Fragmentation of Nimotuzumab for Preparation of ^{125}I-F(ab')$_2$-Nimotuzumab as a Precursor for Preparing ^{125}I-F(ab')$_2$-Nimotuzumab-NLS Radiopharmaceutical for Cancer Therapy

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ABSTRACT

Nimotuzumab is an anticancer agent which belongs to the inhibitor group of Epidermal Growth Factor Receptor (EGFR). This monoclonal antibody has a relatively high molecular weight which makes slow penetration on tumor cell, as concequence, it is less attractive in imaging kinetics, and potentially elicits antibodies respons. Therefore in this study nimotuzumab was fragmented to form bivalent antibody [F(ab')$_2$] and then labeled with ^{125}I to form ^{125}I-F(ab')$_2$-nimotuzumab which can be used further as a precursor for preparing ^{125}I-F(ab')$_2$-nimotuzumab-NLS (NLS = nuclear localizing sequences) radiopharmaceutical for radioimmunotherapy. The aims of this study were to obtain characteristics of ^{125}I-F(ab')$_2$-nimotuzumab by comparing with the ^{125}I labeled-intact nimotuzumab (^{125}I-nimotuzumab). This study was initiated by purifying nimotuzumab by mean of dialysis. The purified nimotuzumab was then fragmented by using pepsin. The F(ab')$_2$-nimotuzumab formed was then purified from its by-products which formed in fragmentation process by using a PD-10 column (consisted Sephadex G25). The intact nimotuzumab and its F(ab')$_2$ fragment were then labeled with the ^{125}I to form ^{125}I-nimotuzumab and ^{125}I-F(ab')$_2$-nimotuzumab. The radiochemical purity are 98.27 % and 93.24 % ,respectively. Stability test results show that, both of ^{125}I-nimotuzumab and ^{125}I-F(ab')$_2$-nimotuzumab more stable at 4 °C than at room temperature storage and 37 °C.

INTRODUCTION

One of the public health problems in the world included in Indonesia is cancer. Every year, cancer patients in the world reached 12 million people and 7.6 million of them died. Therefore we need an appropriate control action, if it is not carried out then in 2030 the estimated 26 million people will suffer from cancer and 17 million of them will die from the disease [1].

Considering the high number of cancer patients in Indonesia. it is necessary to develop effective treatments to kill the cancer cells. One of treatment that has been developed at this time is an anti-cancer therapies using monoclonal antibodies. The anti-cancer therapies work by eliminating cancer cells without damaging normal cells to excess because targeting the specific markers expressed by cancer cells [2].

For almost three decades, antibodies have been used in medicine for imaging and therapeutic purposes because of their selectivity towards specific targets [3]. At least 19 monoclonal antibodies (mAb) in the market that have been approved by the FDA. More than 150 of its in clinical development [4]. However, still there is an obstacle in the use of mAb which is due to its large size molecular weight. Monoclonal antibodies (mAbs) has large size (molecular weight of ~ 150 kDa) thus causing penetrate slowly and need long time in the blood circulation (days to weeks) [5].

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In order to overcome that obstacle a smaller size of mAb which maintains all its property would be desirable.

R.S. Stowers, J.A. Callihan, & J.D. Bryers [6] reported that antibody fragment reduces steric hindrance and gives better tissue penetration which lead to be be a more sensitive antigen detection. Furthermore,antibody fragment also has reduced non-specific binding and lower immunogenicity *in vivo*. According to T.M Behr, *et al.* [7] the advantages of small antibody fragments such as $F(ab')_2$ is that its accumulation in tumor cells will be faster and more homogeneous. In this study, when compared to the use of intact nimotuzumab which has greater molecular weight, nimotuzumab fragments (F $(ab')_2$-nimotuzumab) with a smaller molecular weight is expected to increase its effectiveness in cancer treatment.

Nimotuzumab is a humanized mAb that inhibits EGF binding because it binds to the extracellular domain of the EGFR [8]. Nimotuzumab has been approved in several countries for the treatment of glioma as well as head and neck tumors [9]. Nimotuzumab is in clinical trials for various tumor types including prostate, glioma (pediatric and adult), colorectal, pancreatic, breast cancer, non–small cell lung, cervical, and esophageal [8]. Moreover, it was reported that nimotuzumab had been found to have higher therapeutic effect when combined with the external radiation therapy [10].

