Application Note

Wyatt Technology Corporation

Characterization and Formulation Screening of mAb and Antibody-Drug Conjugates (ADCs) by High-throughput DLS

Aileen La, Ananda Seneviratne†, Gaya Ratnaswamy, Jihea Park

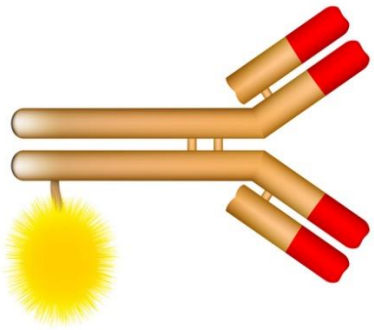
Agensys, Inc., an Affiliate of Astellas Pharma
1800 Stewart Street, Santa Monica, CA 90404

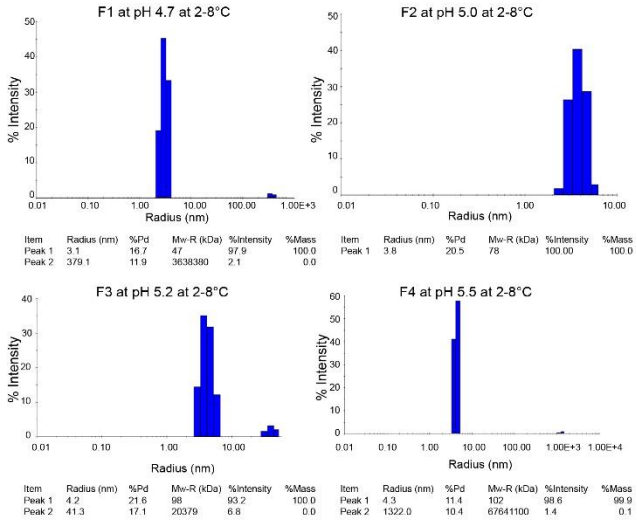
Summary

Antibody-Drug Conjugates (ADCs) are important biotherapeutic candidates that combine highly potent cytoxic drugs with monoclonal antibodies (mAb) for targeted drug delivery in the treatment of cancer or neurodegenerative disorders. However, while the underlying mAb may be a relatively stable molecule, the addition of the drug and linker often destabilizes the protein or adds undesirable intermolecular interactions, so that ADC biotherapeutics are heavily prone to aggregation. Uncontrolled aggregation can lead to a loss in clinical efficacy *in vivo* or, in extreme cases, invoke a serious immunogenic response. Monitoring stability during formulation is therefore essential to ensure that ADC compounds meet commercial, performance and safety targets.

This application note demonstrates how high-throughput dynamic light scattering (DLS) with automated well-plate sampling using the DynaPro Plate Reader and DYNAMICS software enables the rapid and reliable characterization of two ADCs based on monoclonal antibodies (mAb) IgG1 and IgG2. The results show that the attachment of small molecule drugs to the mAb does not appreciably change the molecule's average size (hydrodynamic radius, R_h). However, polydispersity slightly increases, indicating the presence of some high molecular weight species in this region.

Data are also presented to illustrate the use of DLS in formulation screening. Trend studies into pH, temperature and buffer variations show that the automated DynaPro Plate Reader is an effective tool for rapid, high-throughput biotherapeutic screen.





Monitoring how the hydrodynamic radius (R_h) of a protein changes under different formulation conditions with DLS is an essential aspect of stability studies.

†ananda.seneviratne@agensys.com

I. Introduction

Antibody Drug Conjugates (ADCs), also known as immunoconjugates, are a novel class of biotherapeutics which combine IgG1 and IgG2 monoclonal antibodies (mAb) with small molecular cytotoxic drugs, such as maytansines, auristatins, duocarmycin or calicheamicin. As of early 2015, two ADC-based drugs are currently available for targeted cancer treatment, with many more undergoing clinical trials.

