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Article

Comparison of ZnS(Ag) Scintillator and Proportional Counter Tube for Alpha Detection in Thin-Layer Chromatography

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Abstract: (1) Background: Targeted alpha therapy is a new emerging field in nuclear medicine driven by the two advantages of overcoming resistance of cancer-suffering patients against beta therapies and practically application of lower activities of ²¹²Pb- and ²²⁵Ac-labelled peptides to reach the same doses compared to beta therapy due to the high cytotoxic nature of alpha particles. Quality control of the ²¹²Pb/²²⁵Ac-radiopharmaceuticals remains a challenge due to the low activity levels used for therapy (100 kBq/kg) and the formation of several free daughter nuclides immediately after formulation of patient doses; (2) Methods: The routine alpha detection of thin-layer chromatograms (TLC) of ²¹²Pb- and ²²⁵Ac-labelled peptides using a MiniScanPRO+ scanner combined with an alpha detector head was compared with detection using an AR-2000 scanner equipped with an open proportional counter tube. Measurement time, resolution and validity were compared for both scanners; (3) Results: For ²²⁵Ac quality control the values of RCP are within the acceptance criteria 2 h after TLC development, regardless of when the TLC probe was taken. If the TLC probe was taken 24 h after radiosynthesis, the true value of RCP within the acceptance criteria was not measured until 5 h after TLC development. For ²¹²Pb-labelled peptides, the probe sampling did not have a high impact on the value of RCP for the MiniScanPRO+ and AR-2000. A difference was observed when measuring the TLC with the AR-2000 in different modes; (4) Conclusions: The MiniScanPRO+ is fast, does not require additional equipment and can also measure the gamma spectrum, which may be important for some radiopharmaceutical production sites and regulatory authorities. The AR-2000 has a better signal-to-noise ratio and this eliminates the need for additional waiting time after TLC development.

Keywords: ²¹²Pb; ²²⁵Ac; PSMA; TATE; TOC; thin-layer chromatography (TLC); quality control

1. Introduction

Targeted alpha therapy (TAT) has recently come to the forefront of clinical nuclear medicine as commercial sources of alpha-emitting nuclides such as ²¹²Pb ($t_{1/2} = 10.6$ h, Alpha particle energy $E_{\alpha} = 6.1$ MeV (36%) and 8.8 MeV (64%)) and ²²⁵Ac (half-life $t_{1/2} = 9.9$ d, $E_{\alpha} = 5.8$ MeV, 6.3 MeV, 7.1 MeV and 8.4 MeV) are being established [1,2]. Chelators such as TCMC (1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane) [3], lead-specific chelator (PSC) [4], and macropa (MCP) [5] have been developed which form highly stable complexes with the parent nuclides and, very importantly, with some of the daughter nuclides (Figure 1). The newly developed chelators outperform the gold standard chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) [6] in terms of stability and labelling conditions. This has led to the clinical translation of preclinical results [4,7] and the introduction of new quality control standards [8,9].

