IN VIVO EVALUATION OF COPPER-64-LABELED CORE-SHELL NANOPARTICLES UTILIZING MICROPET AND BIODISTRIBUTION

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Keywords: microPET, Cu-64, shell crosslinked nanoparticle, biodistribution

Introduction. Shell crosslinked nanoparticles (SCKs) are amphiphilic core-shell nanoparticles inspired from biological constructs (1-2). They are prepared by the self-assembly and subsequent crosslinking of block copolymer micelles and can be optimized for guest packaging. The easily controlled structural and chemical features play a critical synergistic role in their resulting binding, sequestration, toxicity, and immunogenic properties. To explore the biomedical application of these SCKs, we have designed biocompatible nanoscale scaffolds that will provide multi-/poly-valent presentation of functionalities for detection, diagnosis and intervention of disease states by coupling various target molecules to SCKs, in which radionuclides are included for imaging and/or radiotherapy. Herein, we present an approach to complex ^{64}Cu to the SCK via conjugation of a TETA derivative, and preliminary in vivo evaluation of the ^{64}Cu-labeled nanoparticles.

Methods. The SCKs and nanocages (NCs) (resulting from ozone degradation of the SCK core) were prepared as previously described (3-5). The conjugation of TETA-CONH-$(C_2H_4O)_2C_2H_4NH_2$ (TETA-NH_2) to the SCK or NC was carried out by modifying our DOTA-mAb conjugation procedure. ^{64}Cu-labeling was accomplished by incubation of $^{64}Cu(OAc)_2$ with TETA-NH-SCK (or NC) for 4 h at 70 C, followed by a DTPA challenge and Centricon separation. The radiochemical purity was monitored by FPLC. The biodistribution studies used mature Sprague-Dawley rats (n = 4, *ca.*12 Ci/100 L), and the PET imaging was performed on the first commercially available microPET (Concorde Microsystems, Knoxville, TN) using Balb/c mice (n = 2, 150 Ci/100 L).

Results and Discussion. Because self condensation between the carboxyl groups and the amino groups of TETA-NH_2 molecules occurred when direct conjugation to the nanostructures was attempted, a step was introduced to produce and separate the activated SCK-NHS ester. Through the modified procedure, both SCKs and NCs showed up to 70% radiolabeling yields, and over 95% radiochemical purity after a DTPA challenge and centricon separation, as indicated by FPLC. The preliminary biodistribution data of ^{64}Cu-TETA-NH-NC (57-nm, Mw 1,510,900) and ^{64}Cu-TETA-NH-SCK (13.7-45 nm, Mw 523 - 4,004 KDa) showed rapid ^{64}Cu uptake in liver (NC: 69.23 1.31 %ID/liver at 10 min p.i.; SCK with MW 4,004 KDa: 46.88 3.27%ID/liver at 10 min p.i.), and slow clearance via kidneys and intestine, which was further confirmed by the microPET imaging studies. To lower the liver burden, a PEG-derivatized SCK (MW 4,004 KDa plus added PEG) was prepared (PEG: poly(ethylene glycol)). The microPET images clearly showed both kidneys, which indicates that an increased renal clearance resulted from the PEG derivatization. Future studies include: determination of SCK and NC size effects on the in vivo behavior; further modification of the SCK and NC surface chemistries; and coupling of ligands for specific molecular targeting.

Acknowledgment. This research is supported by a NCI/NASA Biomolecular Sensors contract (N01-CO-27103), and a CBI Program Training Fellowship (MLB) at Washington University funded by the NIH-NRSA (5-T32-GM08785-02). The ^{64}Cu was provided through an NCI grant (R24 CA86307).

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[O-15] ACTIVITY TRAPPING AT HIGH TEMPERATURES IN THE PRODUCTION OF [O-15] CO