The aims of this preliminary study were to prepare ^{125}I-$F(ab')_2$-nimotuzumab and to obtain characteristic data of ^{125}I-$F(ab')_2$-nimotuzumab which was then compared with the ^{125}I-labeled-intact nimotuzumab (^{125}I-nimotuzumab). Next study in the near future will modify this ^{125}I-$F(ab')_2$-nimotuzumab by conjugating it with NLS to prepare ^{125}I-$F(ab')_2$-nimotuzumab-NLS as a therapeutic radiopharmaceutical. A typical NLS peptide motif containing a cluster of four or more cationic residues of lysine (K) or arginine (R) enables the radiopharmaceutical to be in close proximity to the DNA so that the nanometer-range Auger electrons emitted by ^{125}I can cause DNA damage of cancer cells [11].

EXPERIMENTAL METHODS

Material used in this study included Nimotuzumab/TheraCIM obtained from Kalbe Farma. Na^{125}I was prepared by irradiating ^{124}Xe in G.A. Siwabessy Multi Purpose Reactor to produce ^{125}Xe which decayed to ^{125}I. I-125 was then recovered and processed at the Radioisotope and Radiopharmaceutical Centre, National Nuclear Energy of Indonesia (RRC – NNEI) to produce ^{125}I. Pepsin from porcine gastric mucosa purchaced from Sigma-Aldrich. Monobasic sodium phosphate ($NaH_2PO_4.H_2O$), dibasic sodium phosphate ($Na_2HPO_4.2H_2O$), trizma base, tris-glycine, bovine serum albumin, acrylamide, bis-acrylamide, sodium dodecyl sulfate, coomassie blue and bromphenol blue tetramethylethylenediamine (TEMED) were obtained from Sigma. Methanol, chloroform and sodium acetate were obtained from E. Merck. All chemical reagen that used in this study are pro analysis. Protein standart and protein dye were purchaced from Bio-Rad and Iodogen from Pierce. Saline solution and aquabidest sterile were obtained from IPHA. Dialysis cassettes (20 KDa molecular weight cut-off, MWCO) were obtained from Thermo Scientific, PD10 Sephadex G-25 Columns were purchaced from GE Healthcare and Whatman paper No.1 from E.Merck.

Equipment used for analysis are included high performance liquid chromatography, HPLC (Shimadzu) equipped with a size exclusion columns (SEC) BioSuite 250 column (Waters) and an UV-Vis detector (Shimadzu), termomixer (Eppendorf), mini-Protean 3 cell (Bio-Rad), gamma counter (Nucleus), centrifuge (Beckman Coulter), dose calibrator (Capintec), and plate reader (BioTek).

Nimotuzumab dialysi

Nimotuzumab was firstly purified from its excipients by dialysis. Nimotuzumab was injected onto a dialysis cassette (20 KDa molecular weight cut-off) and the cassete was then dialysed with sodium acetate, pH 4.5. This process was carried out at 4 ºC with three times buffer changes for 72 hours [12]. The dialysed nimotuzumab was analyzed by using high performance liquid chromatography (HPLC) equiped with SEC Biosuite 250 column and UV-Vis detector.

Optimization of $F(ab')_2$-nimotuzumab

Pepsin (0.75 mg) was dissolved in 100 µL 0.02 M sodium acetate pH 4.5 which was then added into 3 mL of dialyzed nimotuzumab (5 mg/ mL). The mixture was mixed and heated by using termomixer at 37 °C for 14 hours. Samples were taken at 0, 3, 6, 9,12, 13, and 14 hours. To each sample was added Tris-HCl 10 mM, pH 8.0 to stop fragmentation process. The samples were then analyzed by sodium dedocyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE). Running buffer pH 8.3 used in this analysis [13].

Purification of F(ab')$_2$-Nimotuzumab

Purification of F(ab')$_2$-nimotuzumab was carried using a PD-10 column. Aliquot of raw product (mixture of fragmented nimotuzumab, F(ab')$_2$ and Fc) was loaded onto a PD-10 column pre-blocked and pre-equilibrated with 500 µL 10% BSA and 0.01 M PBS pH 7.4 respectively. The column was then eluted with 0.01 M PBS pH 7.4 [12]. The resulting eluent was collected in 0.5 mL fraction and its purity was analyzed using HPLC.

Preparation of ^{125}I-F(ab')$_2$-Nimotuzumab

The purified Nimotuzumab fragment (F(ab')$_2$-Nimotuzumab) was radiolabelled with Na^{125}I using Iodogen method. The reactant molar ratio was 1: 100: 2 for F(ab')$_2$-Nimotuzumab : iodogen : potassium iodide respectively. The iodination process was initiated by dissolving the Iodogen in chloroform in a test tube. It was then dried using nitrogen gas at room temperature to form a tiny layer Iodogen on the surface of the test tube. Solution of F(ab')$_2$-Nimotuzumab was then added to the test tube which was then was stirred using a vortex which was followed by addition of ^{125}I and potassium iodide. The mixture was stirred and then left to incubate for 2 minutes. The same procedure was also performed for labeling intact nimotuzumab with^{125}I [14 with modification].

