Life Science Analytical Tools for Real Life Problems

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When light interacts with matter (i.e. molecule/sample) many interesting phenomena occur. Light may get absorbed by your sample and depending on the sample properties you will observe light emission, reflection and scattering. HORIBA Scientific builds tools that measure these phenomena allowing our users to gain meaningful information about their samples. This information is used to solve outstanding problems in research or guide scientists to monitor product development in various fields. Life Science, in particular, is an attractive market because, over the years, it has experienced exponential growth, and continues to do so. All the divisions that engage in the scientific study of living organisms including plants, animals and human beings fall under the life science umbrella. This article will highlight the key contributions our products offer in the life science subdivisions - pharmaceutical and health sciences - and will compare their advantages over existing technologies.

Introduction

HORIBA Scientific, over the years, has developed sophisticated high end analytical instruments for research scientists primarily in the physical and chemical sciences. Recently, our efforts have extended to the life science market. This has been an organic transition due to the overwhelming use of fluorescence-based technology in life science and the addition of a new product to our portfolio called “Surface Plasmon Resonance imaging” (SPRi). As we are catering to a new type of customers, we have realized that their needs/expectations for instrumentation and software is very different. Their primary interest in our products is to solve outstanding problems in Life Science. Solving real life problems comes with challenges, but the rewards can be huge. In this article, we will review the utility of fluorescence-, Raman- and SPRi-based technologies in drug formulation, bioprocessing and medical diagnostics.

Drug Formulation

The human body is made up of billions of cells. These cells use different types of proteins to assist them to perform vital functions from processing nutrients and eliminating toxins to regulating physiological reactions. After many years of small molecule drug development, pharmaceutical companies realized that a specific type of Y-shaped proteins called “antibodies” are more effective in treating certain diseases (i.e. cancer) than small molecule drugs (Figure 1). A comparison of the molecular weight (MW) of these products indicate the differences in the scale of complexity which will affect the ease of characterization; the respective MW’s of an antibody vs. small molecule drugs are 150,000 kilodalton (kDa) and 854 dalton (Da) respectively.

The process of discovery and development of antibody-based drugs is extremely challenging and the early assessment of their quality and developability is crucial. One specific challenge is maintaining the stability of antibodies because they have the tendency to clump together (aggregate). Before protein aggregation, it will unfold and the aromatic amino acids like tyrosine and tryptophan (Trp) that are normally buried in a “water-fearing” (hydrophobic) cavity become exposed to water and their local environment becomes hydrophilic. This change in the tertiary structure of the protein can be monitored with Raman which is a spectroscopic technique that provides a unique molecular fingerprint of a sample (i.e. a protein). The Raman spectrum is sensitive to changes to the protein local environment allowing one to follow dynamic fluctuations in the protein secondary and tertiary structure. The unique advantage over other conventional conformational-based technologies like fluorescence and differential scanning calorimetry is that you are able to assess the stability of high concentration formulations without the need to dilute the sample. This allows you to assess the drug

