^{18}F- and ^{11}C-labelling of quantum dots with n.c.a. [^{18}F]fluoroethyltosylate and [^{11}C]methyliodide: a feasibility study

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Abstract Quantum dots functionalized on the outer surface with either amino- or carboxyl functions were labelled with [^{18}F]fluoroethyltosylate and [^{11}C]methyliodide in order to use the positron emitter-labelled fluorescence agents for multimodality imaging techniques, i.e. fluorescence imaging and positron emission tomography. ^{18}F-Labelling of both compounds was realized with yields up to 5% as determined by size exclusion chromatography, which is twice as much as reported in literature before [1]. ^{11}C-Labelling of amino- and carboxyl-QDs proceeded with good yields (up to 45 and 35%, respectively) under optimized reaction conditions. In general for both QD-types and both labelling agents the labelling yield increased with the amount of QDs used in the reaction as well as with reaction time and reaction temperature.

Keywords Quantum dot · PET · ^{18}F · ^{11}C · Size exclusion chromatography

Introduction

Due to their unique properties compared to conventional fluorescence agents semiconductor crystals, so called quantum dots, have attracted rising interest within the scientific community. Quantum dots (QDs) consist of a semiconductor core such as CdSe, CdTe, InP or InAs, often encapsulated in a shell of a material with a larger band gap such as ZnS. The size of the nanocrystals is in the order of

magnitude as the wavelength of electron wavefunction, therefore the energy levels available for electrons inside the crystal are quantized, an effect called "quantum confinement" [2–4]. As a consequence the optical properties of quantum dots differ significantly from those of conventional fluorescence dyes: quantum dots are characterized by a narrow and symmetric peak in the emission spectrum in combination with an absorption continuum, which allows to use an excitation wavelength well separated from the emission maximum. Moreover the emission wavelength, i.e. the colour of the quantum dots, can be easily controlled by the size of the nanocrystals, which enables the researcher to perform so called multiplexing experiments [5], where quantum dots with different emission wavelengths are simultaneously excited by means of a single wavelength. In addition to that quantum dots exhibit a much higher photostability, a longer fluorescence lifetime, and a higher detection sensitivity when compared to conventional fluorescence agents. And they can be easily functionalized on the outer layer either to modify physical properties such as solubility and lipophilicity [6, 7] or to introduce an affinity to specific biological targets such as tumor cells [5], receptors [8], vasculature [9] etc. A number of in vivo animal applications of quantum dots functionalized with different ligands, among these for example antibodies [8, 10, 11], peptides [12, 13] and receptor ligands [14], have been reported during recent years. However, an application of quantum dot based imaging agents in humans is hampered by the fact that there are only very few data available on the properties of such agents or the quantum dots itself in the whole organism in vivo. Positron Emission Tomography (PET) can serve as an ideal tool to generate such data provided that quantum dots can be labelled by positron-emitting nuclides. Quantum dots are currently commercially available from several sources with different

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functionalities on the outer surface, i.e. amino- and carboxyl-functional groups suitable for different further functionalization or radiolabelling.

We here report our first results on the labelling of commercially available quantum dots (amino- and carboxyl-functionalized) with both Fluorine-18 and Carbon-11, two of the most important radioisotopes used for PET.

Experimental

Materials

Quantum Dots with amino functions (Qdot® 705 ITK™ amino (PEG) quantum dots) or carboxyl functions (Qdot® 705 ITK™ carboxyl-quantum dots) were obtained from Invitrogen (Carlsbad, CA). Kryptofix® 2.2.2 (for synthesis), potassium carbonate (puriss.), sodiumtetraborate (for analysis), acetonitrile for azeotropic distillation (quality: for DNA synthesis) and iodine (p.a.) were obtained from Merck (Darmstadt, Germany). Ethylenditosylate (purum), dimethylsulfoxide (DMSO) and dimethylformamide (DMF) (puriss., over molecular sieve) were purchased from Fluka (Buchs, Switzerland). ^{18}F-Separation cartridge 30-PS-HCO$_3$ and the HPLC column for purification of [^{18}F]fluoroethyltosylate, Nucleosil 100-5 C18 HD 250 × 7.8 mm, was acquired from Macherey-Nagel (Dueren, Germany). The column for size exclusion chromatography, TSKgel G2500PWXL 300 × 7.8 mm, was obtained from TOSOH Bioscience (Tokyo, Japan). The solid phase extraction cartridges Strata-X 33μ polymeric reversed phase 200 mg/6 mL were manufactured by Phenomenex (Torrance, CA). Molecular sieve 4 Å and Porapak N (50–80 mesh) were obtained from Alltech (Deerfield, IL), Reduced Nickel catalyst (Shimalite Ni) was purchased from Shimadzu (Kyoto, Japan). Water for injection was obtained from DeltaSelect (Dreieich, Germany).