Purification of ^{125}I-F(ab')$_2$-Nimotuzumab

^{125}I-F(ab')$_2$-Nimotuzumab was purifed from its impurities, free ^{125}I by using a gel filtration column (PD-10) [7]. The reaction mixture from radiolableling process was loaded onto a PD-10 column pre-blocked and pre-equilibrated with 500 µL 10% BSA and 0.01 M PBS pH 7.5. The column was then eluted using 0.01 M phosphate buffer pH 7.5. The eluent was collected in 0.25 mL fraction (up to 50 fraction). Radioactivity of each fraction was then measured by using a dose calibrator. Fractions which have high radioactivity were then tested for their radiochemical purity by means of paper chromatography.

Determination radiochemical purity of ^{125}I-F(ab')$_2$-Nimotuzumab

Radiochemical purity of ^{125}I-F(ab')$_2$-Nimotuzumab was determined by using paper chromatography with Whatman paper No.1 (1.5 x 15 cm) and 85% methanol as stationary and mobile phases respectively [15]. An aliquot of reaction mixture was spotted 2 cm from the bottom of the strip. The strip was cut into 1 cm portion and the radioactivity of each portion was measured using a gamma counter. The radiochemical purity of ^{125}I-F(ab')$_2$-Nimotuzumab was calculated based on the ratio of the count rate of ^{125}I-F(ab')$_2$-Nimotuzumab fraction with the total count rate.The same procedure was also performed to determine the radiochemical purity of ^{125}I-Nimotuzumab.

Stability test of ^{125}I-F(ab')$_2$- nimotuzumab (stored at room temperature, 37 °C and 4 °C)

The stability of ^{125}I-F(ab')$_2$- nimotuzumab which was stored at room temperature, 37 °C and at 4 °C were monitored using an using a paper chromatography with Whatman paper No.1 (1.5 x 15 cm) and 85% methanol as stationary and mobile phases respectively At set intervals (1, 3, 5 days), 2 µl sample was taken and spotted on Whatman paper No. 1 and developed in 85% methanol. The radiochemical purity of ^{125}I-F(ab')$_2$-nimotuzumab was similar to the above-mentioned procedure [12].

RESULTS AND DISCUSSION

The initial phase of this study is dialysis process, in order to purify the sample nimotuzumab of excipients contained in it. The results were then analyzed using HPLC. To compare chromatograms profiles, analysis was performed on samples nimotuzumab before and after dialysis. Dialysis cassette used has a molecular weight cut off 20,000 Da, which means that substances having a molecular weight below 20 000 Da would be out of the membrane cassette while those having a molecular weight above 20,000 will retained in the membrane. Nimotuzumab has a molecular weight of 150,000 Da [16], that remain in the membrane cassettes. While excipients such as sodium phosphate dibasic (143 Da), monobasic sodium phosphate (142 Da), sodium chloride (58.5 Da) and polysorbate 80 (1309 Da) which has a molecular weight below 20,000 Da would be out of membrane cassettes. The results of the analysis using HPLC (Fig. 1) showed that there was no significant difference in nimotuzumab before and after dialysis. The retention times were 6.683 and 6.667 mins before and after dialysis respectively. The excipients having very low molecular weight were not appear because their peaks were to small as compared to the main substance (i.e. nimotuzumab). These phenomena might be due to low concentrations and/ or lack of chromophore which

are able in absorbing UV light in the excipients which in turned giving no absorbances/ peaks in the chromatogram.

