In situ analytical quality control of therapeutic mAbs preparations by confocal Raman spectroscopy

Ayyoub Rayyad [a], Alaa A. Makki [b], Victor Massot [c], Igor Chourpa [a], Franck Bonnier *[a]
[a] Université de Tours, Faculté de Pharmacie, EA6295 NMNS « NanoMédicaments et NanoSondes », France.
[b]University of Gezira, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, P.O. Box 20, 21111, Wad Madani, Sudan
[c] CHU de Tours, Unité de Biopharmacie Clinique Oncologique, Pharmacie, France

*corresponding author: Franck Bonnier, Université de Tours, UFR sciences pharmaceutiques de Tours, EA6295 NMNS « NanoMédicaments et NanoSondes », 31 avenue Monge, 37200 Tours, France; Tel: +33 2 47367307; E-mail: franck.bonnier@univ-tours.fr

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1 Introduction

Analytical Quality Control (AQC) of anticancer drug solutions is a critical step in centralized clinical preparation units to ensure patients receive the correct dose prescribed for their treatment. Enabling quantitative analysis of solutions directly into perfusion bags, i.e. without need for samples withdrawal, is an important concern to address the safety for both patients and staff members. Herein, the analytical performances of Confocal Raman Spectroscopy (CRS) for AQC of monoclonal antibodies (mAbs) in solutions is investigated. An *in situ*-like experimental setup has been used to collect specific molecular signatures from a range of solutions. The data have been treated by multivariate analysis protocols (namely PLSR – Partial Least Square Regression) in order to evaluate the precision and accuracy of the technique. Presently, commercially purchased injectable solutions of Trastuzumab (TRS), Bevacizumab (BVC) and Atezolizumab (ATZ) were studied.

2 Material and methods

2.1 Samples preparation

Commercial mAbs solutions, Trastuzumab (TRS), Bevacizumab (BVC) and Atezolizumab (ATZ), were used, as received and after subsequent dilutions in 0.9 % NaCl, resulting in the concentration range 21g/L – 0.16 g/L for TRS or 25 g/L – 0.19 g/L for BVC and ATZ. Within this concentration range, for each mAb, three independent sets of samples were prepared and identified as SET_01, SET_02 and SET_03.

2.2 Experimental set-up and data acquisition

For the purpose of the study, an experimental design was developed to simulate *in situ* analysis directly into perfusion bags. 400 µl of the solution to analyse was placed in a 96 well plate then a square-shaped piece cut-out from a perfusion bag wall was positioned over the liquid, ensuring full contact to avoid the presence of air bubbles. Each spectrum was collected as an average of 4 accumulations of 90s. For each sample, 10 spectra were collected from different locations on the same depth of 600 µm under the surface of the perfusion bag, since this depth was found to be optimal. Raman data have been pre-processed and analysed using MATLAB® (Mathworks, USA). Spectra were subjected to a Savitzky-Golay smoothing filter (K=14, W=31), followed by a baseline correction (Lieber function, 2nd order polynomial, 10 iterations) and a vector normalization.

3 Results and Discussion

PLSR analysis was performed using SET_01 and SET_02 as training sets (calibration and validation) and SET_03 was used as a test set. PLSR performances are presented in Table 1. R^2 values for both validation set and test set remain above 0.99, highlighting the linear correlation between prepared and predicted

concentrations. RMSECV obtained are respectively 0.3735 g/L, 0.5503 g/L and 0.5024 g/L for TRS, BVC and ATZ. RMSEP of 0.5861 g/L, 0.4968 g/L and 0.6053 g/L.

***Table 1**: PLSR results obtained for the 3 mAbs analysed In Situ*

	Training (SET_01 /SET_02)			Test (SET_03)	
mAb	**RMSECV (g/L)**	**R^2**	**LVs**	**RMSEP (g/L)**	**R^2**
TRS	0.3735	0.9971	5	0.5861	0.9983
BVC	0.5503	0.9936	7	0.4968	0.9979
ATZ	0.5024	0.9955	5	0.6053	0.9963

Figure 1 presents PLS regression coefficients for the 3 mAbs used in this study, which confirm that PLSR models are constructed based on spectral features that are specific to both excipients and mAbs. Figure 2 shows the typical PLSR regression plot obtained for TRS.

