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Optimization of the labeling yield by determination of the Cu^+–acetonitrile complex constant in Cu^+-catalyzed nucleophilic exchange reactions in mixed solvent conditions

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Abstract To apply the Cu^+-assisted nucleophilic exchange based radioiodination of aromatic compounds for more lipophilic compounds the reaction is carried out in mixed solvent conditions. Due to its physicochemical properties acetonitrile is an attractive solvent. Although acetonitrile forms complexes with Cu^+ decreasing the labeling yield. This article describes a method for the determination of the complex constant at labeling temperature based on a Lineweaver–Burk approach, relating the reaction rate constant and the concentration of precursor in presence of different amounts of acetonitrile. The method also allows to calculate the adjusted amount of copper salt in order to obtain the same high labeling yield as obtained in absence of acetonitrile.

Keywords Radioiodination · Mixed solvent · Acetonitrile · Complex constant · Adjusted copper concentration

Introduction

The current awareness of problems in the global world supply of ^{99}Mo, can lead to an increased demand of cyclotron-produced radioisotopes, for instance ^{123}I for the production of radioiodinated SPECT tracers. In this context, the Cu^+-assisted nucleophilic radioiodination method has proven its use for the production of ^{123}I-labeled radiopharmaceuticals, on both a single centre and industrial manufacturing scale [1].

The Cu^+-assisted nucleophilic exchange radioiodination of aromatic compounds in acid reducing conditions can be used with success in mixed solvents when lipophilic compounds, like brain receptor tracers, are involved [2–4].

Due to its physicochemical properties, acetonitrile (ACN) is a common used solvent for nucleophilic exchange reactions in mixed solvent conditions. Moreover acetonitrile is often present in the mobile phase used for HPLC recovery of a no carrier added radioiodinated compound coupled to the azeotropic distillation at low temperature of the ACN/water mixture.

ACN forms as a soft Lewis base stable complexes with the Cu^+-ion, a soft electron-pair acceptor [5–7]. When applying the Cu^+-assisted nucleophilic exchange radioiodination, the complexation of the Cu^+-ions causes a considerable decrease of the labeling yield [8].

As the complex formation constants reported in literature are obtained at room temperature [9] it is needful to develop a method to estimate the Cu^+–ACN complex formation constant at the higher temperatures required to obtain high labeling yields.

Materials and methods

Reagents

All reagents and solvents were obtained from commercial suppliers and were HPLC- or analytical grade and used as such.

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2-Iodo-hippuric acid (OIH) and meta-iodobenzylguanidine sulfate (MIBG) were donated by Mallinckrodt Medical, The Netherlands.

Nitrogen was 5.0-grade and purchased from Hoekloos, The Netherlands.

Radioiodide (Na[^{123}I], no carrier added; specific activity of 8695 GBq/μmol) in 10^{-2} M NaOH was obtained from BV Cyclotron Vrije Universiteit Amsterdam.

HPLC equipment and analyses

The reaction mixtures for the labeling of OIH and MIBG with ^{123}I were analyzed by HPLC: Rheodyne injector (0.1 ml loop), a Jasco pump with a Jasco UV monitor at 230 nm, a flow-through NaI(Tl)-radioactivity detector (Ortecs electronics), a LiChrosorb RP Select B column (Merck), 5 μ, 125 × 4 mm. OIH was analyzed with a MeOH/0.08 M sodium dihydrogenphosphate solution 10/90 (v/v) (pH ∼ 5.2) as mobile phase at a flow rate of 1.2 ml/min and MIBG with a MeOH/0.08 M sodium dihydrogenphosphate solution 35/65 (v/v) (pH ∼ 4.9) at a flow rate of 0.95 ml/min.

All chromatographic date were filed and analyzed using Gina Star software, version 14.0.

Labeling procedure and experiments

- Radioactivity: Radioactivity was measured using a Veenstra dose calibrator, type VDC 404.
- Radioiodination procedure: Labeling experiments were carried out in a 2 ml flat-bottom vial (with PTFE-faced silicone septum and open top crimp cap) with weighed amounts of OIH or MIBG, 2,5-dihydroxybenzoic acid (gentisic acid) and citric acid, dissolved in 0.8 ml aquapure water. Afterwards appropriate volumes of an aqueous $CuSO_4$ stock solution were added and final volume was adjusted to 1 ml using aquapure water.