Synthesis of [^{18}F]fluoroethyltosylate

Fluorine-18 was produced via the ^{18}O(p,n)^{18}F nuclear reaction by irradiation of [^{18}O]H$_2$O with 16.5 MeV protons. [^{18}F]Fluoroethyltosylate was prepared by aminopolyether assisted nucleophilic substitution on ethylenditosylate [15] in an automated synthesis module (TRACERlab FX FN, GE Medical Systems). [^{18}F]Fluoride was separated from the irradiated target water by means of an anion exchange cartridge (^{18}F-Separation cartridge 30-PS-HCO$_3$), preconditioned with 1 mL ethanol and 1 mL water for injection. [^{18}F]Fluoride was eluted from the cartridge with a solution consisting of 15 mg (40 μmol) Kryptofix® 2.2.2, 2.75 mg (20 μmol) potassium carbonate, 800 μL acetonitrile and 200 μL water for injection and subsequently evaporated to

dryness at 95 °C within 6 min under reduced pressure. The cryptand [K ⊂ 2.2.2.]$^{+18}$F$^-$ was reconstituted in dry acetonitrile and reacted with a solution of ethylenebistosylate 16 mg (43 μmol) in 1 mL dry acetonitrile for 3 min at 80 °C. The reaction mixture was diluted with 1 mL water for injection and 0.5 mL acetonitrile, purified by semipreparative HPLC (Nucleosil 100-5 C18 HD, 250 × 7.8, eluent acetonitrile/water, 60/40, v/v, 5 mL/min). The product fraction was diluted with 10 mL water for injection and fixed on a reversed phase cartridge (Strata-X polymeric reversed phase 200 mg/6 mL), and eluted with 2 mL warm (100 °C) DMSO.

Synthesis of [^{11}C]methyliodide

Carbon-11 was produced via the ^{14}N(p,α)^{11}C nuclear reaction by irradiation of N$_2$ + 0.5% O$_2$ with 16.5 MeV protons. The primarily produced [^{11}C]carbon dioxide, [^{11}C]CO$_2$, was converted to [^{11}C]methyliodide, [^{11}C]CH$_3$I, by means of an automated system (MeI MicroLab, GE Medical Systems) as described previously [16–19]. Briefly [^{11}C]CO$_2$ was trapped on molecular sieve 4 Å and converted to [^{11}C]methane, [^{11}C]CH$_4$, in presence of Ni catalyst (Shimalite-Ni reduced 80/100, Shimadzu). [^{11}C]CH$_4$ was reacted with elemental iodine at 760 °C to [^{11}C]CH$_3$I, which is trapped on a Porapak column. By heating the Porapak to 360 °C [^{11}C]CH$_3$I is released by a stream of helium.

Labelling of quantum dots (amino) or (carboxyl) with [^{18}F]fluoroethyltosylate

190 μL of [^{18}F]fluoroethyltosylate in DMSO were introduced into a 1 mL Reactivial (Alltech, Deerfield, IL) and 10, 20 or 30 μL of quantum dots (amino) or (carboxyl) 8 μM in 50 mM borate buffer pH 8.3 or pH 9, respectively, were added. The mixture was heated to 100 or 120 °C under stirring for different reaction times (3, 5, 10, 30 and 120 min). The reaction was quenched by addition of 400 μL water for injection and analyzed by size exclusion chromatography (TSKgel G2500 PWXL 300 × 7.8 mm, 50 mM borate buffer adjusted to pH 9 with 1 M HCl, 0.8 mL/min; detection by fluorescence emission (excitation wavelength 275 nm, emission wavelength 705 nm) and radioactivity detector).

Labelling of quantum dots (amino) or (carboxyl) with [^{11}C]methyliodide

10, 20 or 30 μL of quantum dots (amino) or (carboxyl) 8 μM in 50 mM borate buffer pH 8.3 or pH 9, respectively, were added to 1 mL of [^{11}C]CH$_3$I in DMSO. The mixture was incubated at room temperature or heated to 80 or

140 °C for 10, 30, or 60 min. The labelling reaction was stopped by introduction of the reaction vial into an ice-bath and subsequently analyzed by size exclusion chromatography (TSKgel G2500 PWXL 300 × 7.8 mm, 50 mM borate buffer adjusted to pH 9 with 1 M HCl, 1.3 mL/min; detection by fluorescence emission (excitation wavelength 275 nm, emission wavelength 705 nm) and radioactivity detector.