ADC product development presents a complex formulation challenge in comparison to standard monoclonal antibody drugs. As more small-drug molecules are attached to the mAb base the conjugate becomes increasingly hydrophobic, which may compromise critical performance attributes such as solubility and physical stability. Monitoring and controlling the behavior of the ADC complex in formulation is therefore essential to ensuring the drug meets pharmacological, safety and commercial performance targets.

Dynamic Light Scattering (DLS) is one of the most effective techniques for submicron size analysis of proteins, their aggregates and other nanoparticles. DLS provides rapid measurements of hydrodynamic radius (R_h), degree of polydispersity, temperature of aggregation onset, and colloidal stability. Formulation screening with DLS helps developers rationalize variables such as temperature, pH or concentration in terms of their impact on stability, solubility or propensity to aggregate. Identifying the biological target and formulation conditions most likely to deliver ideal behavior during early phase screening accelerates the development process and greatly reduces the risk of downstream failure.

Traditional DLS measurements are performed in batch mode, making formulation analysis a time- and labor-intensive process. Advances in DLS technology have greatly increased the productivity of formulation screening while maintaining the high performance and accuracy demanded by industry, by performing automated, high-throughput DLS analysis *in situ* in standard 96-, 384- or 1536-well microtiter plates. In this study, the hydrodynamic radii and physical stability of samples of IgG1, IgG2 and ADCs based on those molecules were measured using the DynaPro Plate Reader II high-throughput DLS system under a variety of formulation conditions.

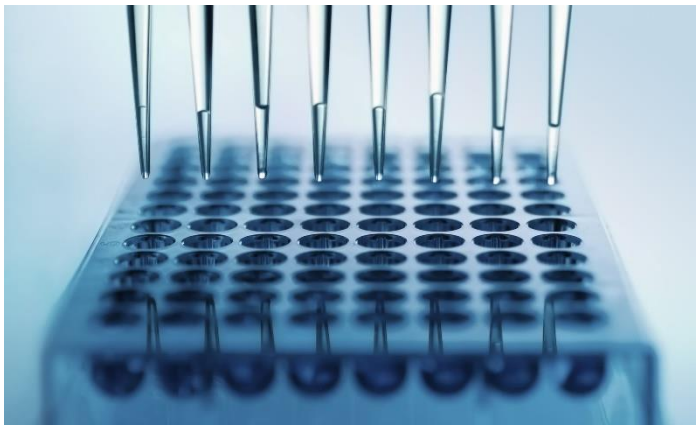


Figure 1. Automated DLS with the DynaPro Plate Reader II improves productivity in formulation stability studies.

II. Materials and Methods

Monoclonal antibodies (IgG1 and IgG2, expressed in two different cell lines) and antibody drug conjugates (IgG1-Drug 1 and IgG2-Drug 1) were characterized using DLS. The R_h, polydispersity (*%Pd*), and presence of any high molecular weight species in solution were measured. Regularization analysis was performed to determine R_h, *%Pd* and the relative mass of the fitted peaks.

A 20–70 µL sample volume was used at a concentration of 1.0 mg/mL. Prior to sample analysis, the well-plate was centrifuged at 3000 rpm for 1 minute to remove air bubbles. Each sample was measured in triplicate in which a single measurement consists of 10 acquisitions for 10 seconds at 25°C. The raw data (auto-correlation functions) was analyzed by cumulants analysis and/or regularization analysis. The distribution plots were obtained from regularization analysis.

Instrumentation

The data were acquired at 25°C using the temperature controlled DynaPro Plate Reader and DYNAMICS v7.1.0 software. A 384-well plate was used to enable automated, high throughout sample analysis.

III. Results and Discussion

Figure 2 shows the dispersion plots and histograms for mAb 1a and mAb 1b (IgG1) and mAb 2 and mAb 3 (IgG2). The results are tabulated in Table 1.

DLS analysis shows that that IgG1 and IgG2 have almost the same R_h while IgG2 has a greater degree of polydispersity than IgG1. This may be attributed to the two additional disulphide bonds present in IgG2 which often lead to greater heterogeneity.