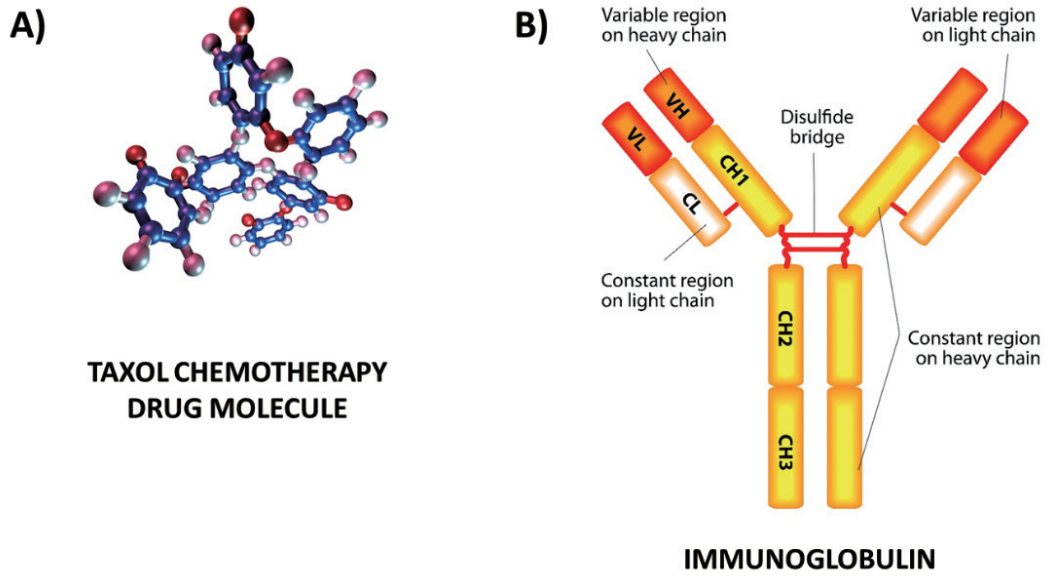

Figure 1 A schematic comparison between small molecule (TAXOL) and protein (antibody IMMUNOGLOBULIN) drugs.

stability in the concentration range that it would be administered to a patient and is important because dilution of the drug can itself influence the stability, lowering the accuracy of the measurement. Other than Raman-based techniques, no other technologies are currently available to assess high concentration samples providing detailed information about protein structure.

A recent study[1] by Dr. Fran Adar, using lysozyme, a small protein found primarily in egg whites, was perturbed by the addition of 20 % ethanol. The Raman spectrum revealed an increase in the relative intensities of Trp band ratio (I_{877}/I_{760}), suggesting a local environmental change around the Trp moiety (Figure 2) involving a change in hydrophobicity.

Alternatively,Prof. Kouhei Tsumoto[2] at The University of Tokyo investigated IgG polyclonal antibody stability as a function of concentration. As the solution concentration increased, the ratio of the tyrosyl doublet (I_{856}/I_{830}, a specific side chain marker) started to increase and eventually was over-saturated at concentrations higher than 80 mg/mL. This outcome implies that at lower concentrations the tyrosine side chain starts to be in close proximity to other neighboring proteins (Figure 3). The other protein stability marker that was examined is related to tryptophan (side chain marker) at 1555 cm^{-1} position which increased in band width as a result of an excluded volume effect (Figure 4). This is because at high concentration there is an increase in disorder (entropy) and the Trp residue avoids the unfavorable steric repulsion against the neighboring side chain residue producing a variety of Trp conformations.

The ability to monitor structural changes in protein drugs at high concentration is extremely important to pharmaceutical scientists as this can provide means for them to measure the propensity for aggregation. Drug safety and drug efficacy are highly compromised by the presence of aggregates, therefore, Raman can be used as a first measure screening tool at different stages of the drug formulation development process.

Bioprocessing

During the manufacturing process of protein based drugs like monoclonal antibodies, pharma scientists use Chinese Hamster Ovary (CHO) cells. Human DNA is used to modify these cells in order to produce therapeutic monoclonal antibodies targeted for treatment of specific human diseases. The modified cells are then introduced into a nutrient liquid medium that is conditioned to promote healthy cell growth and reproduction in a chamber called a bioreactor. Each cell produces a tiny amount of the therapeutic antibody. The bioreactor runs for as much as several weeks or months (depending on production conditions) to produce up to 30 billion cells, and they are then transferred to a larger bioreactor to continue the production of cells. This is repeated several times until reaching the desired quantity of cells.