Results and discussion

Size Exclusion Chromatography (SEC) in general is a powerful tool for the separation of macromolecules. Under ideal conditions the separation mechanism would exclusively be based on entropic effects, i.e. separation is based on the difference in molecular size. However, usually interactions between the column material and the analyte do occur (enthalpic effect) and are tried to be suppressed by additives to the eluent [20]. We examined different SEC column types with regard to their suitability to analyze QDs. Among these were a silica based SEC column, usually eluted with aqueous buffers, a polystyrene based SEC column, that should be eluted with organic solvents and a polymethacrylate SEC column that is recommended for ionic polymers and should be run with aqueous buffers of low ionic strength (manufacturers recommendations). Among these columns tested only the polymethacrylate based SEC column exhibited reproducible signals and an acceptable analyte recovery. Chromatography of QDs with both the silica and the polystyrene based SEC columns suffered from low analyte recovery and/or lack of reproducibility. With the polymethacrylate based SEC column operated with an aqueous buffer we were able to separate the labelling precursors, i.e. [^{18}F]fluoroethyltosylate and [^{11}C]CH$_3$I from the QD fractions. However, it was not possible to separate labelled from unlabelled QDs under the applied chromatographic conditions, which most likely is due to the fact that the size of the analytes do not differ enough from each other to be distinguished by this type of chromatography. Nevertheless, it is possible to determine the labelling yield by this method since the radioactivity channel of the chromatographic system exclusively detects the labelled fraction of the QDs.

Synthesis of [^{18}F]fluoroethyltosylate

[^{18}F]Fluoroethyltosylate was produced by aminopolyether assisted nucleophilic subtitution of [^{18}F]fluoride in an automated synthesis module TRACERlab FX FN. Retention time of [^{18}F]fluoroethyltosylate during semipreparative HPLC was 5.5 min. Radiochemical yield was about 52% corrected for decay within a total synthesis time of 23 min.

Synthesis of [^{11}C]methyliodide

[^{11}C]CH$_3$I was produced with 60% radiochemical yield (corrected for decay) based on produced [^{11}C]CO$_2$ within an overall synthesis time of 15 min.

Labelling of quantum dots (amino) or (carboxyl) with [^{18}F]fluoroethyltosylate

[^{18}F]Fluoroethyltosylate was reacted with both amino- and carboxyl-quantum dots in a Reactivial at 100 and 120 °C for different reaction times (3, 5, 10, 30 and 120 min). The reaction mixture was then analysed by size exclusion chromatography to determine the yield of the labelling reaction.

[^{18}F]Fluoroethyltosylate could not be detected under the afore mentioned chromatographic conditions within a run time of 3 hrs. At a flow of 0.8 mL/min ^{18}F-Labelled QDs were detected at 7.2 min (k' 0.3).

In general, radiochemical yields were higher for amino-quantum dots compared to carboxyl-quantum dots. For both quantum dot types yields were higher at 120 °C compared to 100 °C (Figs. 1, 2). Higher reaction temperatures than 120 °C were not tested so far. Labelling yields increased for both quantum dot types with increasing reaction time (Figs. 1, 2) and with the amount of QDs used in the reaction (Fig. 3).

Even though [^{18}F]fluoroethyltosylate is not the ideal radiolabelling agent for reaction systems containing a considerable amount of water (aquoeus part from quantum
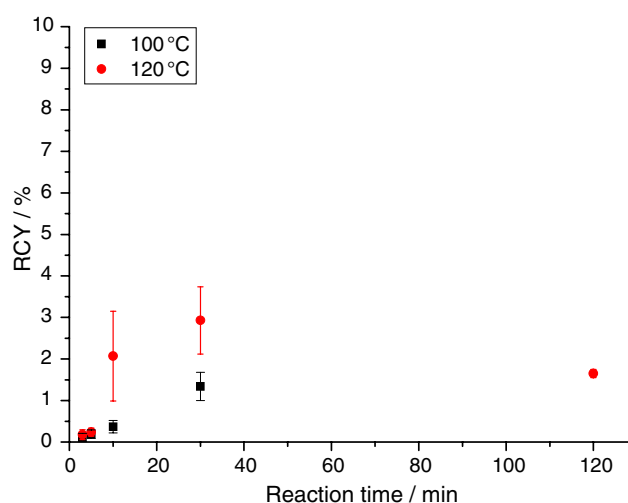