Subsequently, 10–20 MBq Na[^{123}I] (10–15 μl) was added, the vial was crimped and the content flushed with a gentle stream of N_2 during 10 min at room temperature.

The vial was placed in a copper containment containing paraffin oil and heated in a thermo block. After fast cooling of the reaction mixture to room temperature, a sample was taken for HPLC-analysis to measure the labeling yield.

The labeling yield (LY) is defined as the ratio of the amount of the labeled compound to the initial amount of activity calculated from the surfaces of the peaks in the radiochromatogram.

According to the described labeling procedure, two analogue series of labeling experiments were performed using OIH and MIBG as reference substrate.

The influence of ACN on the reaction rate: For the reactions in presence of ACN, the Cu^{2+}-solution is added first to the solution containing the reducing agents allowing complete reduction (1 min) to Cu^{+} before the addition of ACN.

• Labeling experiments using OIH as substrate

(a) *with variable OIH-concentrations*: 5 mg gentisic acid, 7 mg citric acid, and different amounts of OIH, i.e., 0.3, 0.5, 1 and 1.4 mg OIH, were weighed and dissolved in 0.8 ml water (range [OIH] = $9.8 \times 10^{-4} - 4.59 \times 10^{-3}$ M), and subsequently the addition of 50 μl Cu^{2+}-solution (2.6×10^{-4} M). Labeling experiments were carried out in presence of ACN: 0, 0.285 and 0.57 M ACN and final volume was adjusted to 1 ml using aquapure water. The reaction mixtures were heated at 75°C during 20 min.

(b) *adjusted Cu^{2+}-concentration in presence of ACN*: 1 mg OIH (3.28×10^{-3} M), 5 mg gentisic acid and 7 mg citric acid were dissolved in 0.8 ml water and subsequently the addition of 50 μl Cu^{2+}-solution (2.6×10^{-4} M). Labeling experiments were carried out in presence of different amounts of ACN: 0, 0.285 and 0.57 M ACN and final volume was adjusted to 1 ml using aquapure water. The reaction mixtures were heated at 75°C during 20 min. Labeling experiments in presence of ACN were repeated with an adjusted Cu^{2+}-concentration, i.e., 95 and 130 μl Cu^{2+}-solution in case of 0.285 and 0.57 M ACN, respectively.

• Labeling experiments using MIBG as substrate

(c) *with variable MIBG-concentrations*: 5 mg gentisic acid, 7 mg citric acid, and different amounts of MIBG, i.e., 0.5, 0.75, 1, and 1.5 mg MIBG, were weighed and dissolved in 0.75 ml water (range [MIBG] = $1.54 \times 10^{-3} - 4.63 \times 10^{-3}$ M) and subsequently the addition of 120 μl Cu^{2+}-solution (1.281×10^{-3} M). Labeling experiments were carried out in presence of different amounts of ACN: 0 and 0.191 M and final volume was adjusted to 1 ml using aquapure water. The reaction mixtures were heated at 110°C during 30 min.

(d) *adjusted Cu^{2+}-concentration in presence of ACN*: 1 mg MIBG (3.09×10^{-3} M), 5 mg gentisic acid and 7 mg citric acid were dissolved in 0.75 ml water and subsequently the addition of 120 μl Cu^{2+}-solution (1.281×10^{-3} M). Labeling experiments were carried out in presence of 0 and 0.191 M ACN and final volume was adjusted to 1 ml using aquapure water. The reaction mixtures were heated at 110°C during 30 min. The labeling experiment in presence of ACN was repeated with an adjusted Cu^{2+}-concentration, i.e., 205 μl Cu^{2+}-solution.

Results and discussion

We earlier showed that the presence of acetonitrile in the reaction mixture of the Cu^{+}-assisted radioiodination of

MIBG decreased the labeling yield from 99 to 1.5% while changing the concentration of ACN from 0 to 30% v/v (corresponding to 6 M) [8].

The aim of this paper was to propose a method to calculate the apparent complex formation constant of Cu^+ and ACN at a higher temperature required for appropriate labeling, as the complex constant values mentioned in literature are determined at room temperature.