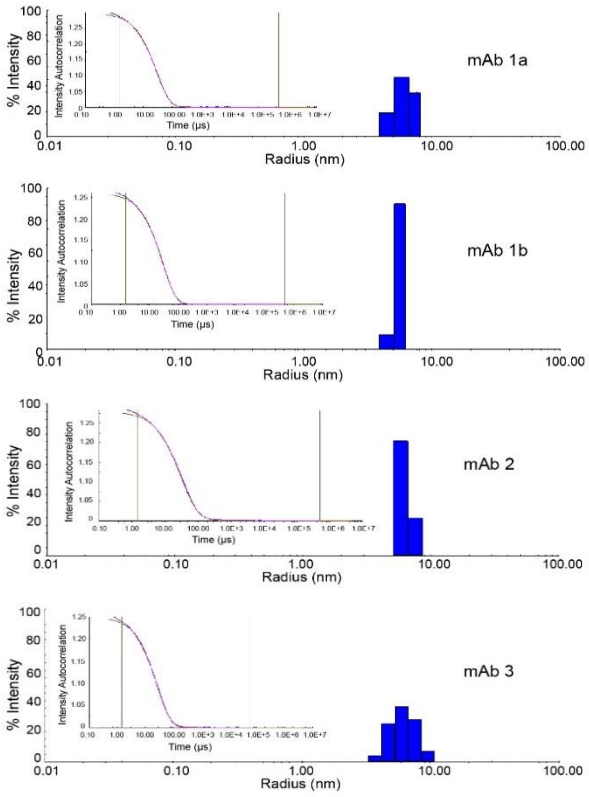


Figure 2. The autocorrelation functions and regularization histograms of mAb 1a, mAb1b (IgG1) vs. mAb 2 and mAb 3 (IgG2).

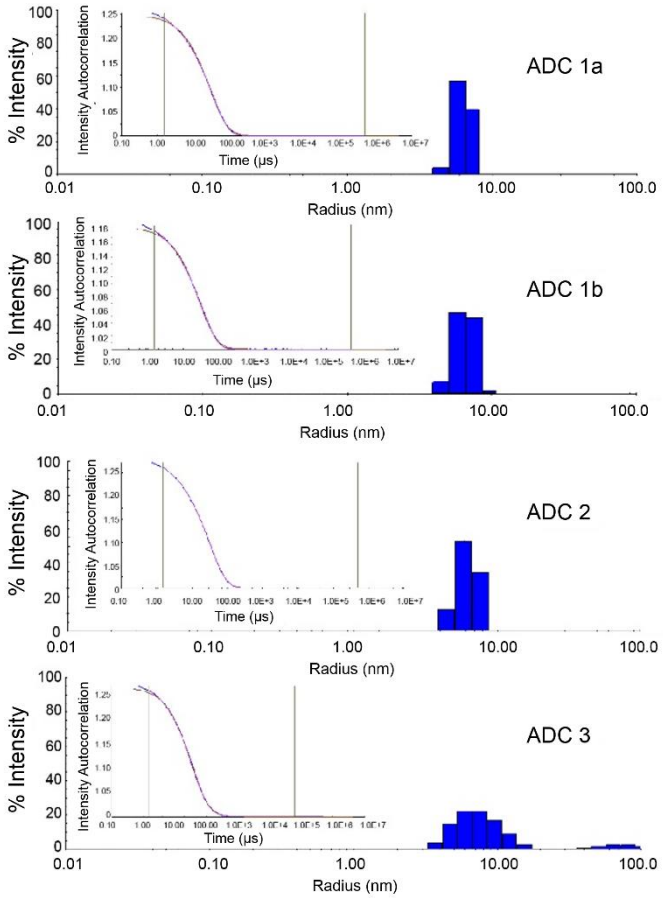


Figure 3. The autocorrelation functions and regularization histograms of ADC 1a, ADC1b (IgG1) vs. ADC2 and ADC3 (IgG2).