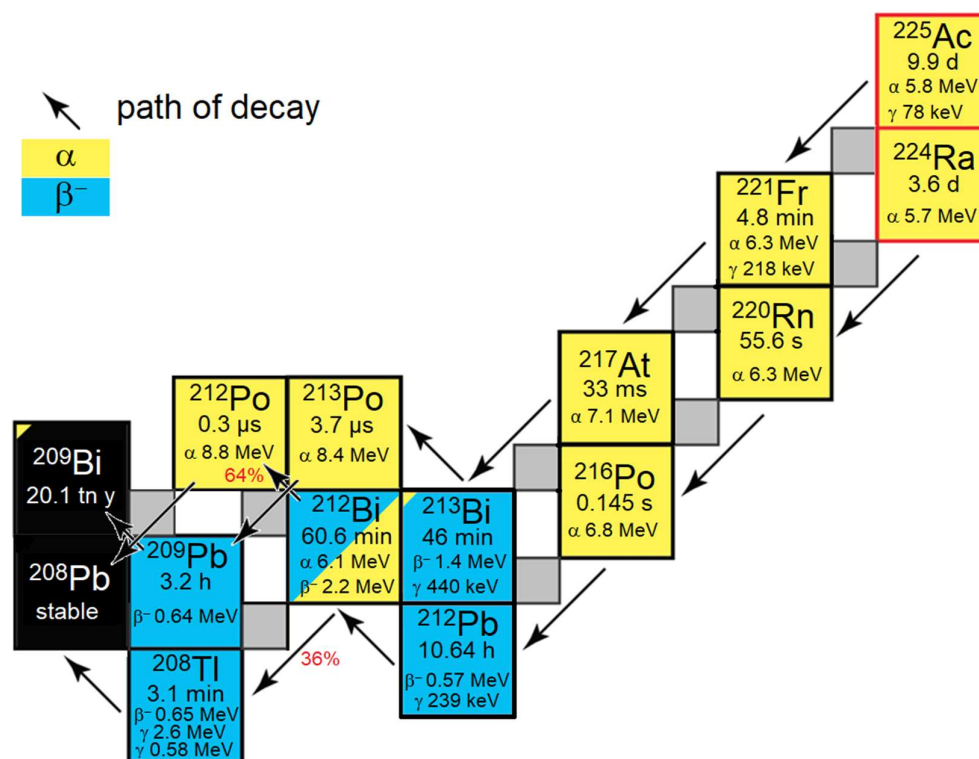


Figure 1. Decay diagram for ^{225}Ac and ^{224}Ra . Shown are their daughter nuclides with half-life (tn = trillion), type of decay and the corresponding decay energy and important γ energies for imaging and identification in γ spectrum analyses. The arrows indicate the main decay of the nuclide. ^{212}Bi has two major decays, the probability of which is given in percent (red) by the arrows.

The ejection of three alpha particles from ^{225}Ac in relatively short time dramatically reduces the activity used for peptide receptor-targeted therapy by a factor of 1000 [10] and the ejection of in sum one alpha particle from ^{212}Pb reduces the activity by a factor of 20–30 [11], while maintaining or even improving the therapeutic outcome. The cytotoxic nature of the alpha particles is mainly due to destruction of Golgi, endoplasmic reticulum, mitochondria or else structures within the cell plasma rather than direct DNA double strand breaks as often cited in the literature [12], since the ^{212}Pb - and ^{225}Ac -labelled peptides certainly not reach the cell nuclei, but are able to pass the cell membrane at least to some extent. Commercially available DOTA-conjugated peptides used for radiotherapy can be commonly labelled with ^{177}Lu or ^{90}Y but practically also with ^{225}Ac . The advantage is clear: lower activities for the patient are associated with lower doses for the personnel and less shielding material. However, the inadvertent use of alpha-emitting nuclides is linked with a significantly higher dose to personnel compared to beta-emitting nuclides. The only obstacle to widely clinical use of ^{225}Ac is the lack of sources. ^{225}Ac can be produced by cyclotrons using ^{226}Ra targets and bombardment with protons [1]. Once this defile is overcome, a wide field of ^{225}Ac therapy is open.

A noteworthy characteristic of purified alpha-emitting nuclides mentioned in this work is, that their daughter nuclides are permanent formed during radiosynthesis and quality control and interfere with the determination of true values for the radiochemical purity (RCP) of radiopharmaceuticals measured by standard methods like thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). While the RCP cannot be measured by HPLC because the free daughter nuclides always show signals at the beginning of a chromatogram [7], TLC is very well suited because the paper sheets can be set aside after development to de-cay of the free daughter nuclides until the true RCP of the radiopharmaceuticals can be measured [13]. A second characteristic is that the purification of these radiopharmaceuticals leads to the separation of non-chelated daughter nuclides from the product and therefore to a different measured activity on dose

calibrators compared to the measured activity in equilibrium of parent and daughter nuclides. This affects the calculated radiochemical yield (RCY), which then appears to be lower than the true RCY. Here, certain time-dependent normalization factors for local dose calibrators are useful, but these can only be determined experimentally by comparison with dose measurements performed with a high-purity Germanium (HPGe) detector.

The MiniScanPRO+ is a scintillation counting system that mechanically moves a TLC strip under a collimated detector. During this process, the system records the count rate as a function of position along the strip. Different types of scintillation detectors can be used with the MiniScanPRO+ to make it possible to detect alpha- (ZnS(Ag)), beta- (plastic), and gamma (NaI(Tl)) radiation. The ionising radiation interacts with the scintillator, which produces light pulses that can be detected by a photomultiplier tube. In addition, the instrument can be equipped with a multi-channel analyser (MCA) and a corresponding spectroscopy quality NaI(Tl) detector for nuclide identification.

Compared to the MiniScanPRO+, the AR-2000 is a position sensitive gas filled proportional counter. During the measurement, a thin wire is continuously flushed with an ionisation gas while a high voltage is applied to the wire. Ionising radiation can interact with the gas molecules to form ion pairs. Free electrons from these ion pairs are accelerated towards the anode by the electric field created by the high voltage applied to the wire. As they move, more gas molecules are ionised, creating more ion pairs. This process generates a large number of ion pairs, resulting in a detectable current pulse, that can be correlated to the position of the event along the wire. The AR-2000 allows separate counting of alpha and beta emissions by adjusting the operating voltage. Due to the significantly higher ionisation produced by alpha particles (more than 10 times that of beta particles), alphas can be selectively counted at a reduced high voltage setting. By reducing the high voltage to 1000 V, the AR-2000 effectively excludes beta-emitting isotopes, allowing only alpha particles to be detected. At the standard high voltage of 1500 V, both alpha and beta emissions are measured. To isolate beta emissions, alpha particles can be easily blocked by using a simple shield, such as paper or a thin layer of plastic (e.g. parafilm). As the AR-2000 uses the imaging of an entire TLC lane compared to the scanning of the MiniScanPRO+, it is possible to visualise a single TLC strip in less than a minute with almost 100 times the sensitivity of the MiniScanPRO+.