In a first mechanistic approach of the Cu^+-assisted nucleophilic exchange in acid reducing conditions, Mertens et al. [10] proposed a Cu^+–arylhalogen complex as the intermediate yielding the radiohalogenated tracer (Fig. 1). They claimed that at higher Cu^+-concentrations the Cu^+-ions can attack and destroy their own aryliodide-complex and thus making the catalyst itself to be rate limiting by a self-inhibition reaction. The concentration of the Cu^+–arylhalide complex in these conditions can be represented by

$$[\ Cu^+-\text{Arylhalide-complex}\ ] = \frac{[RX]_0 \cdot [Cu^+]_0}{K_d + K' \cdot [Cu^+]_0 + [RX]_0} \quad (1)$$

with $K_d = k_{-1}/k_1$, and $K'.[Cu^+]_0$ representing the self-inhibition reaction.

In presence of a Cu^+-complexing substance I, (1) will rearrange to

$$[\ Cu^+-\text{Arylhalide-complex}\ ] = \frac{[RX]_0 \cdot [Cu^+]_0}{\alpha \cdot (K_d + K' \cdot [Cu^+]_0) + [RX]_0} \quad (2)$$

with $\alpha = 1 + [I]$. K_c—with K_c the apparent complex formation constant $(Cu^+\text{-ACN})^+$

At a higher Cu^+-concentration an accurate estimation of the Cu^+–Arylhalide-complex is hindered by the presence of the unknown self-inhibition reaction constant K'.

If $[Cu^+]_0$ is small (conditions as applied in our experiments), the self-inhibition reaction is not significant and the concentration of the complex becomes directly proportional to $[Cu^+]_0$

$$[\ Cu^+-\text{Arylhalide-complex}\ ] = \frac{[RX]_0 \cdot [Cu^+]_0}{\alpha \cdot K_d + [RX]_0} \quad (3)$$

and thus simplifying the calculation. Therefore the $[Cu^+]$-concentrations and corresponding "Precursor/$[Cu^+]$" ratios were chosen in a range were the reaction rate constants vary in a linear way with the $[Cu^+]$-concentration and $K'.[Cu]_0$is negligible.

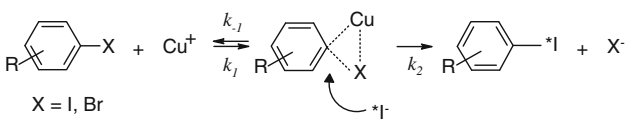

Fig. 1 Nucleophilic radioiodination—reaction mechanism

In a former article [11] we have proven that in presence of only the huge excess of gentisic acid, the reduction of Cu^{2+} to Cu^+ is quantitative and thus $[Cu^+]_0 = [Cu^{2+}]_0$.

$SnSO_4$ was omitted as adjuvant reducing agent in these experiments to avoid any potential interaction with ACN. Citric acid was maintained to adjust the pH-value to 2.3.

Ortho-iodohippuric acid (OIH) was chosen as a test compound as it only requires small concentrations of copper ions to obtain high enough labeling yields.

The Cu^+-assisted labeling reaction follows a pseudo first order reaction [10] and can be represented by

$$-\frac{d[*I^-]}{dt} = k_2\ .\ [\ Cu^+-\text{Arylhalide-complex}\ ]\ .\ [*I^-] \quad (4)$$

For a reaction time of 20 min Eq. 4 can be rearranged as

$$-\text{Ln}\left(\frac{[*I^-]}{[*I^-]_0}\right) = k' \cdot t_{20}$$

$$-\text{Ln}\, f\ [*I^-] = k' \cdot t_{20} = k_{obs}$$

The simultaneous interaction of the aryl-compound and ACN with the Cu^+ ions is comparable with the situation wherein a substrate and an inhibitor compete for reaction with an enzyme.

In our experiments the $[Cu^{2+}]_0$ concentration was kept constant while the k_{obs} values were calculated as a function of increasing concentration of OIH in absence (blank) and in presence of ACN. Two ACN concentrations were assayed, i.e., 0.285 and 0.57 M ACN.