Table 2 and Figure 3 provide a comparison of corresponding IgG1 vs. IgG2 ADC in the recommended lead formulations. A sample exhibiting *%Pd* < 20% is generally considered to be monodisperse. Therefore, a measured level of polydispersity beyond 20% indicates heterogeneity within the sample. The data suggest that although attachment of the small molecule drug to the mAb does not change R_h, the ADC samples become more polydisperse than the mAb samples.

Table 1. The hydrodynamic radii and polydispersities of IgG1 vs. IgG2, calculated by two different methods.

	Type	mAb 1a IgG1	mAb 1b IgG1	mAb 2 IgG2	mAb 3 IgG2
Cumulants	**Average Radius (nm)**	5.3	5.3	5.6	5.0
	% PD	15.3	13.4	25.7	21.5
	Main Peak Radius (nm)	6.1	6.0	6.7	6.2
Regularization	**Main Peak Mass, %**	100.0	100.0	99.6	100.0
	Main % PD	21.6	18.0	31.6	27.3

Table 2. The DLS data of ADC: IgG1 (Mab1a-ADC and Mab1b-ADC) vs IgG2 (Mab2-ADC and Mab3-ADC).

	Type	mAb1a-ADC (ADC1a)	mAb1b-ADC (ADC1b)	mAb2-ADC (ADC2)	mAb3-ADC (ADC3)
Cumulants	**Average Radius (nm)**	5.6	7.2	5.2	6.5
	%Pd	15.7	30.8	23.5	34.5
	Main Peak Radius (nm)	6.5	7.4	6.9	8.0
Regularization	**Main Peak Mass, %**	99.4	99.6	100.0	100.0
	Main %Pd	21.6	18.0	32.3	33.9

Hydrophobicity also increases slightly, indicating the presence of some high molecular weight species.

Formulation Screening

Four mAb formulations were screened using automated DLS to determine their behavior under different conditions. Figure 4 shows how R_h and *%Pd* of the proteins vary as pH is incrementally increased while temperature remains within the range of 2-8°C. At pH 5.5 *%Pd* falls to 11.4 indicating that the sample is mostly monodisperse with less proteins present in the higher molecular weight region.

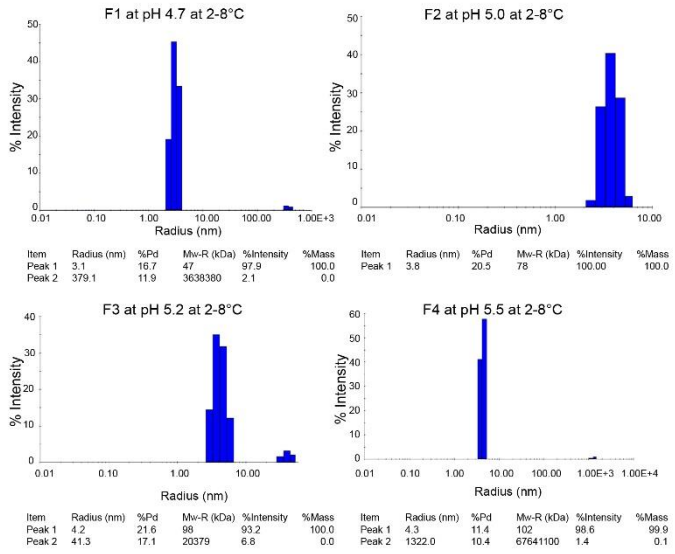


Figure 4. DLS Histograms of R_h for four mAb formulations at different pH values.

Figure 4 shows that R_h increases with pH. The shift can be attributed to increased electrostatic repulsion at higher pH. Additionally a temperature trend study shows that at pH 5.5 the antibodies are relatively stable with a slight increase in R_h around 40°C.

Finally, eight ADC formulations were analyzed in different histidine (F1-F5) and citrate (F6-F8) buffer solutions (Table 3). Figure 5 shows that proteins in three citrate formulations (F6-F8) are more polydispersed, with higher R_h than the histidine formulations. Overall, these data suggest that F5 at pH 5.3 with R_h of 6 nm and *%Pd* of 4.2% is the most stable formulation.