To ensure optimal production, the cells are fed periodically with a cocktail of nutrients. Online and offline monitoring of the bioreactor condition is of prime importance to increase the efficiency of protein production. However, one of the biggest challenges faced is to minimize contaminants introduction as this can affect the efficacy of the protein drug downstream. Raman probe technology offers an attractive solution as it can be inserted directly in the bioreactor (closed-loop feedback) to monitor

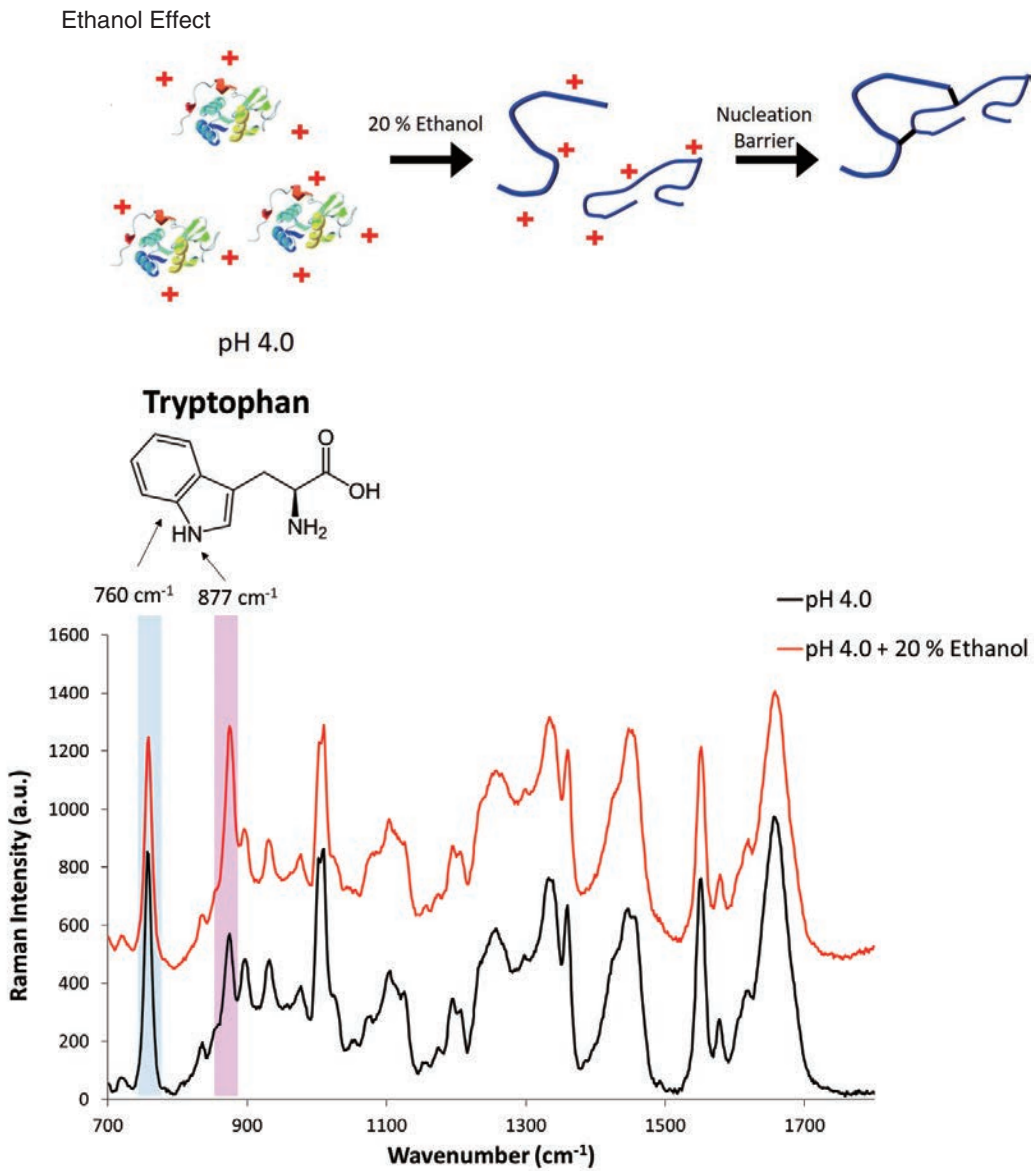

Figure 2 Raman Analysis of lysozyme (200 mg/mL) in 20 mM Citrate-PBS buffer at pH 4.0 before and after the addition of 20 % ethanol. Sample was excited with a 532 nm laser and a grating of 1800 lines/mm was used on the XploRA, a 200 mm focal length instrument.[1]

