Do you care about molar activity?

SRSTT Radiopharmaconnect[1]

[1]Radiopharmaconnect

April 28, 2020

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The term molar activity is not just a concept for radiochemists and radiopharmacists but it should also be taken into account by all disciplines in nuclear medicine.

Here, we give an overview on the most important key principles on molar activity (and specific activity) with a focus on ^{11}C and ^{18}F short-lived radionuclides.

Definition of molar activity ($\mathbf{A_m}$), specific activity ($\mathbf{A_s}$), apparent $\mathbf{A_m}$ and apparent $\mathbf{A_s}$

When the radiotracer and its non-radioactive compound are present in the formulation, we define:

Molar activity ($\mathbf{A_m}$) and *specific activity* ($\mathbf{A_s}$) as the measured activity per mole and per gram of compound, respectively.

If in the formulation non-radioactive impurities are also present, and they might interact with the same biological target as the radioactive compound we define:

Apparent molar activity (*apparent* $\mathbf{A_m}$) and *apparent specific activity* (*apparent* $\mathbf{A_s}$) as the measured activity per mole and grams, respectively, of all compounds (impurities and non-radioactive compound). However, in most cases the main impurity is the precursor.

$\mathbf{A_m}$ and *apparent* $\mathbf{A_m}$ are expressed in Bq/mol (GBq/μmol).

$\mathbf{A_s}$ and *apparent* $\mathbf{A_s}$ are expressed in Bq/g (GBq/μg).

Due to radioactive decay, the time of measurement of $\mathbf{A_m}$, $\mathbf{A_s}$, *apparent* $\mathbf{A_m}$ and *apparent* $\mathbf{A_s}$ must be always stated.

Why is molar activity important? Is high molar activity always the goal?

There are different situations that will determine if high $\mathbf{A_m}$ (or $\mathbf{A_s}$) is a critical factor or not for the study. Three categories can be identified:

High $\mathbf{A_m}$ is critical when studying:

· bioactive or toxic molecules, as the quantity of injected non-radioactive compound can produce undesired pharmacodynamic or toxicological effects.

· low-density receptors. The injection of an excess of the non-radioactive compound can have an effect on the receptor occupancy and, therefore, alter the apparent receptor characteristics. The receptor occupancy with the non-radioactive compound should be kept below 5% in order to avoid pharmacological or pharmacodynamic effects.

· kinetic modelling parameters, as low $\mathbf{A_m}$ can lead to an unfavourable signal-to-noise ratio within the image. $\mathbf{A_m}$ can have a substantial impact on the quantitative analysis and qualitative interpretation of nuclear medicine images.

High $\mathbf{A_m}$ is not critical when studying:

· endogenous compounds normally found in high concentrations in the body (e.g., [^{15}O]water, [^{11}C]acetate, [^{11}C]glucose)

· processes in which the biological target or function is not easily saturated (e.g. glucose metabolism, hypoxia, enzyme activity). Nevertheless, the $\mathbf{A_m}$ can dramatically impact the image outcome, e.g. when the patient eats candies directly before measuring the glucose metabolism.

Intermediate to low $\mathbf{A_m}$ is necessary when studying:

· antibodies, due to liver depletion which reduces the biological half-time of small amounts of antibodies in the bloodstream.

· some peptides or proteins, to optimize the biodistribution properties in terms of reducing radiation burden in specific organs (mostly emunctories) by low organ-to-tumour ratios.

Table 1. Examples of radiotracers falling into the three categories of $\mathbf{A_m}$.

Low $\mathbf{A_m}$	**Intermediate $\mathbf{A_m}$**	**High $\mathbf{A_m}$**
[^{18}O]H_2O	[^{11}C]PIB	[^{11}C]PHNO
[^{11}C]Methionine	[^{11}C]DASB	
[^{18}F]FDOPA	[^{11}C]mHED	
[^{68}Ga]PSMA		

What is the maximum theoretical molar activity?