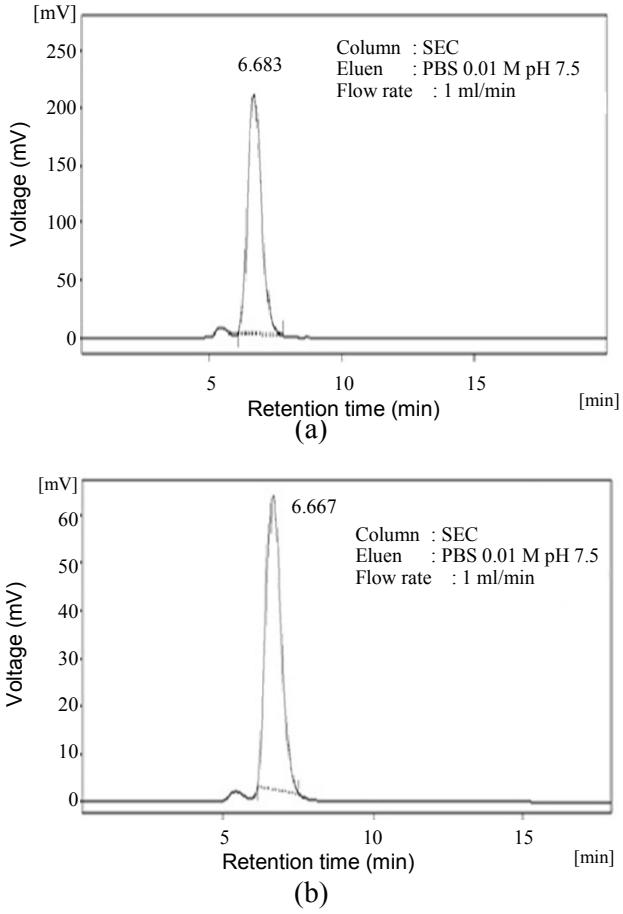

Fig. 1. (a) Nimotuzumab chromatograms before dialysis, (b) Nimotuzumab chromatograms after dialysis.

To determine whether nimotuzumab sample has a molecular weight 150,000 Da, the comparative form of standard proteins used. This standard was also analyzed using HPLC to determine its chromatogram. The results of the standard protein chromatograms presented in Fig. 2.

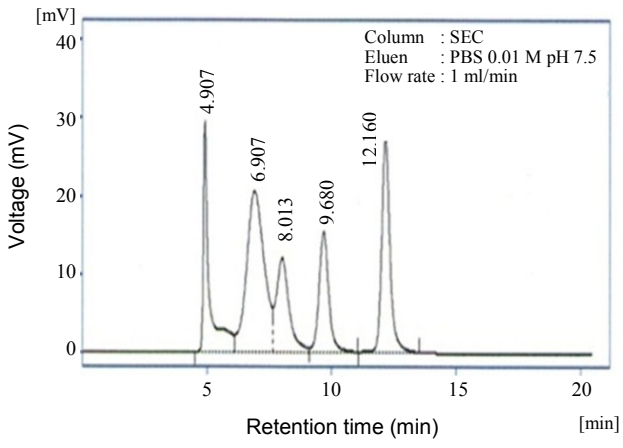

Fig. 2. Protein standard chromatogram.

It can bee seen in Fig. 2 there are five peaks that are associated with standard protein which consisted of thyroglobulin (670,000 Da), γ-globulin (158,000 Da), ovalbumin (44,000 Da), mioglobulin (17,000 Da) and vitamin B_{12} peak (1,350 Da) with retention time (Rt) of 4.907, 6.907, 8.013, 9.680, 12.160 mins respectively. These Rts are meet with basic principle of SEC column used, where a large molecules will be the first to be eluted from the column because a large molecule passing the sidelines gel, while smaller molecules will elute more slowly as it enters the pores of the gel [17]. Based on these data, the positions of nimotuzumab which has a molecular weight of 150,000 Da with a retention time of 6.667 min is almost the same as the γ-globulin which had a retention time of 6.337 min with a molecular weight of 158,000 Da. This suggests that the initial sample nimotuzumab which will be used in this study has a molecular weight corresponding to the reference.

After the dialysis process, the next step is the fragmentation of nimotuzumab using pepsin. Monoclonal antibodies such as nimotuzumab comprises two antigen-binding fragments (Fab) that linked to the constant fragment (Fc) by a flexible polypeptide chain called the hinge region. Each Fab fragment is formed by two pairs of polypeptide chains, namely the heavy chain and light chain [18]. Pepsin can be used to cleave immunoglobuline (IgG) molecules at the C-terminal side of the inter-heavy-chain disulfides in the hinge region, producing a bivalent antigen binding fragment $F(ab')_2$ and an Fc fragment. The $F(ab')_2$ produced still be able to bind with the antigen because this form is bivalent. Furthermore, using this enzyme, most of the Fc fragments undergo extensive degradation and cannot be recovered intact [13,19]. By eliminating the Fc portion it also means removing the ability to bind to Fc receptors, thereby reducing the occurrence of non-specific interactions [3].

Nimotuzumab is a humanized antibody [8], this antibody consists of a small portion of mouse Fab that coupled with human antibodies (95-98%) [20]. Previous work reported that mouse IgG antibody and human antibody were fragmented with pepsin to produce $F(ab)_2$ and Fc within 24 hours and 12 hours respectively [13]. To determine the optimum time for fragmentation of nimotuzumab, which has human and mouse component, incubation time were varied from 3, 6, 9, 12, 13 and 14 hours. Fig. 3 showed the SDS-PAGE images of nimotuzumab with pepsin with fragmentation time varied from 3 up to 14 hours.