When plotting k_{obs} as a function of [OIH], the obtained curves show the typical Michaelis- Menten curve shape [12], i.e., parts of a rectangular hyperbole (Fig. 2), typical for a satiable and reversible interaction with the catalyst.

Plotting the reciprocal k_{obs} values as a function of 1/[OIH], allows to calculate an apparent dissociation constant K_d of the OIH–Cu^+ interaction, in absence of ACN, of 2.863×10^{-3} M, corresponding to an apparent complex formation constant K_c of $\sim 3.49 \times 10^2$.

In presence of ACN, Eq. 3 can be applied,

$$[\ Cu^+-\text{Arylhalide-complex}\ ] = \frac{[RX]_0 \cdot [Cu^+]_0}{\alpha \cdot K_d + [RX]_0}$$

with $\alpha = 1 + [ACN]$. K_c and K_d representing the dissociation constant of the $(Cu–OIH)^+$ complex.

Plotting the reciprocal k_{obs} values as a function of 1/[OIH], three straight lines with different slopes with the same intercept on the *Y*-axis are obtained (Fig. 3). As those lines have the same intercept on the *Y*-axis it can be assumed that the interaction between the catalytic Cu^+-ion and both the arylhalogen compound and ACN is competitive.

α, the ratio of the slopes obtained, respectively, in presence and in absence of ACN, allows to calculate

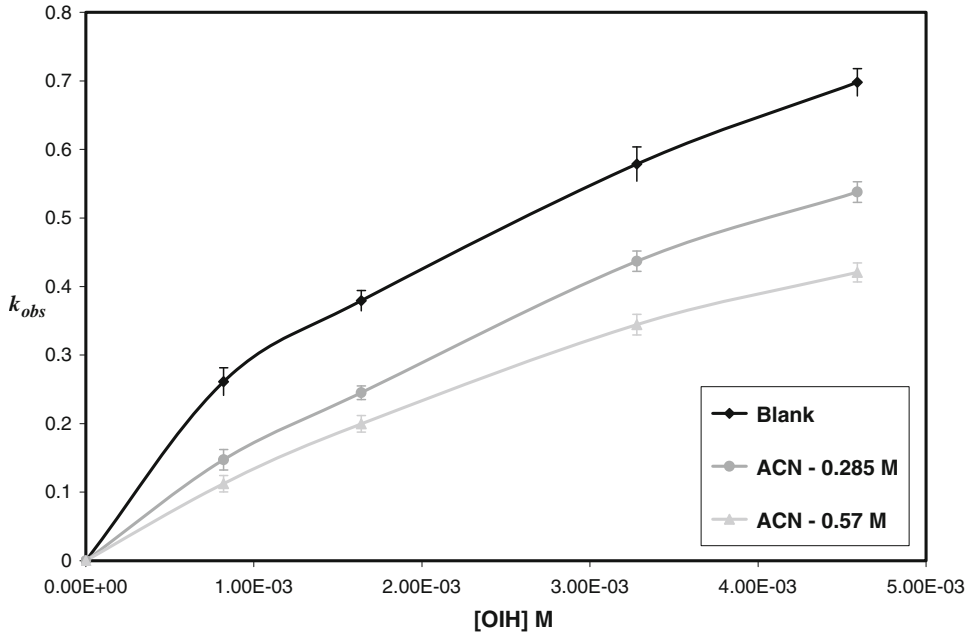

Fig. 2 Influence of ACN on the reaction rate, variable [OIH]; k_{obs} versus [OIH].Values are represented as mean values, $n = 3$, Reaction mixture: X mg OIH, 5 mg gentisic acid, 7 mg citric acid and 3.25 µg $CuSO_4 \cdot 5H_2O$