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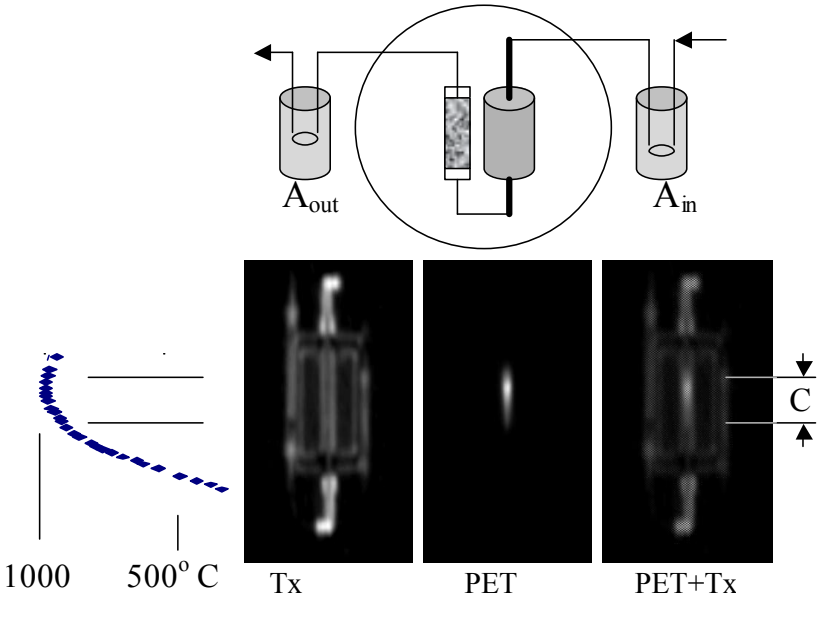
Keywords: Oxygen-15, carbon monoxide

Carbon monoxide (CO) plays a significant role in PET, both as a precursor for formylation reactions and as an *in vivo* tracer of regional blood volume. The symmetric opportunity for labelling with [C-10], [C-11] and [O-14], [O-15] reveals a problem that arises in the oxygen-labeling of CO, where labelled O_2 is passed over activated charcoal at elevated temperatures (900- 1000° C). At the Wolfson Brain Imaging Centre, a flow-through gas target (N_2 +1% O_2; 5 bar) is irradiated with 8 MeV deuterons, with the $^{15}O_2$ flowing to the PET clinic through narrow-bore tubing for conversion to $C^{15}O$, on demand. Major activity losses were noted during $C^{15}O$ production, motivating a series of experiments where the charcoal furnace and a subsequent ascarite trap were mounted in the gantry of the GE Advance PET scanner, bracketed by a pair of dose calibrators monitoring the incoming and outgoing gas-borne activity in matched flow loops. With stable flow and a constant beam on target, the charcoal temperature was ramped from 500° C to 1000° C, where-upon the transmitted [O-15]-CO activity, shown to be radiochemically pure by gas chromatography, was attenuated ten-fold. The bulk of the [O-15] activity was clearly trapped at the upstream end of the 1000° C charcoal converter, shown by careful co-registration of the transmission and emission images (shown below).

Visual inspection of the charcoal converter shows an ash-like deposit at the trapping site. Thermal profiling confirms a uniform temperature (+/- 5%) over the central 50 mm zone containing the charcoal. Work is proceeding with altered carrier concentrations and catalysts (Pt/C) in an effort to reverse the trapping, and retrieve the expected activities.



BASE-PROMOTED DECHLORINATION OF (*R*)-[^{11}C]PK 11195

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Keywords: (*R*)-PK 11195, 'peripheral benzodiazepines', dechlorination

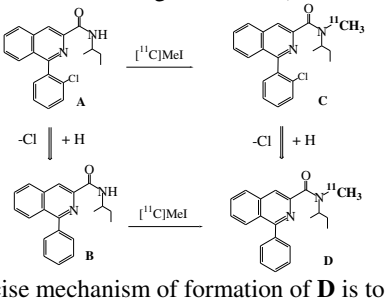
(*R*)-[^{11}C]PK 11195 (**C**, Scheme 1) is a 'peripheral BZR' radioligand that has been used to study various neurological traumas (e.g. cerebral infarction and multiple sclerosis) with PET.

In our preparations of (*R*)-[^{11}C]PK 11195 (obtained by heating 1 mg *desmethyl*-(*R*)-PK 11195 at 80 °C for 2 min with [^{11}C]MeI, in 200 µl DMSO and 20 mg powdered KOH, followed by HPLC purification) we have on several occasions found an unknown radioactive impurity. On our analytical HPLC system (Primesphere C18-HC 5µ, 250 x 4.6 mm, methanol-water 85/15 v/v, 1.8 ml/min) this impurity elutes close (RT = 4.15 min) to the (*R*)-[^{11}C]PK 11195 (RT = 4.0 min). The impurity has represented up to 55% of the radiochemical purity. However, on each occurrence the impurity was not evident in our preparative HPLC chromatogram, obtained on a µ-Bondapak column C18 10µ, 300 x 7.8 mm, eluted with acetonitrile-water 65/35 v/v at 4.25 ml/min ((*R*)-[^{11}C]PK 11195, RT = 6.3 min). This indicated co-elution of the impurity with the (*R*)-[^{11}C]PK 11195. To investigate the identity of the unknown impurity and prevent its occurrence in our final product we have developed more powerful preparative and analytical HPLC methods.