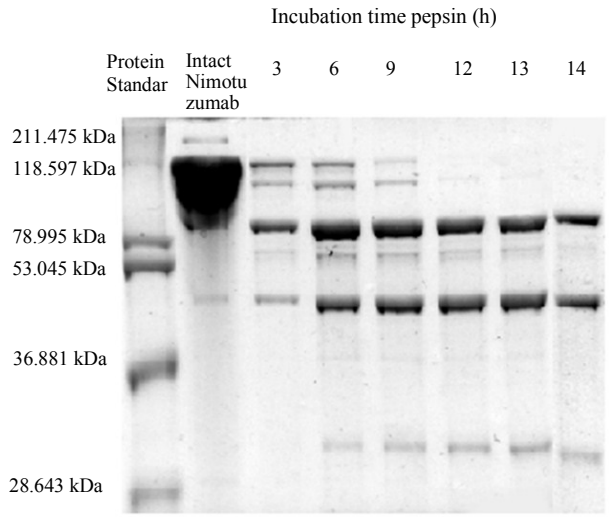

Fig. 3. Time optimization of nimotuzumab fragmentation using pepsin.

It can be seen in Fig. 3 that the initial position of the intact nimotuzumab was between two protein standard with molecular weights of 118,579 Da and 211,475 Da. After the addition of pepsin, the expected molecular weight is about 100,000 Da. Based on the optimization time of fragmentation, it was known that the incubation time for completing the fragmentation of all intact nimotuzumab was 14 hours. It is shown as there was no more protein band at the position of intact nimotuzumab. When compared to protein standard, position of fragment nimotuzumab located between molecular weight 79,995 Da and 118,579 Da. It means that is in compliance with the expected molecular weight. So it can be determined that the time fragmentation of nimotuzumab to produce fragments $F(ab')_2$ was 14 hours.

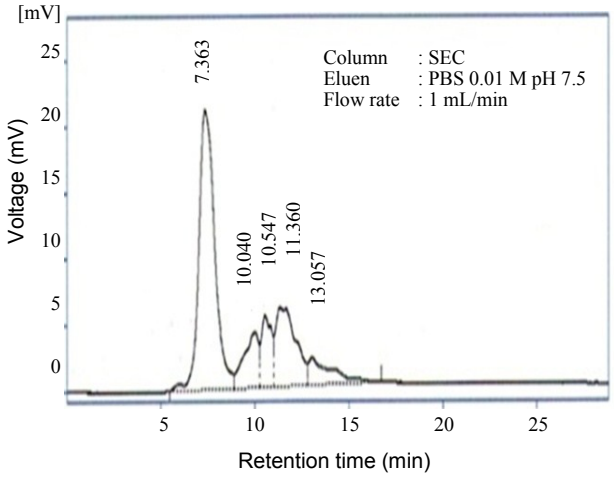

Fig. 4. Chromatogram of $F(ab')_2$-nimotuzumab before purification.

As shown by the SDS-PAGE results, fragmentation process of nimotuzumab with pepsin produce not only $F(ab)_2$ fragment but also by-products such Fc. Chromatogram of the above mentioned raw product which was also analysed with HPLC equipped with SEC BioSuite 250 column (Waters) and an UV-Vis detector (Shimadzu) is presented in Fig. 4.

The chromatogram clearly showed several peaks with Rts of 7.363, 10.040, 10.547, 11.360 and 13.057 mins. The peaks with Rt 7.363 mins was expected to be $F(ab)_2$-nimotuzumab (~ 100,000 Da) as its Rt is betwen Rt of γ-globulin 6.907 mins (158,000 Da) and ovalbumin 8,013 min (44,000 Da) as presented in Fig. 2. Profile chromatograms before purification is needed to determine the effectiveness of the purification performed. The purification method used to separate $F(ab')_2$ with Fc is filtration using PD-10 column of Sephadex G-25.

In order to purify $F(ab)_2$ from other by-products, raw product of fragmented nimotuzumab was passed through a PD 10 column (consist of Sephadex G25 M) pre-blocked and pre-equilibrated with 500 µL 10% BSA and 0.01 M PBS pH 7.4 respectively. Each of fraction from the retrived eluent (0.250 mL/ fraction) was then analysed with with HPLC equipped with SEC BioSuite 250 column (Waters) and an UV-Vis detector (Shimadzu). Figure 5 shows a chromatogram of fragmented nimotuzumab after purification process. It can be seen that purification process by using a PD-10 column was quite effective to eliminate by-product such as Fc portion.