In this work the results of two different TLC scanners (MiniScanPRO+ and AR-2000; Eckert&Ziegler), were compared on the suitability of RCP measurements of ^{212}Pb - and ^{225}Ac -labelled radiopharmaceuticals for clinical application.

2. Results

Radiochemistry

The radiolabelling of the different precursors was performed with >95% radiochemical yield (RCY) by using previously optimised reaction conditions. Considering this fact, the measurement of the radiochemical purity (RCP) by TLC should only show discrepancies due to different probe development times and measurement times. The following test parameters were used:

- Amount of activity required on TLC (1–100 kBq)
- Wait time for true RCP after TLC development immediately after synthesis
- Wait time for true RCP after TLC development 24 h after synthesis
- Wait time for true activity after synthesis and purification for calculation of true RCY
- Acceptance criteria: Radiochemical purity >90% right after synthesis and TLC development (prospective), >97% 2–5 h after TLC development (retrospective)

True activity (in Becquerel) for ^{225}Ac -labelled peptides was measured 2 h after the end of synthesis. 1 h after synthesis, the measured activity value was >95% of the true activity. True activity for ^{212}Pb -labelled peptides was measured 4 h after synthesis. 2 h after synthesis the measured activity value was >85% of the true activity. The relatively short half-life of ^{212}Pb leads to significantly lower activity at the equilibrium (e.g. 10 MBq at the end of synthesis are only 7.7 MBq at equilibrium 4 h later). Therefore, the true activity of the freshly purified product should always be calculated using a normalisation factor rather than waiting for equilibrium.

For ^{225}Ac -labelled peptides there was found a rule for determining the true RCP using the MiniScanPRO+: 2 h after TLC development the values of RCP are within the acceptance criteria regardless of when the TLC probe was taken within the first 30 minutes after radiosynthesis (Table 1). If the TLC probe was taken 24 h after radiosynthesis, the true value of RCP within the acceptance criteria was not measured until 5 h after TLC development (Figure 2).

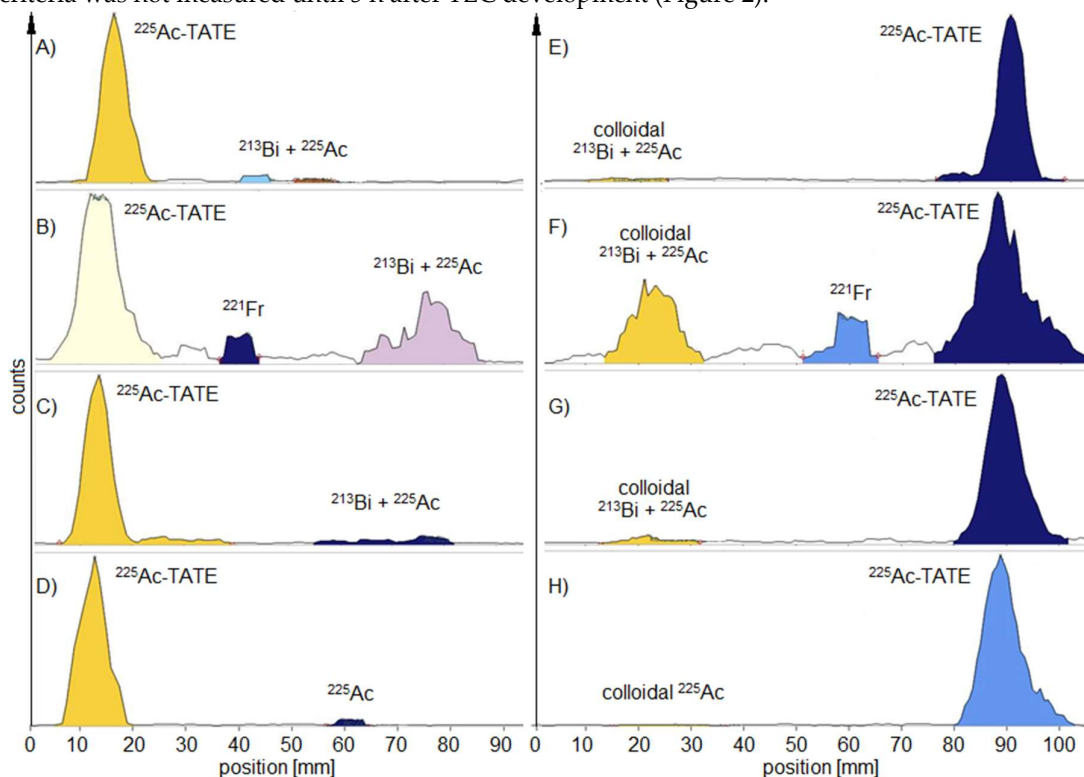


Figure 2. Representative radio-TLCs measured with the MiniScanPro+ at different time-points: A) citrate E) NH_4Ac – TLC probe taken within 5 minutes after synthesis – measurement immediately after development; B) citrate F) NH_4Ac – TLC probe taken 24 h later – measurement immediately after development; C) citrate G) NH_4Ac – TLC probe taken 24 h later – Measurement 2 h after development; D) citrate H) NH_4Ac – TLC probe taken 24 h later – measurement 24 h after development.

