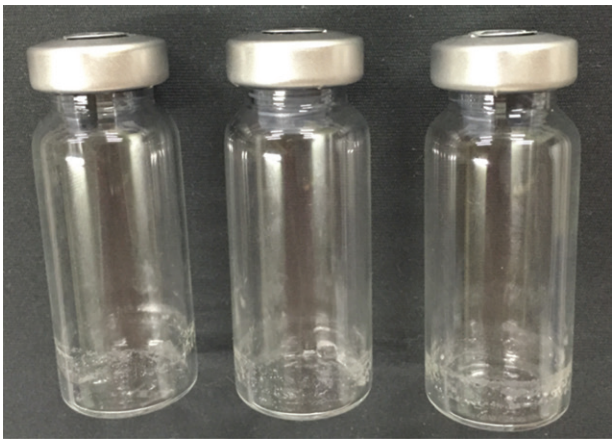

Fig. 3. Freeze-dried DOTMP kit vials for ^{68}Ga radiolabeling.

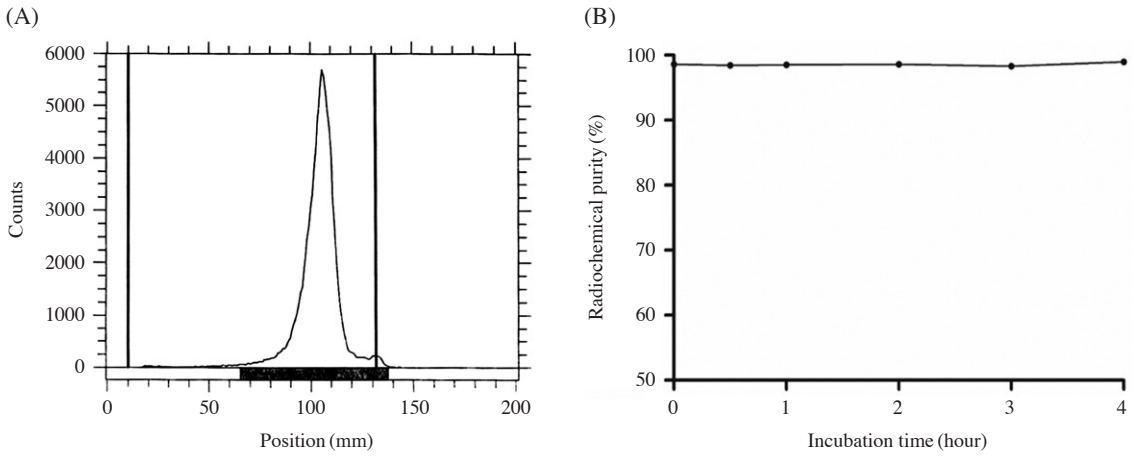

Fig. 4. Preparation of 555 MBq of ^{68}Ga-DOTMP (A) using freeze-dried DOTMP kit, and the stability at room temperature (B). The ^{68}Ga-DOTMP was prepared with high radioactivity over 98%, and the radiochemical purity was maintained for 4 hours.

roendocrine tumors (Archana *et al*. 2014). The optimized labeling condition is useful to avoid possible side effects caused by the high levels of unlabeled peptide.

A prepared freeze-dried kit vial was shown in Fig. 3. The composition of the kit was 400 μg of DOTMP, 19.27 mg of ammonium acetate and 17.62 mg of ascorbic acid. The off-white, zonary lyophilized composition could be seen in the bottom of the freeze-dried vials.

Fig. 4 shows that the radiochemical yield of the freeze-dried DOTMP kit with ^{68}Ga (~555 MBq) was >98%, and the radiochemical purity was maintained for 4 hr after labeling which was a considerable amount of time for imaging using the 68-min short half-life of ^{68}Ga. The total preparation time of ^{68}Ga-DOTMP using freeze-dried kits was less than twenty minutes from the ^{68}Ga elution to the evaluation of labeling yield.

Although the loss of ammonia during lyophilization was concerned (Archana *et al*. 2014), the kit was successfully applied for the preparation of up to 555 MBq (15 mCi) of ^{68}Ga-DOTMP with >98% radiochemical purity. PET/CT imaging of bone metastases with 462 MBq of ^{68}Ga-BPAMD showed intense accumulation in multiple osteoblastic lesions in the central skeleton, ribs, and proximal extremities (Kazuma and Hideo 2011). The freeze-dried DOTMP kit in this study was evaluated to be labeled with ~555 MBq ^{68}Ga, and it would be enough to prepare a patient dose of ^{68}Ga-DOTMP. Because of the structural similarity between DOTMP and BPAMD which are the DOTA derivatives, the formulation of the freeze-dried DOTMP kit could be applied to prepare ^{68}Ga-BPAMD.

As the fast and simple labeling procedure is important for a clinical application, the fast analysis of labeling yields is also significant (Fellner *et al*. 2012). Although another analysis methods such as HPLC usually takes 10~15 min, the TLC methods can be performed in less than 10 min. For this reason, the labeling yield was evaluated by TLC methods in this study, and two TLC systems increased the accuracy. From the elution of ^{68}Ga to evaluate the radio-labeling yield, the preparation of ^{68}Ga-DOTMP (RCP>98%) was performed within twenty minutes which is of great advantage in the clinical application.

A suitable freeze-dried kit is useful to prepare the desired patient doses of ^{68}Ga-DOTMP through a simple step procedure in hospital radio-pharmacies. In addition, the use of the kit enable to prepare ^{68}Ga-DOTMP immediately before administration, which would reduce the possibility of radiolysis during transportation and storage (Tapas *et al*. 2014). The availability of the kit will increase the use of ^{68}Ga-DOTMP in the fields of nuclear medicine.

CONCLUSION

In this study, we described the development of the ready to use DOTMP kit for labeling with ^{68}Ga to be used in hospital radiopharmacy along with ^{68}Ge/^{68}Ga generator. The kit consists of DOTMP in ammonium acetate and ascorbic acid, and could be labeled with ^{68}Ga, in consistently high labeling yield (>98%) within twenty minutes. The easy and efficient labeling of this kit with ^{68}Ga make them suitable for preparing ^{68}Ga-DOTMP for imaging of bone metastasis.

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