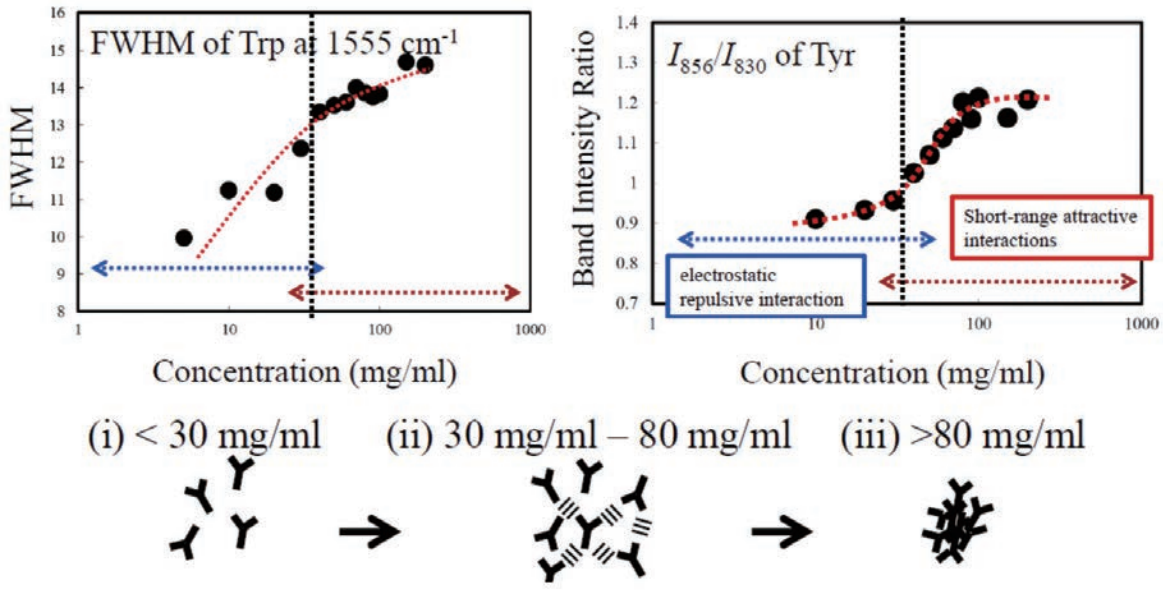

Figure 3 Comparison of the Trp band at 1555 cm^{-1} full width half maximum (FWHM) width (top left) and I_{856}/I_{830} tyrosyl ratios (top right) at concentrations ranging from 10 to 200 mg/mL. A schematic representation of the microenvironment around IgG molecules changes as the concentration increases (bottom (i)-(iii)).[2]

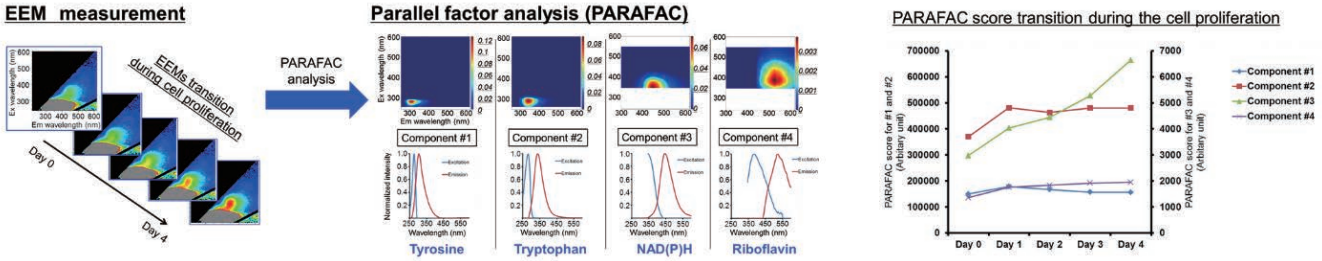

Figure 4 Monitoring cell proliferation over 4 days using ATEEM. The EEM data were analyzed using PARAFAC analysis. As a result, four spectral components were successfully extracted and assigned to tyrosine, tryptophan, NAD(P)H and Riboflavin.[3]

continuously in real time viable cell density, glucose, ammonium, lactate, glutamate, glutamine and total cell density. With this knowledge, feeding strategies can then be optimized which will have a positive outcome on yield and concentration.