Table 1. Comparison of TLC measurements for determining radiochemical purity of ^{225}Ac peptides.

TLC development	TLC measurement	Product peak [%] MiniScanPRO+				Product peak [%] AR-2000			
		^{225}Ac -PSMA-I&T		^{225}Ac -TATE		^{225}Ac -PSMA-I&T		^{225}Ac -TATE	
		Citrate	NH_4Az	Citrate	NH_4Az	Citrate	NH_4Az	Citrate	NH_4Az
Immediately after synthesis	immediately	94.7	90.4	94.1	96.6	97.6	95.5	99.6	99.2
	2 h later	98.1	99.2	n.d.	n.d.	99.9	99.5	n.d.	n.d.
	immediately	55.7	85.7	66.9	75.6	82.9	84.8	86.4	77.8
24 h after synthesis	2 h later	91.3	94.3	90.0	94.8	96.5	94.3	96.5	97.5
	5 h later	98.1	96.6	n.d.	n.d.	98.4	96.1	n.d.	n.d.
	24 h later	98.7	97.5	96.7	98.9	98.4	96.3	98.9	99.4

With the AR-2000, the waiting time for true RCP from a probe taken up to 24 h later was reduced to <2 h after development. Furthermore, the AR-2000 measured RCP values within the acceptance criteria for the first 2 hours of TLC probe sampling when applying alpha detection. In addition, ^{213}Bi -labelled PSMA was also found on the TLC with a higher signal when using beta detection compared to alpha detection measurements (Figure 3).

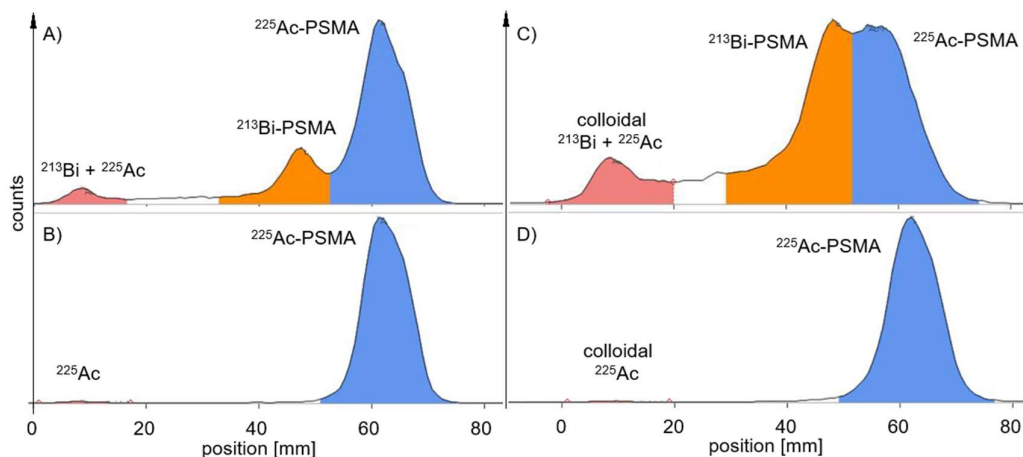


Figure 3. Representative radio-TLCs measured with the AR-2000 at different time-points and measurement modes - probe taken immediately after synthesis: A) alpha detection, 5 minutes after development; B) alpha detection, 4 h after development (^{213}Bi is decayed) C) beta detection, 5 minutes after development – free ^{213}Bi and ^{213}Bi -PSMA have higher signals in beta mode (440 keV); D) beta detection, 4 h after development.

For ^{212}Pb -labelled peptides, the probe sampling did not have such an effect on the values of RCP (Table 2) for the MiniScanPRO+ and AR-2000 (Figure 4). A difference was observed when measuring the TLC with the AR-2000 in different modes. In alpha detection, values of RCP were measured within the acceptance criteria on TLC with citrate, when the probe was taken immediately after synthesis up to 2 h after synthesis. In beta detection, values of RCP were measured within the acceptance criteria on TLC with ammonium acetate/methanol (NH_4Ac), when the probe was taken immediately after synthesis up to 2 h after synthesis (Figure 5). Interestingly, on NH_4Ac sheets the daughter nuclide ^{212}Bi showed a high signal out of the acceptance criteria in alpha detection, whereas in beta detection the sum of free ^{212}Pb and free ^{212}Bi was measured and the result was within the acceptance criteria. Therefore, it can be assumed, that the out of specification impurity (up to 25%) in alpha detection is mainly consisting of colloidal ^{212}Bi , which was not efficiently chelated during the reaction (Figure 5C). However, most of the ^{212}Pb can be found in the product peak, when measuring the TLC in beta detection (Figure 5D).