Theoretical $\mathbf{A_m}$ values for PET radionuclides are very high (e.g. 341.1 TBq/μmol for carbon-11, 63.3 TBq/μmol for fluorine-18) however these values are very far from those obtained in laboratories (e.g. 10-5000 GBq/μmol for carbon-11, 10-1000 GBq/μmol for fluorine-18).

Table 2. Half-life and maximum theoretical $\mathbf{A_m}$ of some PET radionuclides.

Radionuclide	Half-life (minutes)	Μαξιμυμ τηεορετιςαλ $\mathbf{A_\mu}$ (TBχ/μμολ)	Examples of radiotracers pr
^{11}C	20.4	341.1	4.9 ± 2.4 ([^{11}C]Raclopride) at E
^{18}F	109.8	63.3	4.4 ([^{18}F]DCFPyL) at EOS
^{15}O	2.0	3394.0	
^{13}N	10.0	699.3	
^{68}Ga	68.0	102.3	

Low $\mathbf{A_m}$ might be due to a dilution process with stable nuclides.

$\mathbf{A_m}$ is decreased by:

(i) the generation of the radioactive precursor in the cyclotron. Impurities potentially come from the target gas, the valves, pressure regulators, seals, target body, target windows or even residues from cleaning solvents.

(ii) the transfer of the irradiated gas/liquid to the hot cells. Long-time processes decrease $\mathbf{A_m}$. Keeping the lines pressurized with high purity gases and avoiding fluorine-rich materials during the preparation of ^{18}F radiotracer should help to maintain $\mathbf{A_m}$.

(iii) the synthesis process. The use of high purity reagents and precursors is the most important factor. Reagents should be prepared and stored under adequate inert conditions to prevent contamination. The synthesis unit should be also cleaned and dried thoroughly and kept isolated from atmosphere/contaminants;

$\mathbf{A_m}$ can be increased either by:

(i) increasing beam time and/or current (up to a certain level), loading/unloading the target before irradiation, discarding the first 1-3 irradiations of the day and keeping the target under pressure between runs.

(ii) decreasing the amount of non-radioactive sources that might potentially contaminate the reaction. Contaminants from the atmosphere (e.g. atmospheric CO_2 during the preparation of ^{11}C radiotracer *via* [^{11}C]CO_2), or undesired chemicals released from tubes, etc. or the presence of impurities in reagents could contribute to decrease the $\mathbf{A_m}$.

How to calculate the molar activity.

The methods for measuring the $\mathbf{A_m}$ will depend on the physical state of the radioactive material. Gaseous and liquid materials can often be analysed with the use of radiogas chromatography, high pressure liquid

chromatography (HPLC), ion chromatography or mass spectrometry.

Here we give an example of a procedure to calculate the $\mathbf{A_m}$ for a ^{11}C-radiotracer using the HPLC system.

What you need: HPLC, analytical column, pipettes, falcon tubes, ^{12}C-reference compound, PET dose calibrator, a solution of a ^{11}C-radiotracer, laptop, solvents.

1) Develop an HPLC method to identify the reference compound using an analytical HPLC column and define the:

- UV wavelength (measured at the maximal UV absorption);
- mobile phase;
- column temperature;
- flow rate;
- injection volume (e.g. 20 µL).

2) From a stock solution of the ^{12}C-reference compound (e.g. 1 µM), prepare seven standard solutions in a non-volatile solvent (e.g. 200 nM, 100 nM, 50 nM, 25 nM, 12.25 nM, 6.13 nM, 3.06 nM). From the concentration of the standard solutions calculate the amount (µmol) present in the HPLC injected volume (e.g. 20 µL). Inject the standard solutions into the HPLC resulting in chromatograms with a single peak - note: you should inject three times each standard solution.