A challenge that scientists face during bioprocessing is the ability to measure free amino acids in the so called "supernatant" of the bioreactor. Knowledge of the presence, identity, and concentrations of amino acids during cell culture processes is critical for optimizing nutrient levels for better cell growth and improved product yield. Typically, a comprehensive analysis generally requires a combination of time consuming chromatographic separations and mass spectrometry (i.e. LC-MS). Recently, a unique new technology was developed by HORIBA called Absorption and Transmission fluorescence Excitation Emission Matrix (A-TEEM) that has found great use for offline profiling of free amino acid levels, which are important indicators for bioreactor condition. A-TEEM allows you to produce individual excitation and emission spectra for all fluorescent sample components while simultaneously also revealing information on components that absorbed light but did not fluoresce. Inherently, this technology like Raman provides a unique molecular fingerprint of your sample.

Yuichi Kitagawa[3] and co-workers illustrated the utility of A-TEEM to monitor several important components in CHO reactors that provide meaningful insight on the bioreactor condition. As shown in Figure 4, cell proliferation was monitored over 4 days, PARAllel FACtor analysis (PARAFAC) was used to resolve the various components/species in the EEM data. Based on their spectroscopic signature, the components were identified as tyrosine, Tryptophan, NAD(P)H and Riboflavin. From the 4 components identified, NAD(P)H experienced a steady increase as it is a known biomarker for changes in cell environment conditions.[4] The new A-TEEM system not only allows you to profile free amino acids in cell culture media but reduces time and cost in comparison to conventional methods.

Medical Diagnostic

The culprit behind a young child's allergic reaction to cow's milk is Alpha-Lactalbumin (LAC). Therefore, developing robust diagnostic platforms that can detect the presence of this allergen in blood can improve diagnosis. Critical criteria for a diagnostic platform are to achieve good sensitivity, reproducibility and specificity. All these criteria are highly influenced by choice of capture ligand. Surface Plasmon Resonance imaging (SPRi) is a technology that allows you to screen an array of ligands against a specific target in real time and label free. In addition to its screening capabilities the system enables you to obtain kinetic parameters of your sample. Recently, Dr. Renzo Vanna and co-workers[5] used the new SPRi system called XelPleX to screen a microarray of peptides with 3 different configurations (linear, branched and tandem) against LAC antibody (Figure 5).

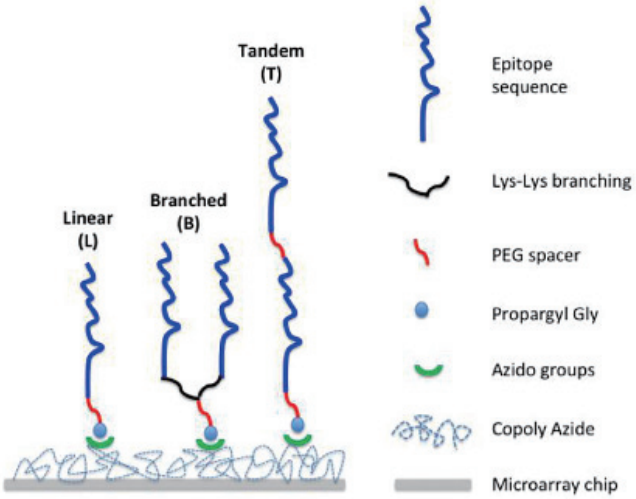

Figure 5 A schematic representation of the three different configuration of peptides (ligands) used on the microarray chip: linear (L), branched (B) and Tandem (T).[5] (Reproduced with permission from ref.5, Copyright 2017, Elsevier)