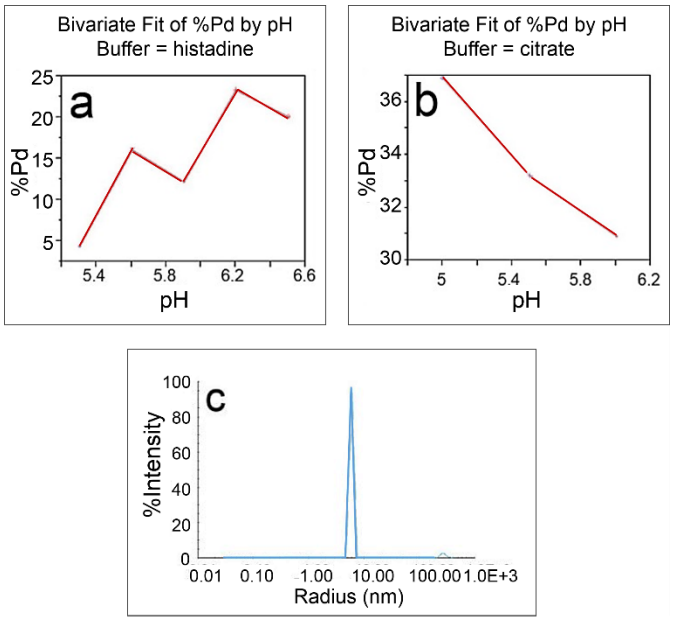


Figure 5. Formulation screening: *%Pd* of histidine formulations (a) vs. citrate formulations (b) and the histogram of the F5 formulation (c).

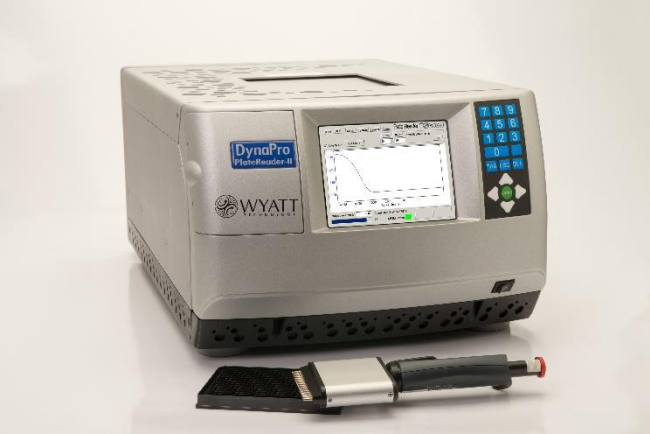
Table 3. DLS data of eight ADC formulations in Histidine (F1-F5) and citrate buffers (F6-F8). These data suggest that proteins in formulation F5 are the most stable.

Formulation ID	Buffer Type	pH	R_h	%Pd	%Mass
F1	histadine	6.5	5.985	20.0	99.8
F2	histadine	6.2	6.056	23.3	99.8
F3	histadine	5.9	5.526	12.1	99.9
F4	histadine	5.6	5.448	16.0	99.7
F5	histadine	5.3	5.931	4.2	99.6
F6	citrate	5.0	8.338	36.9	99.8
F7	citrate	5.5	8.084	33.2	99.8
F8	citrate	6.0	7.818	30.9	99.5

IV. Conclusions

DLS is a rapid and useful bioanalytical method for biotherapeutic characterization and formulation screening. By providing R_h and polydispersity measurements over a series of conditions, DLS enables developers to closely monitor and control the stability of their formulation throughout every stage of the development process. Additional studies may be carried out to determine the thermal and colloidal stability profiles of each candidate and buffer condition.

High-throughput screening of formulations and drug candidates is made possible by the DynaPro Plate Reader. This instrument reduces the labor intensity and cost of investigative trend studies through automation and its unique ability to carry out DLS measurements *in situ* in microtiter plates.



The DynaPro Plate Reader enables reliable testing of thousands of combinations of candidate biotherapeutics, excipients and buffer conditions in formulation studies.