Two-dimensional fluorescence as an analytical tool in bioprocess measurement and control

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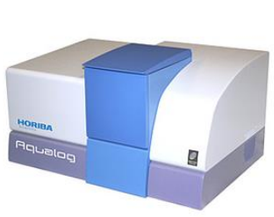
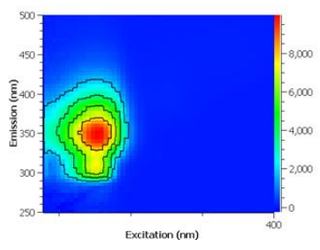
Introduction

Fluorescence spectroscopy is widely used in a variety of industries including biological sciences. Two dimensional (2D) fluorescence excitation-emission matrix (EEM) spectroscopy has been described in the literature for over 30 years to characterise or "fingerprint" biological samples. Historically, spectra were slow to collect and required multiple corrections. The advent of Charge Coupled Device (CCD) technology and data correction methodology of common errors such as the Inner Filter Effect (IFE) has meant renewed interest in 2D-Fluorescent spectroscopy as a technique. The HORIBA Aqualog is a 2D-Fluorescence instrument that uses innovative ATEEM (Absorbance-Transmission fluorescence Excitation and Emission Matrix) acquisition technology to collect spectra.





Class	Fluorophore	Excitation λ (nm)	Emission λ (nm)
Amino acids	Phenylalanine	260	280
	Tyrosine	275/280	300, 330-350
	Tryptophan	280, 290	350
Co-enzymes	NADH	290, 350	440, 460
	FAD, Flavines	450	515, 535
Vitamins	Vitamin A	325	510
	Pyridoxin	332, 340	390, 400
	Riboflavin	370, 440	520, 530

Figure 1. Left – HORIBA Aqualog ™; Middle – An example EMM spectra of natural fluorophores found in bioprocessing ; Right – Excitation-emission table biological relevant fluorophores adapted from Graf et al. [1]

The HORIBA Aqualog was originally developed for use in the water treatment industry, but has found applications in other industries including biologics. In biologics manufacture, there is growing interest in novel process analytical technology (PAT), that can be deployed to allow automation of biologics manufacture. A number of papers have suggested 2D-Fluorescent spectroscopy as a potential technology to be adopted by the industry [1]. CPI is involved in testing of biologics PAT technology for the Innovate UK (IUK) funded project 93825 "Integrating Continuous Technologies Rapid Delivery of Cost Effective Biotherapeutics to Patients". In this poster we describe the use of 2D-Fluorescence to characterise six monoclonal antibody in-process purification samples, as a first step to its adoption as a PAT instrument in biologics manufacture.

Monoclonal antibody (mAb) production

The generation and purification of monoclonal antibodies is a well established technique that consists of a number of discrete steps (see Figure 2). Samples of i) Cell media, ii) Bioreactor – Protein A Load, iii) Protein A – Flow Through, iv) Protein A - Wash I, v) Protein A – Wash II and vi) Post Viral Inactivation, were taken for analysis using the HORIBA Aqualog instrument.

Method:- Samples were recorded using the HORIBA Aqualog ™ in ATEEM mode. Each samples was diluted to an absorbance of ~ 0.5. The Excitation Emission Matrix (EEM) used excitation from 800 – 239 nm with 3 nm steps and Emission from 800 -250 nm with 5 nm steps. The Integration Time was set at 0.01 secs. Data was corrected for IFE and Raman first order and second order scattering at 16 and 32nm and then normalised to 1. Parallel Factor Analysis (PARAFAC) data analysis was performed using the Solo software (Eigenvector Research Inc., USA).

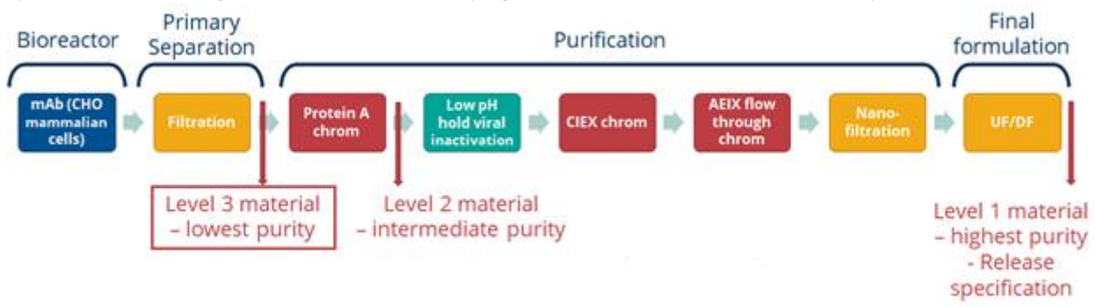


Figure 2. An Overview of the Biomanufacturing process including upstream and downstream steps to final product

The raw 2D-fluorescence data for each of the six in-process samples is shown in Figure 3.