Table 2. Comparison of TLC measurements for determining the radiochemical purity of ^{212}Pb peptides.

TLC development	TLC measurement time	<i>Product peak [%] MiniScanPRO+</i>		<i>Product peak [%] AR-2000</i>	
		^{212}Pb -VMT- \ast -NET		^{212}Pb -VMT- \ast -NET	
		Citrate	NH_4Az	Citrate	NH_4Az
entry	immediately	95.5	96.9	93.1	94.7
	6 h later	95.3	94.8	96.5	90.0
	18 h later	95.8	96.8	96.7	94.7

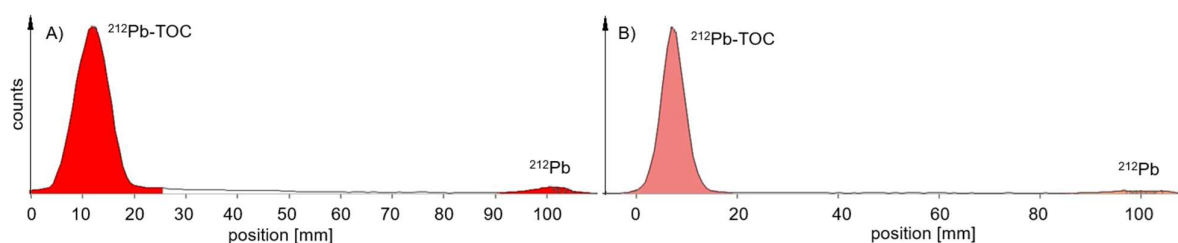


Figure 4. Representative radio-TLCs measured 5 minutes after development in citrate buffer - probe taken immediately after synthesis; A) measured with MiniScanPRO+ B) measured with AR-2000 in alpha mode, ^{212}Pb -signal is lower at the front compared to measurement A).

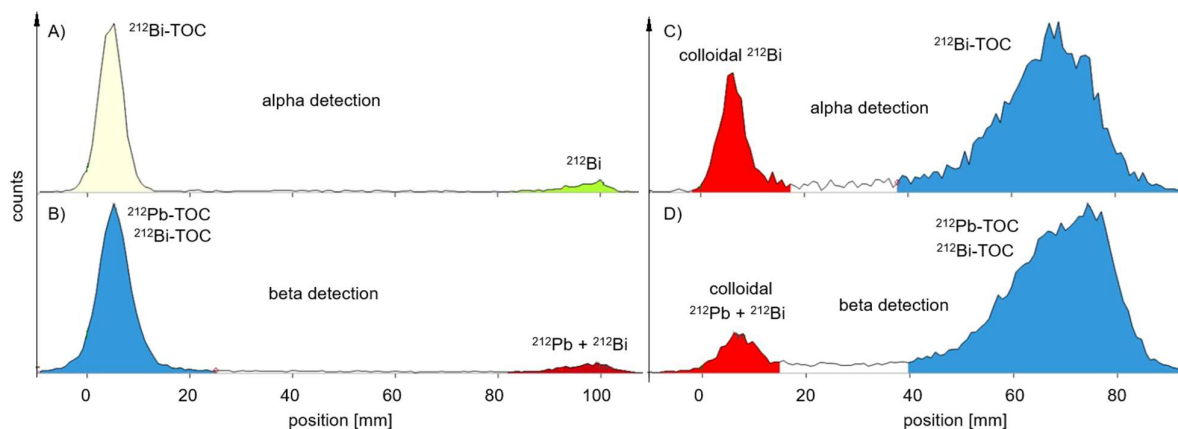


Figure 5. Representative radio-TLCs measured 5 minutes after development with AR-2000 - probe taken immediately after synthesis; A) TLC in citrate buffer with alpha detection – measuring 8% free ^{212}Bi ; B) TLC in citrate buffer with beta detection – measuring 7% free ^{212}Pb (238 keV) and free ^{212}Bi (2.2 MeV); C) TLC in NH_4Ac with alpha detection – measuring 24% colloidal ^{212}Bi ; D) TLC in NH_4Ac with beta detection – measuring 8% colloidal ^{212}Pb and ^{212}Bi .

3. Discussion

The term “true radiochemical purity” is defined in this paper as the RCP of the product measured when all daughter nuclides are in equilibrium. Kelly et al. made the same definition for 26 h after TLC development [13]. However, for ^{225}Ac , mostly a waiting time of one to two half-lives of ^{213}Bi (46–92 minutes) after TLC development is usually sufficient to find radiochemical purities >97%. Therefore, the synthesis protocol has to have two quality requirements for RCP for safety reasons. In routine synthesis, the first quality requirement is RCP >90% (assigned as prospective), because generally all products which reached this quality in the experiments, had RCPs >97% after 2 h waiting time and remeasurement of the TLC (Table 1). The second quality requirement is for measured product RCPs <90% (assigned as retrospective), which needs to be >97% RCP after 2 h. This has the following consequence for routine quality control: if the measured RCP is >90% (prospective), a second measurement is unnecessary and the quality control can be finished. If the measured RCP is <90%, the quality control has to be elongated to >2 h and the TLC has to be remeasured (retrospective), after the daughter nuclides are decayed. In most of the experiments an RCP <90% was due to later probe sampling more than 5 minutes after the radiosyntheses were finished.