3) From the UV detector chromatogram, determine the area under the curve (AUC) of the peak corresponding to the compound (**Fig. 1A**). Enter these values and amount of reference compound (µmol) into a computer spreadsheet. Create a calibration curve by plotting the AUCs as a function of injected amount of reference compound (in µmol) (**Fig. 1B**). Analyse the data by applying a linear regression analysis (linear least squares fit). This yields the equation $y = mx + y_0$, where **y** is the area, **m** represents the slope, and $\mathbf{y_0}$ is a constant that describes the background. The amount of compound (**x** expressed in µmol) present in a radioactive sample may be calculated from this equation.

4) Synthesize and isolated the ^{11}C-radiotracer.

5) Measure an exact volume of a solution containing the ^{11}C-radiotracer and amount of radioactivity via dose calibrator and decay correct the activity at end of synthesis (EOS) (or at end of delivery - EOB). Calculate the activity in GBq in the HPLC injection volume (e.g. in 20 µL $\mathbf{activity_{inj}}$).

6) Inject a sample (20 µL) of a solution containing the ^{11}C-radiotracer into the HPLC using the same method used to derive the calibration curve. Obtain the area from the UV-chromatograms.

7) Using the appropriate calibration curve, this area is converted to the number of moles of compound injected ($\mathbf{amount_{inj}}$, in µmol).

8) Divide the $\mathbf{activity_{inj}}$ by $\mathbf{amount_{inj}}$ to obtain the $\mathbf{A_m}$ in GBq/µmol (and stated that it is corrected at EOS or EOB).

9) To obtain the $\mathbf{A_s}$ in GBq/µg, divide $\mathbf{A_m}$ by the molecular weight of the compound.

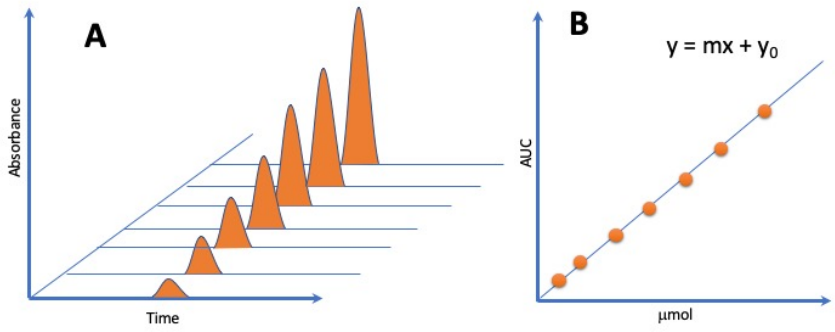

Figure 1: **A)** HPLC UV-chromatograms of standard concentrations of a reference compound. **B**) Calibration curve of AUC vs. amount of reference compound (µmol).

Note: The calibration curve has to be repeated at anytime if any of the HPLC parameters are changed due to maintenance (e.g. change of UV lamp), or a new HPLC column is used, or after a determined time period (e.g. this is stated in your standard operating procedure).

Closing remarks and critical aspects

The time-dependency of the molar activity makes it a necessity to provide a time-cut-off for the applicability of a radiopharmaceutical to avoid side-effects for the last patient. Besides, also the maximal injectable volume in terms of potential unwanted pharmacodynamics or side effects should be stated in the release criteria.

In most cases, only the $\mathbf{A_m}$ is stated, as the apparent $\mathbf{A_m}$ is very difficult to determine as precise information on impurities affecting the target region must be available. Nevertheless, the apparent $\mathbf{A_m}$ is the more critical one in terms of toxicology, interdependencies and signal-to-noise ratio. A starting point is to include the residual amount of precursor in the calculation of the maximal applicable dose and the time-cut-off. Interestingly, this is already standard procedure for peptides, proteins and antibodies as here the ratio of the activity per unlabelled compound is determined, as the separation of precursor and radiopharmaceutical is not possible. Therefore, the borders between the definitions of $\mathbf{A_m}$ and apparent $\mathbf{A_m}$ are fluid for these cases.

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