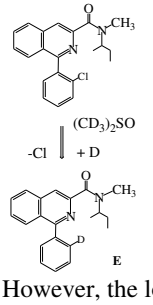
Our new preparative HPLC method (Luna C18 3µ, 20 x 2 mm, acetonitrile-water 28/72 v/v, 1.7 ml/min) gives a baseline separation between the radioactive impurity (RT = 6.5 min) and (*R*)-[^{11}C]PK 11195 (RT = 8.5 min) and shows that the impurity represents between 2 and 20% (n = 15) of the radioactivity in our reaction solutions. With the improved analytical HPLC method (Luna C18 3µ, 20 x 2 mm, acetonitrile-water 32.5/67.5 v/v, 1.65 ml/min) the impurity RT was at 2.4 min with (*R*)-[^{11}C]PK 11195 at 3.1 min.

HPLC-MS of the reaction solution showed the impurity to have *m/z* 319 [100%] with no chlorine isotopic pattern, proving the compound to be a hydro-*des-chloro* analogue of PK 11195 (**D**, Scheme 1). Compound **B** *m/z* 305 [100 %] was also found in the reaction solution and is probably similarly derived from the *desmethyl* precursor (**A**). LC-MS analysis verified this compound was absent in our precursor. This indicates that there are two possible routes of formation of the labeled impurity **D,** as shown in Scheme 1. Further indication of the generation of this product was obtained by heating PK 11195 (80 °C, 5 min) with KOH in deuterated-DMSO (n = 4). This was shown by LC-MS to produce the deuterated product **E** , Scheme 2, (*m/z* 320 [100%]). No detectable amount of **E** was obtained with heating PK 11195 (80 °C, 5 min) with just deuterated-DMSO (n =2).

Scheme 1



Scheme 2



The precise mechanism of formation of **D** is to be determined. However, the loss of the chloro group under these conditions may indicate that it is labile to substitution. This may be exploited to introduce other isotopes (possibly ^{18}F) into this aromatic system. This study also indicates their is a need for great care in the purification of (*R*)-[^{11}C]PK 11195 for PET investigations.

Acknowledgment: Dr V.W. Pike (MIB, NIMH, Bethesda, MD, USA) for helpful discussion

NEW POLYMER-SUPPORTED IONIC LIQUID; IONIC RESIN AS A CATALYST FOR NUCLEOPHILIC FLUORINATION AND [^{18}F]FLUORINATION

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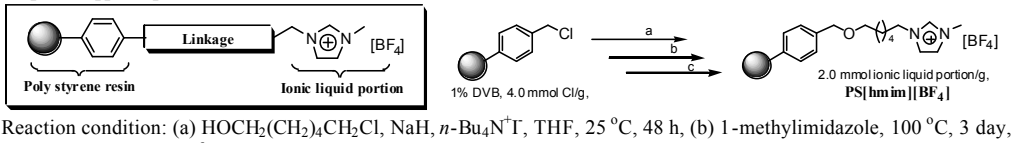
Keywords: Fluorination, Fluorine-18, Labeling method, Cesium fluoride, Ionic liquid, Resin, PET

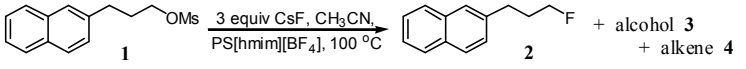
Recently, we reported a highly efficient method for nucleophilic fluorination using KF and no-carrier-added [^{18}F]fluorination in ionic liquids such as [bmim][BF_4]. In this method, ionic liquids play an important role in significantly enhancing the reactivity of KF as well as in reducing the formation of by-products, e.g. alkenes and/or alcohols [1,2]. This method provides a very rapid and efficient means for nucleophilic [^{18}F]fluorination of alkyl mesylate or alkyl halides to their corresponding [^{18}F]fluoroalkanes, without the need for strictly anhydrous conditions. However, despite these merits, we have faced the difficulty in the extraction of polar products from the ionic liquid in case of polar target compound. To solve this problem, we designed the new immobilized ionic liquid and synthesized the polymer-supported 1-*n*-hexyl-3-methylimidazolium tetrafluoroborate (PS[hmim][BF_4]) as shown in Figure 1. Table 1 illustrates that PS[hmim][BF_4] is a better catalyst than any other catalysts for fluorination using CsF. Thus, fluoroalkanes **2** can be obtained through just simple filtration due to easy removal of PS[hmim][BF_4] after the reaction.

