Evaluation of Di-Amino Phenol Substituted EDTA for use in Radiolabelling Proteins with ^{64}Cu.

P. F. Schmidt, S.V. Smith and N. M. Di Bartolo.
Radiopharmaceutical R & D, Ansto, P O Box 1, Menai, 2234 NSW, AUSTRALIA.

In recent years considerable effort has been directed towards synthesis of bifunctional chelating agents for coupling radiometals to proteins for tumour targeting. Of particular interest is the design of chelators for radiolabelling with copper. Two isotopes of copper have been identified as showing potential in the development of diagnostic and therapeutic agents. Copper-64 ($t_{½}$ = 12.4 h, β^+) for use in PET imaging and ^{67}Cu ($t_{½}$ = 12.4 h, γ = 185 keV, β^-_{max} = 390 keV) for use in therapy. This study involves a high yielding synthesis of a novel di-amino-phenol substituted EDTA (DAHA-EDTA) ligand and its radiolabelling chemistry with ^{64}Cu produced at the national medical cyclotron (NMC).

High activity levels (upto 59.2 GBq EOB) of ^{64}Cu is co-produced during the production of ^{67}Ga from enriched ^{68}Zn. Waste eluent from the NMC ^{67}Ga production was evaporated to dryness and found to contain by products such as ^{57}Ni, ^{57}Co, ^{64}Cu, ^{67}Cu and ^{55}Co. A new method involving low acid concentration aqueous/organic mixtures with an anion exchange (AG 1-X8, BioRad) have been used to isolate the carrier-free ^{64}Cu. The specific activity of the ^{64}Cu (5×10^{14} Bq/g) was found to be higher than that produce by Australian radioisotopes (ARI).

The synthesis of the ligand involves the refluxing of EDTA anhydride in the presence of 4-nitro-2-amino-phenol in acetonitrile to produce the di-nitro derivative (DNHA-EDTA) in > 95% yield. The DNHA-EDTA is then reduced in the presence of activated palladium charcoal with sodium borohydride under an inert atmosphere at room temperature. The reaction mixture was acidified and the catalyst removed to obtain the final product, DAHA-EDTA.

Copper complexes of DAHA-EDTA were prepared over a range of pH's (pH 3 - 7). Complexation of the copper was assessed by ITLC-SG analysis (Methanol : Water (9:1)). Complexation of the copper is greater than 99% within 1 min at room temp. Serum stability of the unsubtituted di hydroxy derivative (DHA-EDTA) is good with only 2.1% dissociation of the copper in 72 h.

Labelling of proteins (B72.3, DD-3B6/22 and streptavidin) has been achieved with the DAHA-EDTA ligand. The procedure involves the addition of the copper / ligand complex to the protein at > 3mg/ml in the presence of excess 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The reaction mixture is left to incubate for 1 h at 37°C and radiolabelled protein is then isolated using size exclusion chromatography.