

High Capacity H₂ATSM Kits for On-Site Synthesis of Hypoxia-Specific Agent ⁶⁴Cu-ATSM

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Copper Radioisotopes in PET

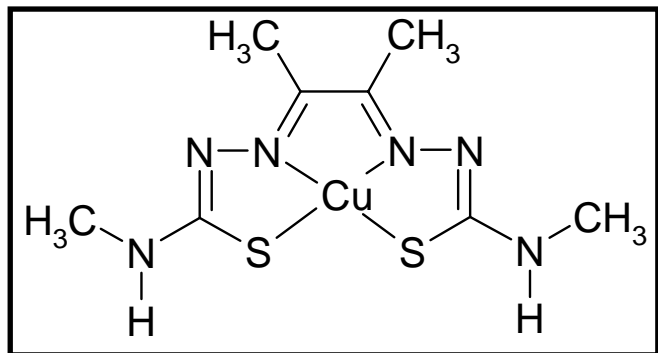
Isotope	$T_{1/2}$	Decay Modes	β^+ Range (mm) ¹	Production Method	Specific Activity (mCi/ μ g)
⁶⁰ Cu	23.4 m	β^+ (93%) EC (7%)	3.09	⁶⁰ Ni(p,n) ⁶⁰ Cu	---
⁶¹ Cu	3.41 h	β^+ (60%) EC (40%)	1.32	⁶¹ Ni(p,n) ⁶¹ Cu	---
⁶² Cu	9.7 m	β^+ (98%) EC (2%)	4.39	⁶³ Cu(p,2n) ⁶² Zn \rightarrow ⁶² Cu (⁶² Zn generator)	>70000 313,000 UL
⁶⁴ Cu	12.7 h	β^+ (19%) β^- (38%) EC (43%)	0.57	⁶⁴ Ni(p,n) ⁶⁴ Cu	\leq 200 3856 UL

¹ H. A. Williams, *et al.*, *Eur. J. Nucl. Med. Mol. Imaging*, 2005:32, 1473-1480.

⁶²Cu and ⁶⁴Cu are distributable PET radioisotopes.

⁶²Cu and ⁶⁴Cu have complementary properties for PET imaging.

Radiocopper Bis(thiosemicarbazone)s



H_2 ATSM ligand shown
complexed to copper

- MW (ligand) = 260.38
- Equilibrium H_2O solubility
~0.6 ppm
- Cu binding capacity
~0.24 μg Cu/ μg ligand

- Bis(thiosemicarbazone) ligands have very high affinity for radiocopper ($K > 10^{17}$).
- Cu-ATSM has shown clinical selectivity for hypoxic tissue.
- Traditional synthesis of ^{64}Cu -ATSM has relied upon reversed-phase purification before dosing.
 - Significant operator doses result
 - EtOH in injection
 - Sterility issues
- Kit-based synthesis highly preferred in clinical setting.
 - Low operator dose and no special equipment
 - No chemical purification required
 - Sterility assured

Bis(thiosemicarbazone) Synthesis Kits



PTI Instant Synthesis Kits:

- **0.4 μg H_2ATSM**
- **2.0 μg H_2ETS**
- **2.0 μg H_2PTSM**
- **4.0 μg H_2ATSM
(Big Vessel)**

- Four bis(thiosemicarbazone) instant synthesis kits are currently produced at PTI for investigational and consumer use: H_2PTSM , H_2ETS and H_2ATSM (2 sizes).
- H_2ATSM kits containing either 0.4 or 4.0 μg of ligand are currently available.
- All kits prepared under GMP conditions.
- Cu binding capacity of H_2ATSM kits:
 - 0.4 μg = 0.1 μg Cu
 - 4.0 μg = 1.0 μg Cu
 - 15.0 μg = 3.6 μg Cu (under development)

Why are Larger Capacity H₂ATSM Kits Necessary?

- The 0.4 μg H₂ATSM kits are used in conjunction with PTI's ⁶²Cu microgenerator for commercial and investigational clinical use.
- The 4.0 μg H₂ATSM kit is available for use with ⁶⁴Cu.
- Multicenter ⁶⁴Cu-ATSM clinical trials for prognostic evaluation of various cancers are being planned.
 - Washington University Mallinckrodt Institute of Radiology
 - Clinical protocol calls for injected doses of up to 25 mCi ⁶⁴Cu.
 - Numerous remote sites included in study
- ⁶⁴Cu specific activity becomes problematic at these doses.