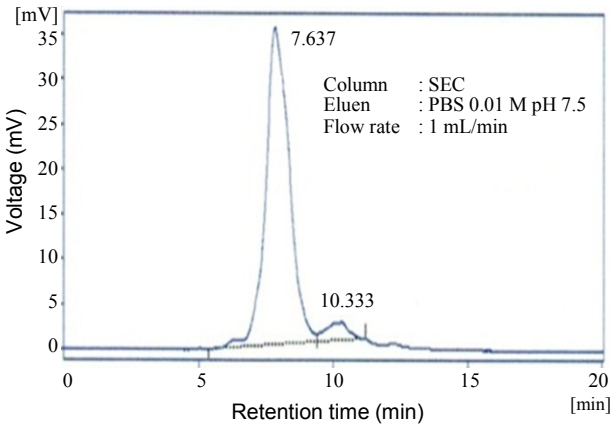

Fig. 5. Chromatogram of $F(ab')_2$-nimotuzumab after purification.

In addition to retention time, from analysis using HPLC, the percent areas of the samples were also obtained. Before purification there were five peaks with the percent area of $F(ab')_2$ was 54.5%. After the purification, there are two peaks with the

percent of area $F(ab')_2$ amounted to 94.7%. Although a small portion remained (Fc fragments = 5.3%) in a purified fraction, but there has been an increase in the percentage purity of the fragment $F(ab')_2$, which is quite significant when compared to the prior column passed.

The radioiodide used for labeling must be analyzed in its radiochemical purity before conjugated with nimotuzumab. To determine the radiochemical purity of $Na^{125}I$ was used paper chromatography (stationary phase : Whatman paper No. 1, mobile phase : methanol 85%).

The radiochromatogram of $Na^{125}I$ was shown in Fig. 6 indicating that the radiochemical purity of $Na^{125}I$ was 97.78%. The requirements set by Medy Physic Inc. for radiochemical purity of I^- is not less than 95% [21]. Based on the reference, $Na^{125}I$ used in this study have met the requirements set for radiochemical purity and can be used for further studies.

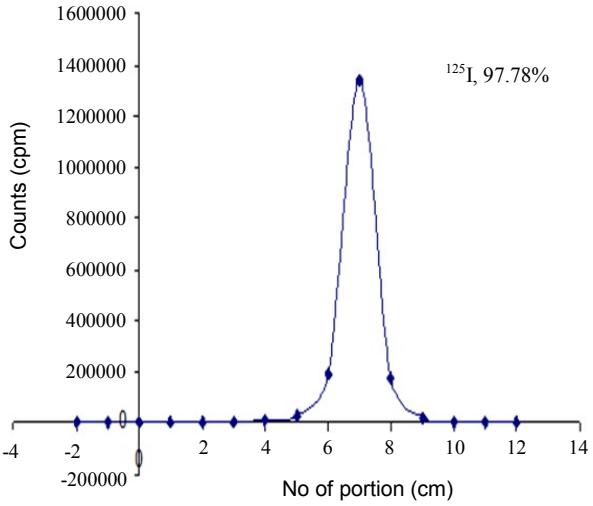

Fig. 6. Radiochromatogram of $Na^{125}I$. A gamma counter was used in the fraction measurements.

In this study, in addition to radiolabeling of ^{125}I-$F(ab')_2$-nimotuzumab, radiolabeling of the intact nimotuzumab was also performed as a comparison. Radiolabeling of $F(ab)_2$-nimotuzumab and intact nimotuzumab with ^{125}I was carried out by firstly oxidizing ^{125}I with Iodogen to form $^{125}I^+$. M. Behe, M. Gotthardt, and T.M. Behr reported that a non reactive form like iodide (I^-) must be activated by an oxidizing agent to a reactive cationic species (I^+) [22]. Its allows a spontaneous electrophilic substitution on phenolic aryl group of tirosin or imidazole group of histidine. The radiolabelled $F(ab)_2$-nimotuzumab and intact nimotuzumab were then purified from unreacted $^{125}I^+$ and other impurities by using PD-10 column (consist of Sephadex G25 M pre-blocked and pre-equilibrated with 500 µL 10% BSA and 0.01 M PBS pH 7.4 respectively).