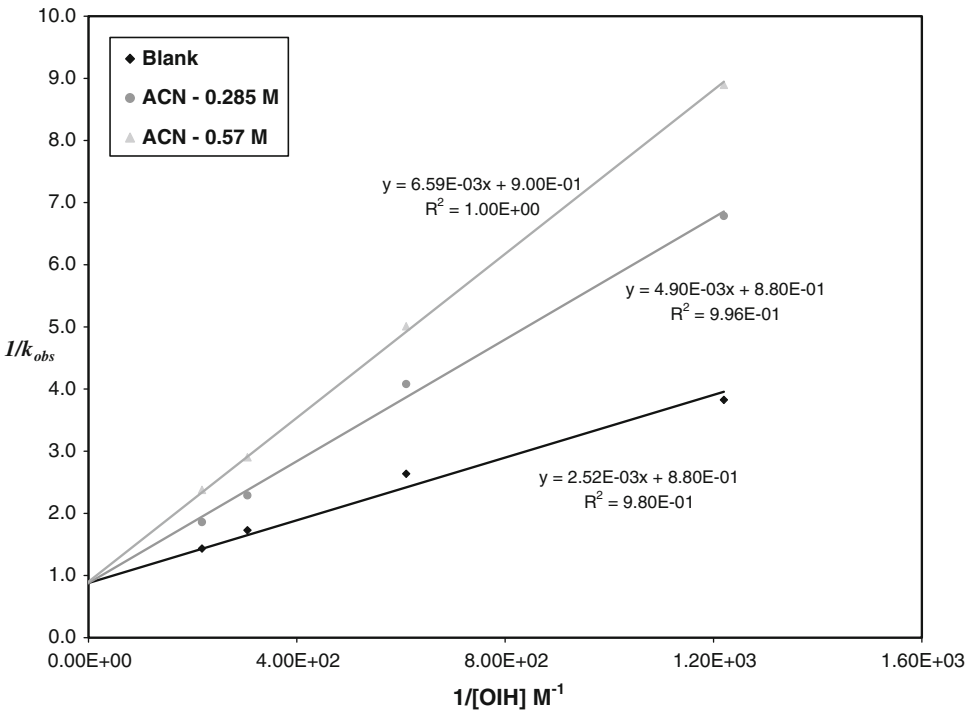

Fig. 3 Influence of ACN on the reaction rate, variable [OIH]; $1/k_{obs}$ versus 1/[OIH]. Values are represented as mean values, $n = 3$, Reaction mixture: X mg OIH, 5 mg gentisic acid, 7 mg citric acid and 3.25 µg $CuSO_4 \cdot 5H_2O$

the respective K_d values of 0.303 M ± 0.025 ([ACN] = 0.285 M) and 0.354 M ± 0.025 ([ACN] = 0.570 M) of the $(Cu–ACN)^+$-complex.

This corresponds to a mean K_c value of 3.1 ± 0.1.

The complex formation constant of Cu^+ with the aryl-halide entity is about 110 higher than with ACN.

This method also gives the opportunity to apply an "adjusted" Cu^+-concentration in presence of ACN, with a labeling yield as high as in absence of ACN, by multiplying the initial Cu^{2+}-concentration by α.