The AR-2000 had a better signal-to-noise ratio for every activity tested on the TLC. It was possible to determine the true radiochemical purity >97% immediately after synthesis without waiting time. On the MiniScanPRO+ it was possible to determine radiochemical purity >90% immediately after synthesis. A short waiting time of 20–40 minutes after TLC development was

required to obtain radiochemical purities >95%. Longer waiting times >2 h resulted in the measurement of true radiochemical purities of >97%.

Measurement of each compound was possible with each dilution tested (10 kBq, 100 kBq) and system (MiniScanPRO+ and AR-2000). In addition, the AR-2000 produced readable spectra even at 1 kBq per TLC, whereas the MiniScanPRO+ had a disturbingly high background at this low activity level. The optimal MiniScanPRO+ measuring time was set to 5 mm/s. A slower measuring time might produce better spectra but would not reach the high sensitivity of the AR-2000.

The AR-2000 requires an ionization gas for measurement and lacks an MCA. One flask of this gas holds at least two years, if measurements are performed twice a week. Therefore, the operation of the AR-2000 with ionisation gas is very convenient.

Interesting is the fact, that ^{225}Ac -labelled radiopharmaceuticals showed not such a high radiolysis in the final diluted and sterile solutions (Table 1, 24 h values) as speculated by the literature [14]. Of course, if on ^{225}Ac nuclide decays, the ejected alpha particle or its repulsion could hit the rest of the molecule and destroys it by a certain percentage. Since the alpha particle can eject in every possible direction of a sphere, the possibility that it hits directly the molecule it is attached to or else molecules which are in the solution is very low. However, if the ^{225}Ac nuclide decays, it is for sure away from the molecule. But the long half-life of ^{225}Ac means, that on nuclide could stay intact for up to 9.9 days. Fact is, that the strong dilution (8–10 mL) of a very small number of radiolabelled molecules (e.g. 5 MBq ^{225}Ac -PSMA-I&T consists of 50 μg or 33 nmol PSMA-I&T molecules) in the final solution leads to a very low radiolysis, which was observed experimentally in this work. Therefore, a centered production of ^{225}Ac -radiopharmaceuticals and distribution for over 24 h might be possible. With up to 5 h waiting time for determination of the true RCP by TLC after 24 h, the ^{225}Ac -radiopharmaceuticals could also be tested for quality on arrival in the clinic in the early morning for application to patients during the daytime. Further, new chelators like PSC and MCP are known to bind the respective radionuclides even at room temperature. Therefore, a certain safety amount of unlabelled precursor within the final solution could lead to a rechelation of daughter nuclides like ^{212}Bi (to PSC) or ^{213}Bi (to MCP). Here, it would be interesting to know, if the strong dilution of the final solution interferes with the theoretical rechelating process. However, a lower dilution could lead to a higher radiolysis. The optimal volume for low radiolysis and high rechelating rate within the final solution will be examined experimentally in the near future.

For ^{225}Ac -labelled radiopharmaceuticals, DTPA may be added to the final solution to prevent a high fraction of free daughter nuclides like ^{213}Bi within the final solution, especially, when the quality control is elongated to >2 h by the above-mentioned procedure. The bounded ^{213}Bi -DTPA might pass faster through the kidneys as compared to free ^{213}Bi , which binds to the kidney physiologically [15]. However, without DTPA in the final solution, most of the patients show good renal function up to the fourth cycle of ^{225}Ac -therapy in our clinic. However, some patients show less renal function already before the fourth cycle of ^{225}Ac -treatment and adding DTPA to the final solution might elongate the good renal function for those patients. In the literature, a DTPA content of 0.1% should be within the final solution [10], which either can be added to the dilution agent during automated syntheses [8] or directly into the final product vial, which might be the better choice. DTPA within the dilution solution might act as scavenger for free ^{225}Ac and could chelate bounded free ^{225}Ac from the purification cartridge and rinse it as ^{225}Ac -DTPA into the product solution. Then, the quality control by TLC would show more ^{225}Ac in the non-product fraction resulting in a wrong true RCP and a complicated situation.

The MiniScanPRO+ has the ability to measure a gamma spectrum with a spectroscopic grade NaI(Tl) detector (Figure 6). This feature measurement is important, if radio nuclidic purity have to be measured for approval of the product. The sensitivity of the MCA is adequate to differentiate between important γ signals of ^{212}Pb and ^{225}Ac , but actually this test is unnecessary for quality control in most of the cases, since the radio nuclidic purity is also given by the certificate of quality and purity provided by the distributors of the radionuclides.