The graph of fraction ^{125}I-nimotuzumab after passing through PD-10 columns is shown in Fig. 7a, indicating that the fraction 14 is a fraction with the highest activity. It was assumed that this fraction is a fraction that can form complexes ^{125}I-nimotuzumab with a maximum radiochemical purity as compared to the other fractions. To prove the assumption, radiochemical purity was tested using paper chromatography. Then its activity was measured using gamma counter, the measurement results are shown in Fig. 8a. Graph of Fraction ^{125}I-$F(ab')_2$-nimotuzumab after passed the PD-10 columns shown in Fig. 7b. Based on that Figure, it was found that the fraction number of 15 is the fraction with the highest activity. Radiochemical purity of the fractions 15 ^{125}I-$F(ab')_2$-nimotuzumab was 93.24% (Fig. 8b).

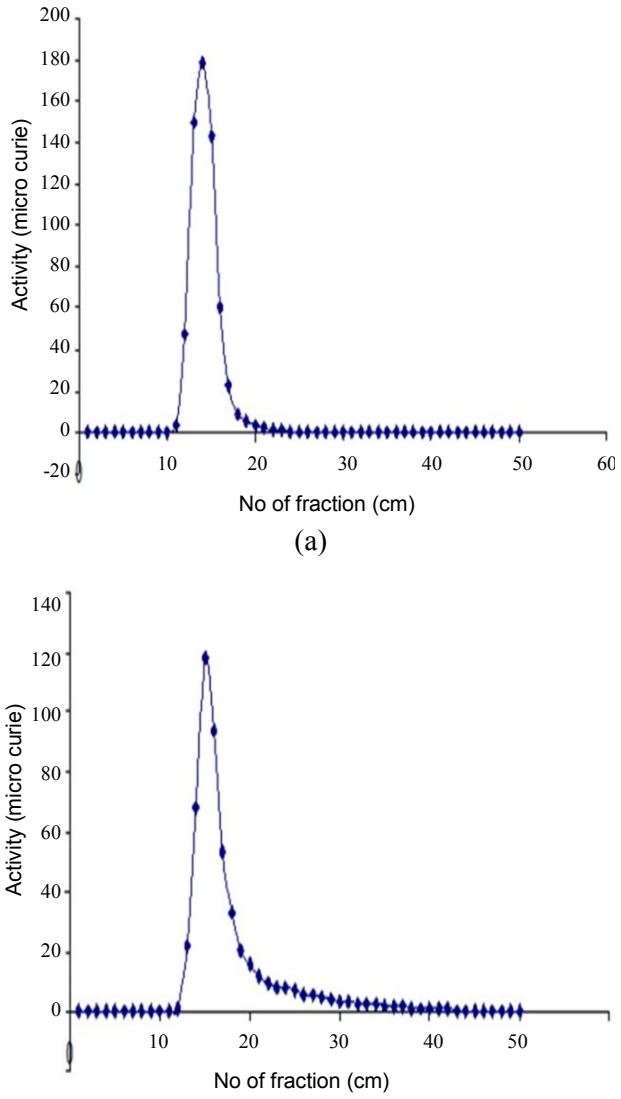

Fig. 7. Graph Fraction of ^{125}I-nimotuzumab (a) and ^{125}I-$F(ab')_2$-nimotuzumab (b) after passed the PD-10 column. A dose calibrator was used in the fraction measurements. Eluent: 0.01 M phosphate buffer pH 7.5.