Fig. 1 [^{18}F]Fluoroethylation of amino-QDs: radiochemical yield (RCY) in % versus reaction time, QDs (amino) used 80 pmol; reaction temperature 100 and 120 °C
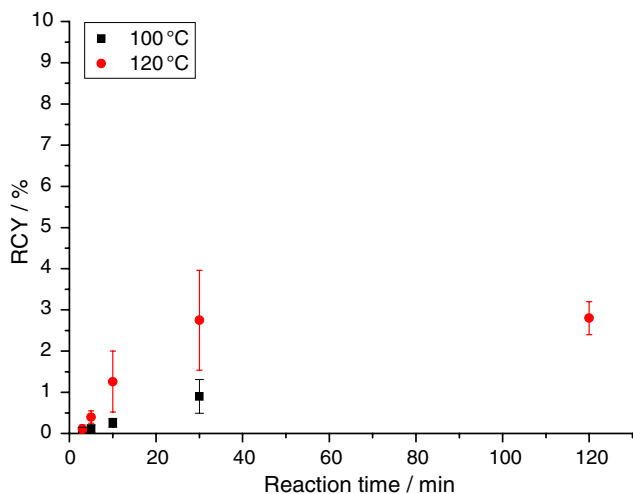
Fig. 2 [^{18}F]Fluoroethylation of COOH-QDs: radiochemical yield (RCY) in % versus reaction time, QDs (COOH) used 80 pmol; reaction temperature 100 and 120 °C
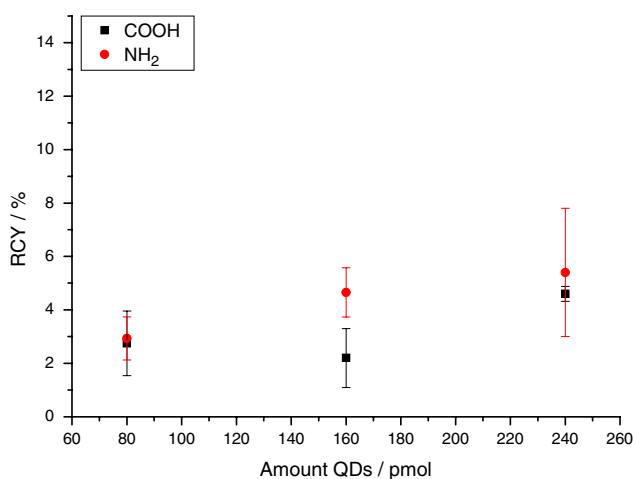

Fig. 3 [^{18}F]Fluoroethylation of amino- and COOH-QDs: radiochemical yield (RCY) in % versus amount of QDs used; reaction time 30 min; reaction temperature 120 °C

dots), it was possible to generate a detectable amount of [^{18}F]fluoroethylated-quantum dots (both types) in the reaction mixture. Moreover, the yields that were obtained by this method were quite comparable to those reported by Duconge et al. [1], who used [^{18}F]FPyME (1-[3-(2-fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione), a maleimido [^{18}F]reagent, to label thiol-QDs. [^{18}F]FPyME initially was developed to label proteins and peptides in water containing reaction mixtures via thiol-alkylation. When applied to thiol-QDs a radiochemical yield of about 2% was reported. In comparison to that, the ^{18}F-labelling of amino-QDs via [^{18}F]fluoroethylation is even superior, with radiochemical

yields reaching up to 5% under optimized conditions (20 µL amino-QD, 120 °C, 30 min).

Labelling of quantum dots (amino) or (carboxyl) with [^{11}C]methyliodide

[^{11}C]CH$_3$I was reacted with 10, 20 or 30 µL quantum dots (amino) or (carboxyl) both at room temperature as well as at 80 and 140 °C for 10, 30 or 60 min reaction time. The radiochemical yield (corrected for decay) was determined by SEC. [^{11}C]CH$_3$I eluted under the afore mentioned chromatographical conditions (flow 1.3 mL/min) at 34 min (k' 5.9). In accordance with the theory of SEC the labelled and unlabelled QDs (large molecules/compounds compared to [^{11}C]CH$_3$I) eluted well before [^{11}C]CH$_3$I at 4.5 min (k' 0.3). Although it was not possible to separate labelled from unlabelled QDs, the determination of the fraction of the reaction mixture corresponding to [^{11}C]CH$_3$-labelled QDs is achievable, since the radioactivity channel exclusively detects the labelled compounds.