^{64}Cu Specific Activity is Problematic

- Maximum ^{64}Cu specific activity is ~ 200 mCi/ μg at EOB.
- At EOB, 25 mCi ^{64}Cu represents 0.13 μg Cu.
- 4.0 μg H₂ATSM kit capacity is ~ 1 μg Cu (7.7-fold excess)
- ^{64}Cu specific activity is often much lower (~ 100 mCi/ μg)
- Overnight shipment to remote clinical sites lowers specific activity by ~ 4 fold (2 half-lives).
- Low specific activity material shipped to remote sites challenges kit capacity and can lower labeling yield.
 - For 100 mCi/ μg at EOB = ~ 25 mCi/ μg next day.
 - For 25 mCi dose = 1.0 μg Cu \sim kit binding capacity.

Preliminary ^{64}Cu Binding Studies of 4 μg Kits

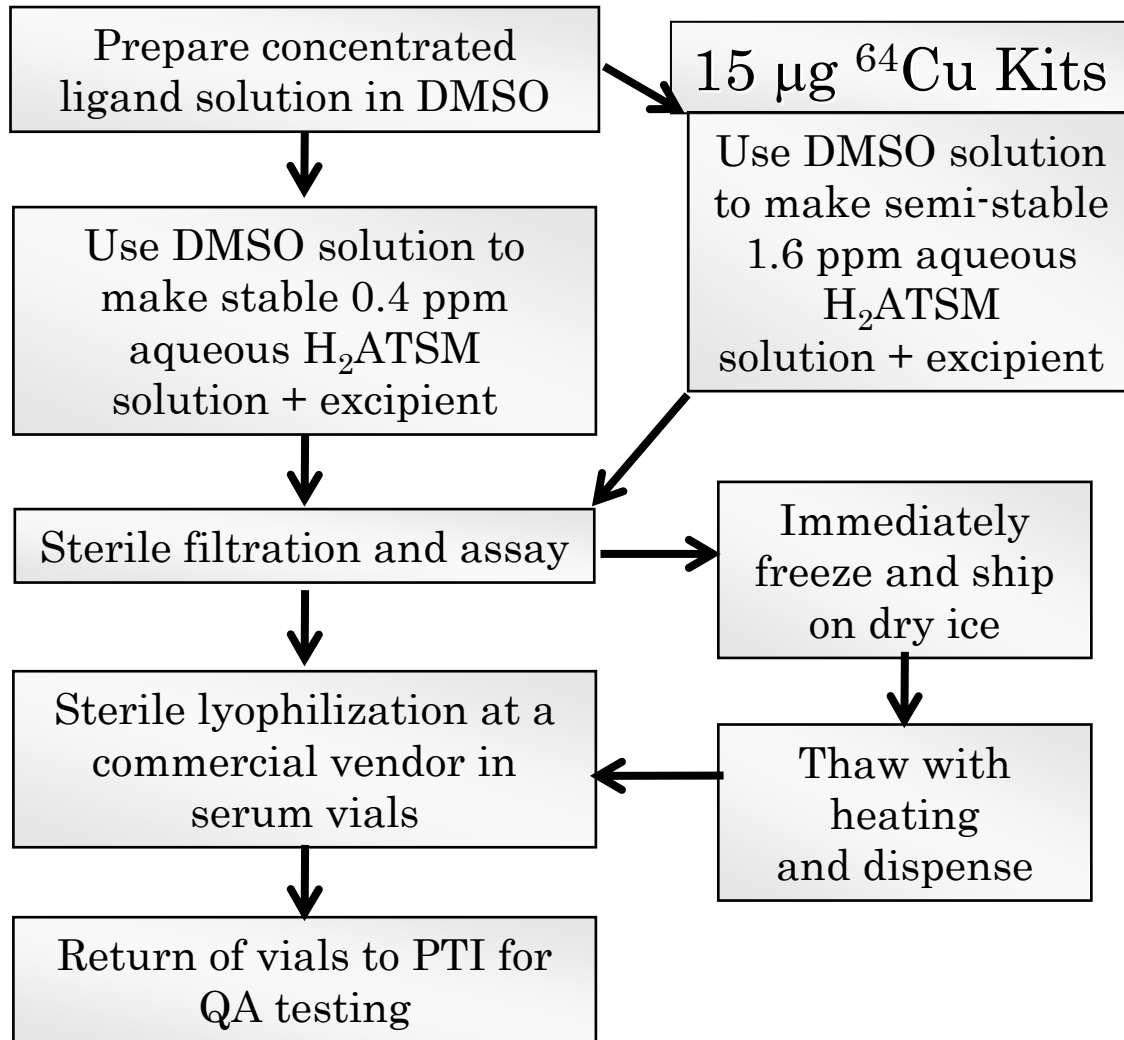
^{64}Cu Activity (mCi)	Specific Activity (mCi/ μg)	Total Cu (μg)	% of Kit Binding Capacity	Radiochemical Labeling Yield (%)
28.5	125	0.23	24	96.5
17.6	125	0.14	14	97.1
31.8	30.6	1.04	~100	92.4