Figure 1. Polymer-supported ionic liquid and synthesis of polymer-supported ionic liquid: PS[hmim][BF_4]



Reaction condition: (a) $HOCH_2(CH_2)_4CH_2Cl$, NaH, *n*-$Bu_4N^+I^-$, THF, 25 °C, 48 h, (b) 1-methylimidazole, 100 °C, 3 day, (c) NaBF4, acetone, 25 °C, 48 h.

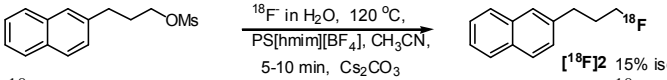
Table 1. Fluorination of Mesylate **1** with Various Metal Fluorides under Various Reaction Conditions.[a]



entry	PS[hmim][BF_4] (equiv)	CH_3CN (mL)	time (h)	yield of product (%) [b]			
				1	**2**	**3**	**4**
1	1	6	2	-	98	-	-
2	0.5	3	2.5	-	98	-	-
3	-	3	48	79	16	-	-
4	18-crown-6	6	5	trace	88	-	7[c]
5	[bmim][BF4] (0.5)	3	3	27	68	-	-

[a] All reactions were carried out on a 1.0 mmol reaction scale of mesylate **1** using 3 mmol of CsF. [b] Isolated yield. [c] NMR determined.

Scheme 2. [^{18}F]Fluorination Using PS[hmim][BF_4]



[^{18}F]Fluorination of **1** using PS[hmim][BF_4] only provided [^{18}F]**2** in 15% radiochemical yield after reaction at 120 °C for 5-10 min, and 75% of $^{18}F^-$ was attached to PS[hmim][BF_4]. Further studies are underway to solve this problem in our laboratories.

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ADIABATIC RESONANCE CROSSING FOR ACCELERATOR PRODUCTION OF NEUTRON-RICH ACTIVATED NANOSPHERES FOR BRACHYTHERAPY

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Keywords: brachytherapy, nanoparticles, microtubes, Rhenium, Lutetium.

Adiabatic Resonance Crossing (ARC) has been proposed by Nobel laureate Carlo Rubbia as a new method to enhance neutron capture for the activation of radioisotopes for medical and industrial applications. The ARC method, which was developed at the European Organization for Nuclear Research (CERN), allows using the neutrons produced on an accelerator target as an efficient radioisotope-production alternative to the use of nuclear reactors.

The ARC method, when coupled with small sized cyclotrons currently used for PET isotopes production (16-19 MeV, 100 µA), can be efficiently used to produce therapeutic doses of radiopharmaceuticals for brachytherapy. We have chosen to activate Rhenium-186, Rhenium-188, Lutetium-177 and Holmium-166. Activation rates can be efficiently calculated through advanced numerical calculations.

Activations are performed on ferrite nanospheres (500 nm diameter) that can contain virtually any radioactive material. The isotope to activate inside the nanosphere can be chosen according to the kind and the size of tumour to treat.

Ferrite nanospheres mixed into a sterile liquid solution, are efficiently injected with the help of implanted perforated microtubes using a new and fast technique. Microtubes are 20 to 50 cm long, 200 µm external diameter (100 µm internal) and are perforated with 50 µm holes at one extremity, where the treatment has to be done. The sterile solution is activated into the ARC target for a few hours before injection, already in its final galenic form, into sealed Aluminium capsules.

Particles can spread uniformly up to several millimetres from the microtube, thanks to their reduced size, and the high-pressure injection technique. Considering the additional range of the decay particle emitted by the active radioisotope, larger areas can be efficiently and uniformly treated.

Nanospheres diffuse efficiently into the irradiated tissue thanks to their size, but, without a conglomerating effect, they tend to subsequently migrate into the body. Due to their magnetic properties, ferrite nanoparticles should form micro conglomerates that remain into the irradiated tissues and do not enter into the main blood circulation, reaching healthy organs. Injection can be repeated several times for an increased therapeutic efficiency.

The advantage of this technique is its reduced cost, its extreme rapidity (a few seconds) and the possibility of efficiently treating large volumes of tissue. Microtubes can be implanted during surgery or with the help of echography, scintigraphy or MRI (Magnetic Resonance. Imaging) in the non-operable metastasis and they can stay for long time in the organism.

The viability of this technique has now to be proved through pre-clinical studies. We believe this technique can be extremely efficient for the treatment of a variety of non-operable cancers.