For both types of QDs the labelling yield increased with the amount of QDs applied in the labelling reaction (Fig. 4) as well as with the reaction time (Fig. 5) and the reaction temperature (Fig. 6) applied. However, there was no significant gain in yield observed when increasing the temperature from 80 to 140 °C. In general, the reaction temperature exhibited a less pronounced influence on the radiochemical yield than reaction time and amount of QDs used. Under ideal conditions (30 pmol QDs, 60 min reaction time) ^{11}C-labelling of amino- and carboxyl-QDs proceeded with good yields (up to 45 and 35%, respectively).
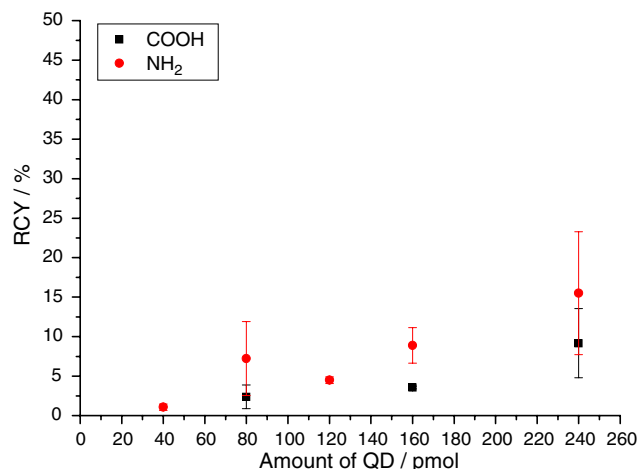

Fig. 4 [^{11}C]Methylation of amino- and COOH-QDs: radiochemical yield (RCY) in % versus amount of QDs (amino or COOH) used; reaction time 10 min; reaction temperature 30 °C
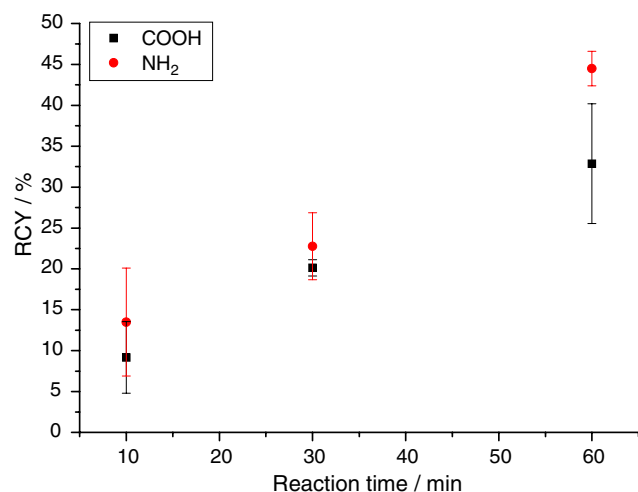
Fig. 5 [11C]Methylation of amino- and COOH-QDs: radiochemical yield (RCY) in % versus reaction time; reaction temperature 30 °C; amount of QD (amino or COOH) used 240 pmol
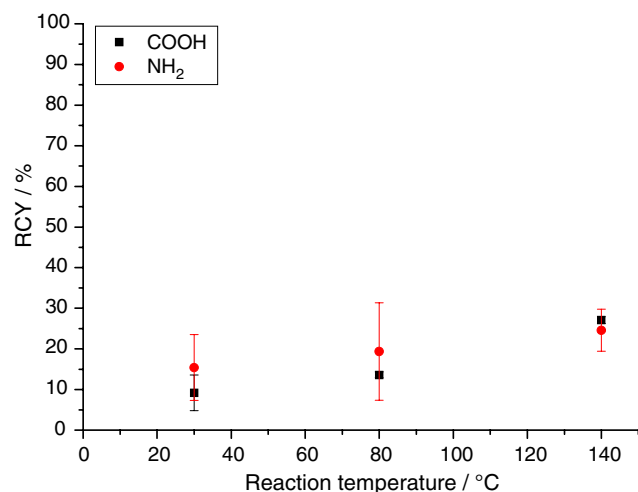

Fig. 6 [11C]Methylation of amino- and COOH-QDs: radiochemical yield (RCY) in % versus reaction temperature; amount of QD (amino or COOH) used 240 pmol; reaction time 30 min

Conclusion

Labelling of commercially available amino- and carboxyl-quantum dots with the positron-emitters Fluorine-18 and Carbon-11 was demonstrated. ^{18}F-Labelling was realized by [18F]fluoroethylation using [18F]fluoroethyltosylate as fluorination agent and proceeded in reasonable yields of up to 5%, which is almost twice the amount that was reported for an alternative [18F]fluoroalkylation of quantum dots[1]. ^{11}C-Labelling of QDs was accomplished by [11C]methylation using [11C]methyliodide and was found to proceed in good yields up to about 45%. To our knowledge this is the first report on a successful ^{11}C-labelling of QDs, except for a conference presentation (BrainPET 2007, Osaka, Japan) [21], that contained no experimental details on the radiolabelling method as well as no results on the radiolabelling yield.

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