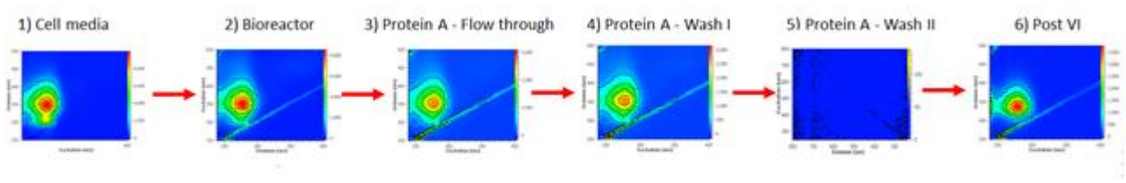


Figure 3. – Raw 2D-Fluorescence Spectra from the six in-process samples

Results

Using 4-component PARAFAC analysis the collected raw data could be modelled with greater than 98 % accuracy. Each identified component could be assigned to a present biological fluorophore, specifically tyrosine (Ex. 275 nm Em. 300 nm), trypophan 1 (Ex. 278 nm Em. 330 nm), tryptophan 2 (Ex. 278 nm Em. 350 nm) and Vitamins and Co-factors (Ex. 356 nm Em. 455 nm). The graphical plots of the model Emission, Excitation and Component loadings for each of the 6 samples is shown in Figure 4. The data shows the red shift of tryptophan's in the post-VI purified mAb. This shift in tryptophan emission reflects a change in the local environment of the tryptophan's present in solution. For example this shift is commonly used to track the folded state of a protein.

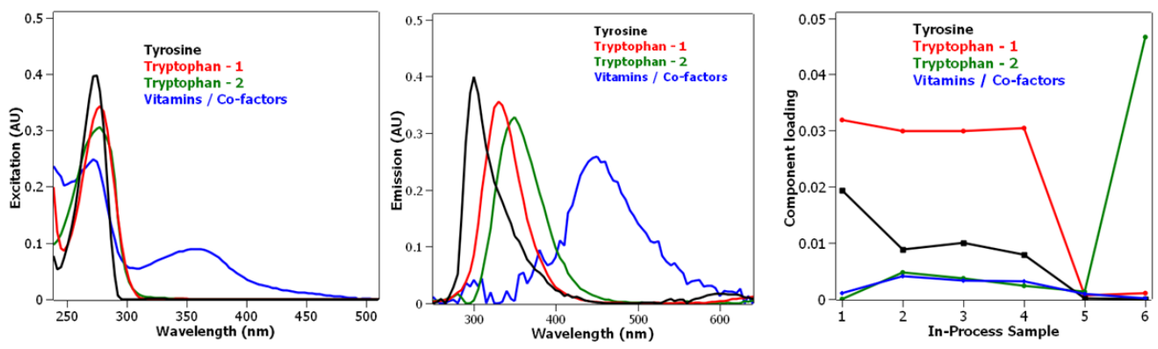


Figure 4. The PARAFAC model Left) Excitation, Middle) Emission and Right) Component loading of the six In-Process 2D-Fluorescence samples

Discussions

In this study 2D fluorescence spectroscopy was performed to characterise six in-process samples. 2D-fluorescence has a number of advantages as an analytical technique, being quick and simple to perform, highly sensitive and data rich. This means it could be used as a standalone technique or to complement other spectroscopies, such as Raman, UV-Vis or infra-red. This experimental work is a first step in understanding the potential of 2D fluorescence as a process analytical technology (PAT) instrument. Further data replicates would confirm the robustness of reproducibility in a defined production run. If successful, the system could be integrated for continuous biologics manufacture. Literature suggests 2D fluorescence is also useful in the analysis of cell media, bioreactor growth, protein identification and protein aggregation studies [2].

References

1) Eng Life Sci. 2019;19:352–362; doi: 10.1002/elsc.201800149
2) Improving Speed and Safety of Biopharmaceutical Manufacturing with HORIBA Analytical Solutions, Readout. No. 55 October 2021

CPI thank HORIBA Scientific UK for the short-term loan of the Aqualog instrument

Acknowledgements

Funding: UKRI Innovate UK
Partners: HORIBA

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