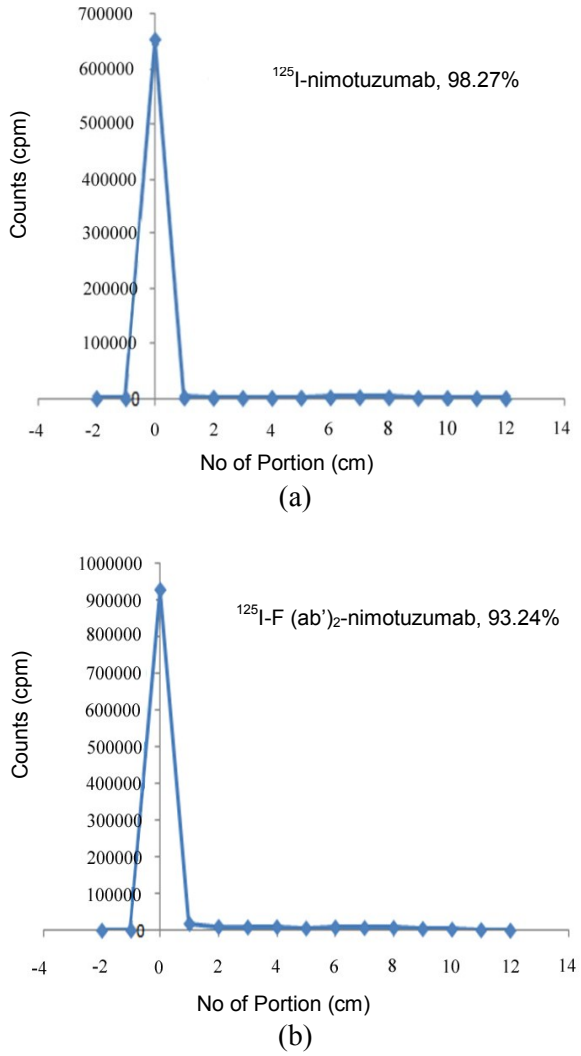

Fig. 8. Radiochromatogram of fraction 14 ^{125}I-nimotuzumab (a) and fractions 15 ^{125}I-F(ab')$_2$-nimotuzumab (b) A gamma counter was used in the fraction measurements.

As it can be seen on Fig. 8, that radiochemical purity of ^{125}I-F(ab)$_2$-nimotuzumab (93.14%) was found slightly lower compared to that of ^{125}I-intact nimotuzumab (98.27%). This might be attributed by the F(ab)$_2$-nimotuzumab used in this experiment which had a purity of only 94.7% and impurity (other protein with a molecular weight < 100 KDa) of ~ 5.3% (Fig. 5). When F(ab)$_2$-nimotuzumab was radiolabelled this impurity was also radiolabelled. This can be seen in existence of tailing in chromatograms of purification of ^{125}I-F(ab)$_2$-nimotuzumab from free $^{125}I^+$ (Fig. 7b).

To date there is no standard regarding the radiochemical purity limits allowed for monoclonal antibodies and/ or their fragments radiolabeled with ^{125}I. However, several reports as follow might be used as a guide. Wen-sheng Huang *et al*., [23] used ^{125}I- and ^{131}I-1H10 monoclonal antibody with radiochemical purity ≥ 90%. While, United States Pharmacopeia (USP) 35 in 2012 states that radiochemical purity of albumin marked or radiolabelled with the ^{125}I , may not be less than 97% [24].

Stability test of ^{125}I-F(ab')$_2$-nimotuzumab as well as ^{125}I-nimotuzumab were performed in order to determine whether these complexes were stable in various storage temperatures (4 °C, room temperature and 37 °C).

Table 1. Percentage of radiochemical purity ^{125}I-F (ab')$_2$-nimotuzumab and ^{125}I-nimotuzumab at 3 variations storage temperature.

Incubation Time (day)	% Radiochemical Purity					
	^{125}I-nimotuzumab			^{125}I-F(ab')$_2$-nimotuzumab		
	Room Temperature	37 °C	4 °C	Room Temperature	37 °C	4 °C
1	97.07	97.07	97.07	92.29	92.29	92.29
3	96.70	95.40	96.55	79.81	83.91	86.98
5	94.14	95.25	96.63	78.53	83.48	87.37

It can be seen in Table 1 that both of ^{125}I-F(ab')$_2$-nimotuzumab and ^{125}I-nimotuzumab maintained their radiochemical purity when stored at any above-mentioned temperature for one day. However radiochemical purity both complexes started to decrease after incubated up to five days. After five days the radiochemical purity of ^{125}I-F(ab')$_2$-nimotuzumab decreased to 78.53, 83.48 and 87.37% when stored at room temperature, 37 and 4 °C respectively. While the radiochemical purity of ^{125}I-nimotuzumab decreased to 94.14, 95.25 and 96.63% when stored at room temperature, 37 and 4 °C respectively. Based the above-mentioned results appeared that both complexes, F(ab')$_2$-nimotuzumab and ^{125}I-nimotuzumab, were relatively stable at 4 °C.

CONCLUSION

Nimotuzumab fragment, F(ab')$_2$-nimotuzumab, with a purity of 94.7 %, had been sucssesfully prepared by fragmenting of intact nimotuzumab with pepsin for 14 hours and purification process by using a colomn chromatography. Radiolabeling of F(ab')$_2$-nimotuzumab with ^{125}I by using a iodogen as oxidator resulted ^{125}I-F(ab')$_2$-nimotuzumab with radiochemical purity of 93.24% after a purification process by using a colomn chromatography. Stability test results showed that ^{125}I-F(ab')$_2$-nimotuzumab as well as ^{125}I-nimotuzumab retained their radiochemical purities when stored either at 4,

37 °C or room temperature for one day. While either ^{125}I-F(ab')$_2$-nimotuzumab or ^{125}I-nimotuzumab was found relately stable when stored at 4 °C for up to five days. Radiochemical purity either ^{125}I-F(ab')$_2$-nimotuzumab or ^{125}I-nimotuzumab however decreased significantly when stored at room temperature and 37 °C for up five days.

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