Assuming that the complex formation between Cu^+ and ACN can be represented by

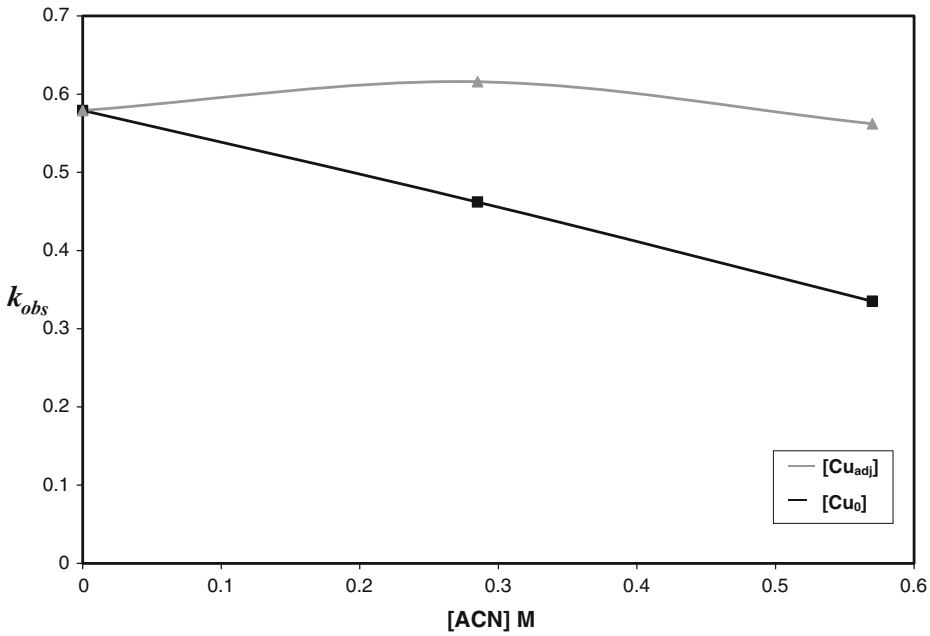

Fig. 4 Adjusted Cu^{2+}-concentration in presence of ACN, k_{obs} versus [ACN]. Values are represented as mean values, $n = 3$, Reaction mixture: 1 mg OIH, 5 mg gentisic acid, 7 mg citric acid and X µg $CuSO_4 \cdot 5H_2O$

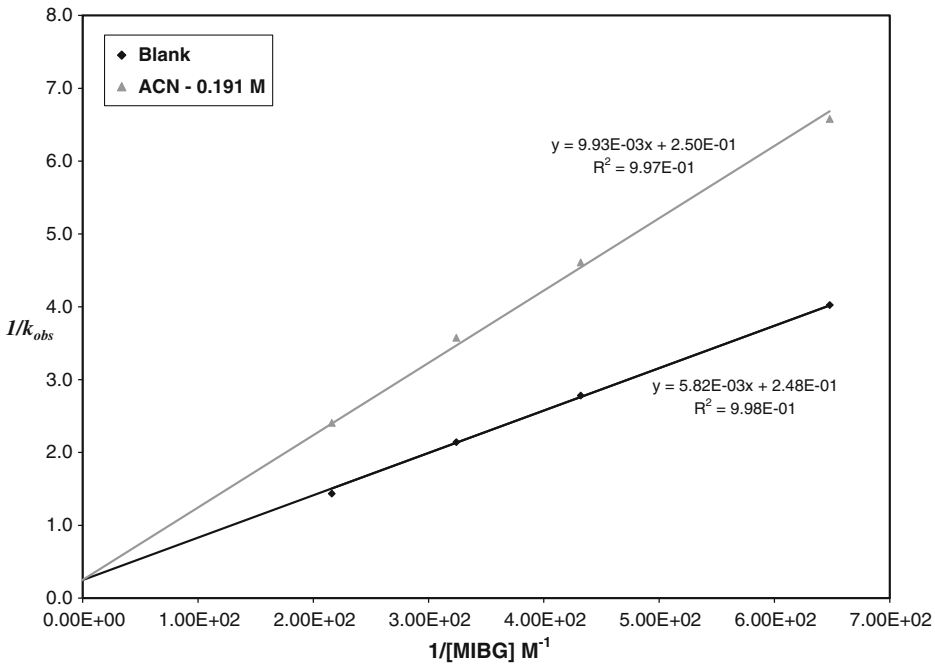

Fig. 5 Influence of ACN on the reaction rate, variable [MIBG]; $1/k_{obs}$ versus 1/[MIBG]. Values are represented as mean values, $n = 3$, Reaction mixture: X mg MIBG, 5 mg gentisic acid, 7 mg citric acid and 38.4 µg $CuSO_4 \cdot 5H_2O$

$$Cu^{+} + ACN \leftrightarrow (Cu{-}ACN)^{+} \text{and,}$$
$$[ACN]_0 > > [Cu^{+}]_0$$
$$\left[(Cu - ACN)^{+}\right] = [Cu^{+}]_0 - [Cu^{+}]$$
$$\text{then, } [Cu^{+}] \cdot \left\{1 + K_c \cdot [ACN]_0\right\} = [Cu^{+}]_0$$

The actual $[Cu^{+}]$ in presence of ACN can now be written as,

$$[Cu^{+}] = \frac{[Cu^{+}]_0}{\alpha}$$

or to obtain the concentration of free $[Cu^{+}]$ required for an optimal labeling yield, the amount of copper sulfate in the reaction mixture has to be multiplied by α.

As a proof of principle the labeling experiments in presence of ACN were repeated with an "adjusted" Cu^{2+}-concentration, being the initial Cu^{2+}-concentration multiplied by the corresponding α values, i.e., 1.94 and 2.61, respectively. The results depicted in Fig. 4 show, using the "adjusted" amount of copper salt, k_{obs} reached the same values as obtained in absence of ACN.