- Kits reconstituted with 25 mM NaOAc/100 mM NaCl prior to adding ^{64}Cu in 0.1 M HCl (Isotonic solution).
- Labeling yield determined by Oasis[®] cartridge method (Waters)

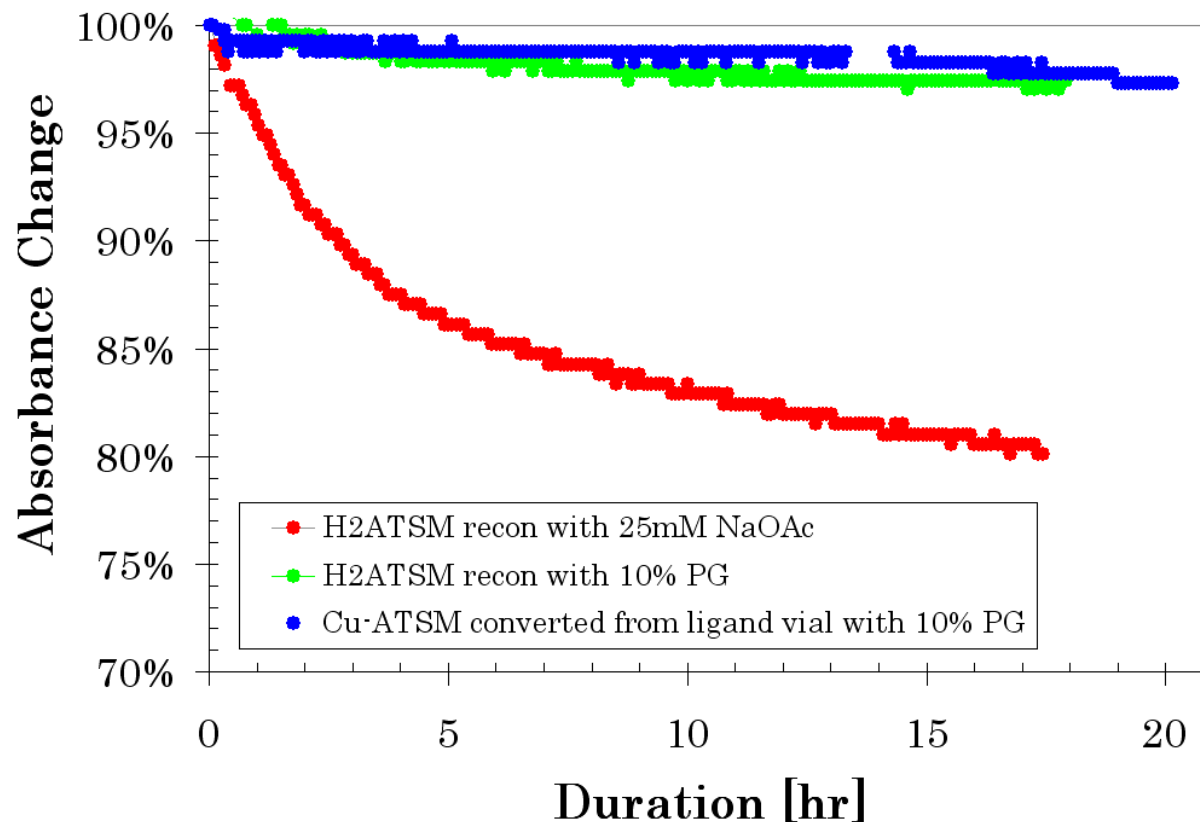
$$\% \text{ yield} = A_{\text{cartridge}} / (A_{\text{saline}} + A_{\text{cartridge}})$$

Preparation of H₂ATSM Synthesis Kits

- Aqueous solubility of H₂ATSM is limited
- Lyophilization volumes
0.4 µg = 1 mL
4.0 µg = 10 mL
15.0 µg = 37.5 mL!!!
- Improved methodology required for larger kits to overcome low equilibrium solubility.
- Preliminary evaluation of 15 µg kits shows satisfactory solubility of ligand and Cu complex.



Time-Dependent Solubility of H₂ATSM and Cu-ATSM in 15 μg Synthesis Kits



Conclusions

- H₂ATSM ‘instant synthesis’ kits for coordination of radiocopper can be readily prepared in various sizes.
- Kits give excellent radiochemical labeling yields in isotonic saline solution with no required workup and relatively low operator dose.
- Developmental kit containing 15 µg H₂ATSM can be successfully prepared and fully reconstituted to provide all ligand available in solution.
 - Pilot run of ~300 kits shows good reproducibility
 - Comparable to GMP 0.4 µg H₂ATSM kits
 - Sterile and pyrogen-free
- Independent ⁶⁴Cu radiochemical labeling to be conducted.

Acknowledgments

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