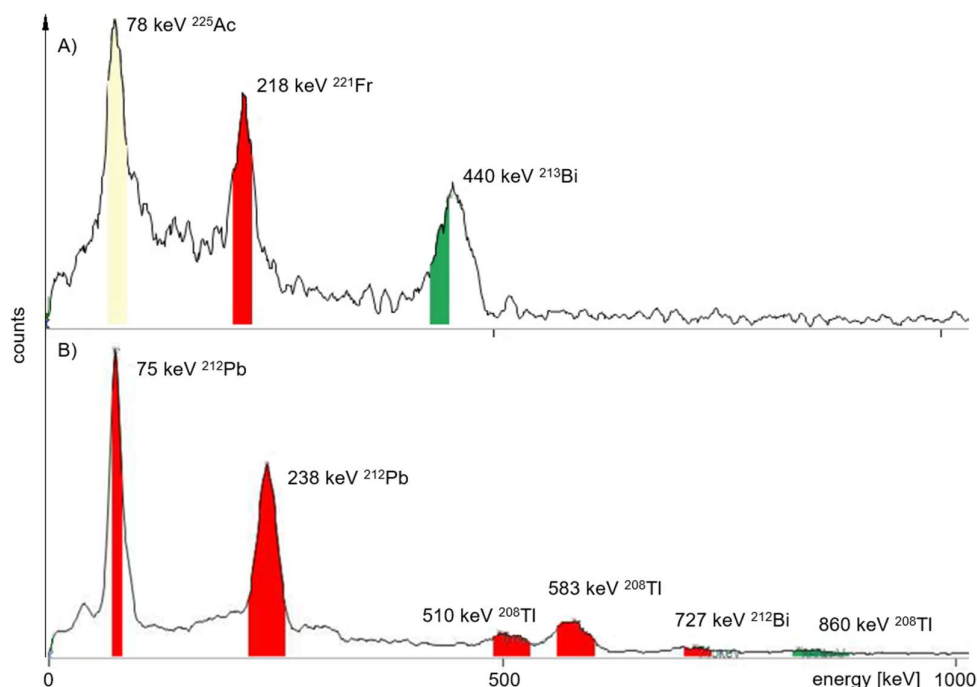


Figure 6. Respective gamma spectra measured with the MCA of MiniScanPro+ for verification of the radio nuclidic purity. Gamma energy found given in keV (percentage of probability): A) ^{225}Ac : 78 keV (3%), ^{221}Fr : 218 keV (12%), ^{213}Bi : 440 keV (26%); B) ^{212}Pb : 75, 238 keV; ^{208}Tl : 510 (22.6%), 583 (85.0%), 860 (12.5%) keV; ^{212}Bi : 727 (6.7%) keV.

Theoretically, spectra of ^{225}Ac -peptides that are measured at the start of TLC (e.g. high percentage ^{225}Ac -TATE only when developed with citrate) and the front of TLC (e.g. low percentage free ^{225}Ac and high percentage free ^{221}Fr + ^{213}Bi) might differ in the shape and intensity of the individual gamma signals at 78 keV (^{225}Ac), 218 keV (^{221}Fr) and 440 keV (^{213}Bi). Experimentally, no difference in the peak height of the signals between start and front of the TLC were found, even with high activity in the range of ~ 37 MBq, as the difference between bound and free ^{225}Ac must be highest. Therefore, with the MCA of the MiniScanPro+, it was not possible to skip the waiting time for determination of the true RCP by performing calculations on the percentage of the product peak by a certain nuclide quotient obtained from the gamma spectrum. Here, only a HPGe might have sufficient sensitivity to detect differences in the peak height of the γ signals between start and front fractions of the TLCs.

To date, the measurement of the radio nuclidic purity is only needed, if it is a requirement by the regulatory authorities for production of alpha-emitting radiopharmaceuticals. To date, there is no such monography for alpha-emitting radiopharmaceuticals, but this would change within the next years as the use of alpha-emitting radiopharmaceuticals in clinic is rising and companies already working on approvals for such alpha-emitting radiopharmaceuticals. An example for beta-emitting radionuclides is ^{177}Lu , which needs the approval of $>99.9\%$ radio nuclidic purity, since $^{177\text{m}}\text{Lu}$ is an important impurity during production of ^{177}Lu and should not exceed 0.1% (Ph. Eur. 11.0 07/2017:2798 corrected 9.3).