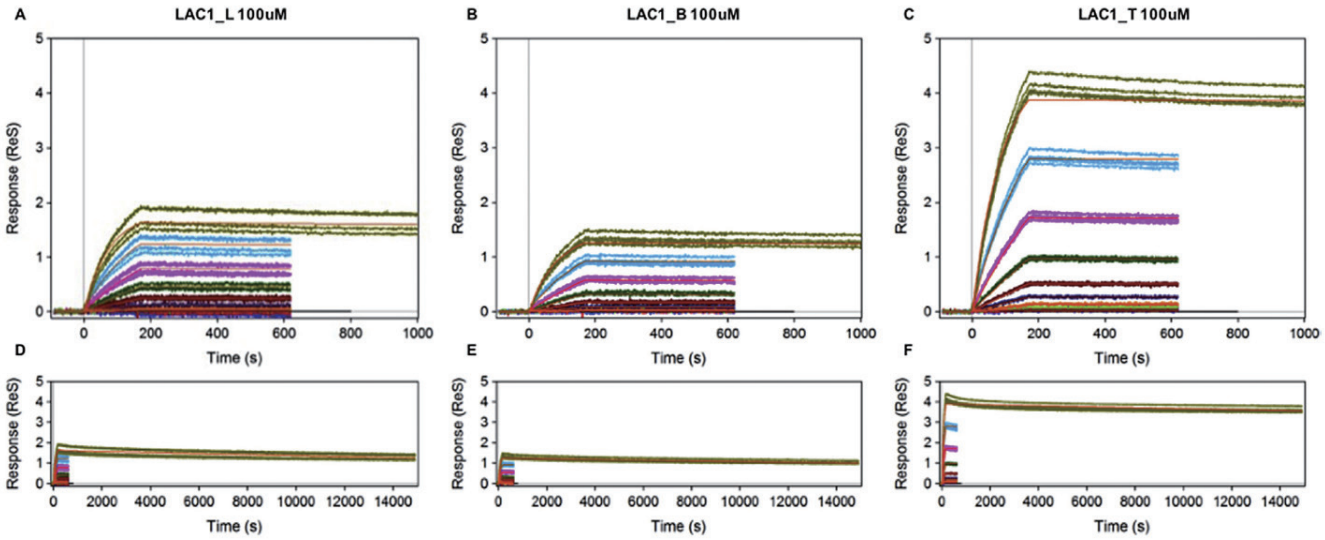

Figure 6 SPRi study of alpha lactalbumin (LAC) antibody binding to the microarray peptide pre-functionalized with various orientation of alpha lactalbumin IgE epitopes LAC1_L, LAC1_B, LAC1_T. The SPRi association (A-C; association rates were deduced from 3 min of association) and dissociation curves (D-F, dissociation rates were deduced from 4 h of dissociation) after the injection of 8 different concentrations (0.98-500 nM) of the antibody (colored plots).[5] (Reproduced with permission from ref.5, Copyright 2017, Elsevier)

It was found that the tandem configuration (Figure 6) was best at improving the binding capacity with respect to the linear and branched peptides. The reason for this was attributed to a slower dissociation phase leading to the conclusion that the tandem configuration experiences multivalent interactions (that is, the analyte binds to multiple peptides). This information cannot be attained by any traditional method like Enzyme-Linked ImmunoSorbent Assay (ELISA) because these other methods only provide you with binding affinity values. A SPRi system on the other hand allows you to gain insights into the mechanisms behind the affinity values as discussed above.

Conclusion

This article provides the reader a quick glance to our product solutions for real life problems in pharmaceutical and health sciences. The power of label-free technologies like Raman, SPRi and A-TEEM illustrate the added-value over competing technologies. The common thread in these technologies is the ability to reduce time and cost for the highlighted applications. The future for these technologies is promising and we foresee more growth and penetration in the Life Science market.

Figure 7

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