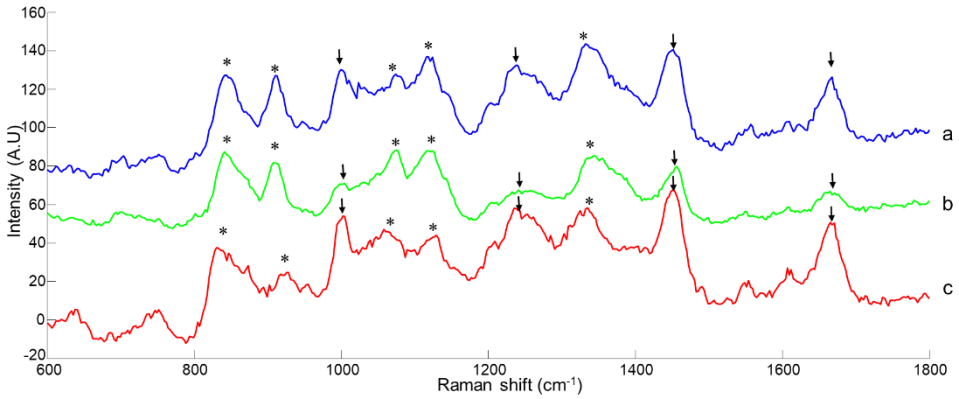

***Figure 1:** PLSR regression coefficients for mAbs a: TRS, b: BVC and c: ATZ, spectra are offset for clarity. ↓ Protein bands, * Excipient bands.*

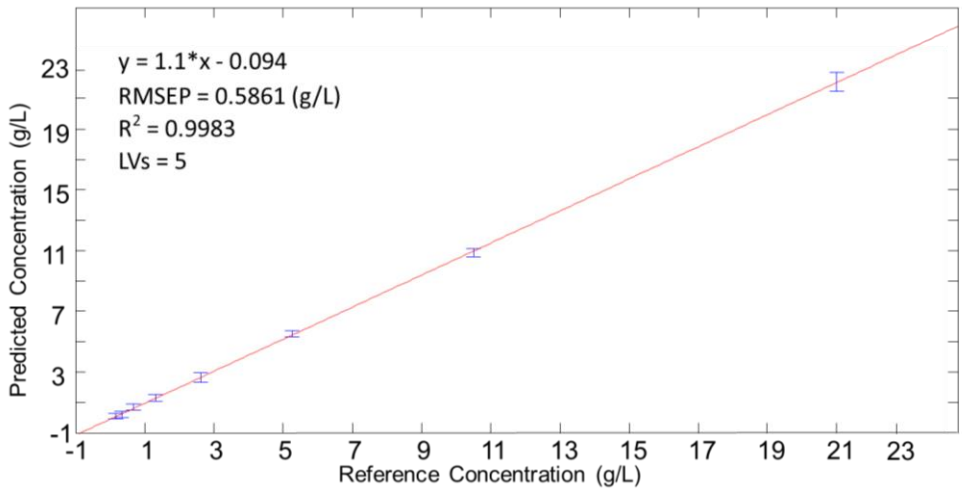

***Figure 2**: PLSR Regression plot for Trastuzumab.*

4 Conclusion

Based on quantitative results from PLSR, it is demonstrated that CRS has precision and accuracy concomitant with requirements for ACQ of mAbs. It has to be highlighted that these results were obtained *in situ*, in confocal Raman microscopy conditions that mimic solutions in perfusion bags. Therefore, this study opens promising perspectives for clinical ACQ, namely in terms of : (i) reducing risk for patients because of possible errors in manipulation; (ii) increasing safety for hospital staff by reducing their exposition to the drugs; (iii) improving the workflow and accelerating release of treatments from centralised preparation units to patient's bedside.