4. Materials and Methods

All reagents and solvents were purchased in highest purity from commercial suppliers and were used without further purification. PSC-PEG₂-TOC (VMT- α -NET) and $^{224}\text{Ra}/^{212}\text{Pb}$ -generator (VMT- α -GEN) were obtained from Perspective Therapeutics Inc., Coralville, Iowa, USA. RCP was monitored by thin-layer chromatography (TLC) on iTLC-SG plates (Agilent, Santa Clara, California, USA). Measurement of the radio nuclidic purity (RNP) and evaluation of the radio-TLC was performed

with a thin-layer scanner (MiniScanPRO+, Eckert&Ziegler Eurotope GmbH, Berlin, Germany) equipped with a Model 43-2 alpha detector ZnS(Ag) scintillator (Ludlum Measurements, Sweetwater, Texas, USA) and a build-in multi-channel analyser (MCA) for gamma spectroscopy or with a proportional gas-counter (AR-2000, Eckert&Ziegler Eurotope GmbH, Berlin, Germany) equipped with an ionisation gas (90% Ar / 10% CH₄ or 90% Ar / 10% CO₂). CORGON-10 was purchased from Linde Gas (Dresden, Germany). Radio-HPLC was performed on a Shimadzu HPLC system (Thermo Scientific, Dreieich, Germany), equipped with a reverse phase column (Analytical: Merck Chromolith RP-18e; 100 × 4.6 mm plus a guard column 5 × 4.6mm and a UV-diode array detector (220 nm). The solvent system used was a gradient of acetonitrile:water (containing 0.05% TFA) (0–13 min: 0–60% MeCN) at a flow rate of 1.6 mL/min unless otherwise stated. The pH was measured with a reflection photometer (QUANTOFIX Relax, Macherey-Nagel GmbH & Co. KG, Düren, Germany).

4.1. Radiochemistry

Automatic ²²⁵Ac-radiolabelling of DOTA-TATE or DOTAGA-PSMA-I&T was performed according to an established protocol [8]. Solid ²²⁵Ac was dissolved in 100–500 µl of 0.04 M HCl_{suprapure}. Briefly, 10–20 µg/MBq of precursor in H₂O_{suprapure} was added to a 5 mL conical vial together with 2 mL 0.2 M NaAc/AcOH buffer (pH 5.7, 99,99% trace metal).

Automatic ²¹²Pb-radiolabelling of PSC-PEG₂-TOC was performed according to an established protocol (DOI : 10.1186/s41181-024-00305-8). In brief, 0.1 µg/MBq of precursor in H₂O_{suprapure} was added to a 5 mL conical vial together with 100 µL EtOH_{absolute}, 290 µL 1 M NaAc/AcOH buffer (pH 4, 99,99 % trace metal) and 2 mg sodium ascorbate (Ph.Eur.) in 100 µl H₂O_{suprapur}.

4.2. Thin-Layer Chromatography

- RCP was determined by TLC either by cutting the developed TLC in two pieces and measuring their activity using a surface contamination monitor (CoMo-170, Nuvia Instruments, Dresden, Germany) or by TLC scanner.
- For the detection of colloidal ²²⁵Ac hydroxide, TLC was performed in 1 M NH₄Ac:MeOH 1:1 on silica gel-aluminum sheets for ²²⁵Ac-PSMA-I&T and on ITLC-SG for ²²⁵Ac-TATE.
- For the detection of free ²²⁵Ac TLC was performed on silica gel-aluminum sheets in 0.1 M citrate buffer (pH 5.0)
- For the detection of colloidal ²¹²Pb hydroxide, TLC was performed in 1 M NH₄Ac:MeOH 1:1 on ITLC-SG
- For the detection of free ²¹²Pb, TLC was performed on ITLC-SG in 0.1 M citrate buffer (pH 5.0)
- RNP and exact volume activity were determined using an HPGe detector
- 10 µL of the product solution was diluted with 990 µL sterile water (1:100) and used for endotoxin level determination using EndoSafe PTS
- The pH was determined using a Quantofix pH meter

5. Conclusions

Two TLC scanners were compared for their ability to measure the RCP of radiopharmaceuticals radiolabelled with ²¹²Pb and ²²⁵Ac. Both scanners have their advantages and disadvantages. The MiniScanPRO+ is fast, does not require additional equipment and can also measure the gamma spectrum, which may be important for some radiopharmaceutical production sites and regulatory authorities. Unfortunately, measurements of the gamma spectrum revealed no difference in the peak area between high and low percentages of ²²⁵Ac in the individual spots at start and front of the TLC, presumably due to the low sensitivity of the MCA.

The AR-2000 requires ionization gas to operate. With independent alpha and beta detection, it was possible to produce clearer TLCs with a better signal-to-noise ratio than the MiniScanPRO+. This eliminated the need for additional waiting time after TLC development. Quality control may be faster than with the MiniScanPRO+ if the waiting time for probe sampling is longer than five minutes after radiosynthesis. However, the AR-2000 cannot measure a gamma spectrum, but as mentioned above,

this additional measurement may not be required by regulatory authorities as the radionuclides themselves already have a certificate of quality and purity when they are delivered.

In our opinion, the best quality control setup would consist of both scanners as they support each other. Otherwise the decision depends on the local regulatory authorities and the head of quality control, who have to consider which scanner would better fit into a safe routine quality control of alpha radiopharmaceuticals.

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Conflicts of Interest: The MiniScanPRO+ and AR-2000 were provided by Eckert&Ziegler especially for evaluation of α -particles on TLC. J.W. is employed by Eckert&Ziegler, which has financial interest in distribution of TLC scanners. The authors declare no further conflict of interest.

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