As a proof of concept this approach was applied on the isotopic exchange labeling at 110 °C with MIBG, a tracer for routine labeled in our laboratory. Figure 5 reveals the LWB plots obtained for the blank reaction and in presence of 0.191 M of ACN.

The K_d value for the aryliodide–Cu^+ complex formation amounts to 2.33×10^{-2} M which is about eight times higher than the value obtained for OIH. This difference in affinity can be attributed to a better resonance-stabilized aryliodide–Cu^+ complex intermediate in case of OIH, due to its conjugation with the ortho-carbonyl group, while the meta-position in MIBG is deactivated. In presence of 0.191 M ACN the α value of 1.7 allows to calculate an apparent complex constant of 3.68 for the $(Cu–ACN)^+$ complex. It must be noticed that in the labeling conditions of MIBG, the concentration of $[Cu^+]$ is situated in the range where the self-inhibition reaction already occurs (non published results), which can explain a somewhat lower value for K_c at 110 °C, *vis à vis* 75 °C.

Also for MIBG, using an "adjusted" amount of copper salt by multiplying the original Cu^{2+}-amount with 1.7, restored the initial labeling yield as in absence of ACN.

Conclusion

In the Cu^+-assisted nucleophilic exchange radioiodination, the interaction of the catalyst Cu^+ with the arylhalide and ACN is competitive and allows to calculate the apparent complex constant by applying Lineweaver–Burk (LWB) plots. Moreover an almost quantitative labeling yield can be restored by adjusting the copper sulfate concentration simply by multiplying the original amount by the factor α obtained from the LWB plots.

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References

1. Eersels JLH, Travis MJ, Herscheid JDM (2005) Manufacturing I-123-labelled radiopharmaceuticals. Pitfalls and solutions. J Labelled Compd Radiopharm 48:241–257
2. Mertens J, Eersels J, Vanryckeghem W (1987) New high yield Cu(I) assisted ^{123}I radioiodination of 15(*p*-I-phenyl)-9-methyl-pentadecanoic acid, a potential mycocardial tracer. Eur J Nucl Med 13:159–160
3. Yu J, Zolle I, Mertens J, Rakias F (1995) Synthesis of 2-[^{131}I]Iodo-phenylmetyrapone using Cu(I)-assisted nucleophilic exchange labelling: study of the reaction conditions. Nucl Med Biol 2:257–262
4. Acton PD, Choi S-R, Hou C, Plössl K, Kung HF (2001) Quantification of serotonin transporters in nonhuman primates using [^{123}I]ADAM and SPECT. J Nucl Med 42:1556–1562
5. Pearson RG (1973) Hard and soft acids and bases. Edit Dowden, Hutchinson & Ross, Stroudsburg
6. Meyer M, Albrecht-Gary AM, Dietrich-Buchecker CO, Sauvage JP (1999) Π–Π stacking-induced cooperativity in copper(I) complexes with phenanthroline ligands. Inorg Chem 38:2279–2287
7. Perrin DD (1978) IUPAC chemical data series, stability constants of metal-ion complexes, part B; no 22. Pergamon Press, Oxford
8. Eersels JLH, Herscheid JDM (2007) Addition of organic solvents might inhibit radioiodination in Cu(I)-assisted nucleophilic exchange. J Labelled Compd Radiopharm 50(Suppl):S270
9. Kamau P, Jordan RB (2001) Complex formation constants for the aqueous copper(I)-acetonitrile system by a simple general method. Inorg Chem 40:3879–3883
10. Mertens J, Gysemans M (1991) Cu^+ assisted nucleophilic exchange; application and mechanistic approach. In: Emram AM (ed) New trends in radiopharmaceutical synthesis, quality assurance, and regulatory control. Plenum Press, New York
11. Eersels JLH, Mertens J, Herscheid JDM (2010) The Cu^+-assisted radioiodination *Kit*: mechanistic study of unexplored parameters concerning the acidity and redox properties of the reaction medium. Appl Radiat Isot 68:309–313
12. Lehninger AL (1978) Biochemistry, the molecular basis of cell structure and function, 2nd edn. Worth Publishers, New York