Use of AUC in AAV Analysis in a GMP Setting

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The recent increase in the development of adenovirus associated virus (AAV) based gene therapies has created a large demand for the analysis of AAV-based drugs for the determination of percent empty and percent full capsid as part of drug substance and drug product quality control. The need for this analysis is triggered by fatalities encountered in early adenovirus-based gene therapy trials due to fatal immune responses.[1] Subsequent research focused on AAV-based therapies, which were known to provoke little or no immune response. Nevertheless, the early therapy failures are still a concern such that two recent guidances from the U.S. Food and Drug Administration require the need for controlling the presence of empty capsids as product related impurities to reduce the risk of (unnecessary) immune response.[2,3] AAV empty capsids, in addition to stimulation of innate and adaptive immune responses, may compete with full capsids for receptor binding on target cells, which could necessitate an increase in the required vector dose.[4]

Many analytical methodologies are available to determine the percent empty and percent full capsid species in a sample. The most common are: 1) anion exchange chromatography (AEX) exploring the difference in the isoelectric points between empty and full capsids; 2) size-exclusion chromatography (SEC) with ultraviolet (UV), dRI (differential refractive index) and multi-angle light scattering (MALS) detection determining the empty to full capsid ratios based on the monomer peak's average molecular weight and total concentration; 3) charge density mass spectrometry (CDMS) measuring the charge and mass-to-charge ratio of individual ions simultaneously, thereby allowing direct determination of the mass of empty, partially filled and full capsids, inclusive of their quantitation; and 4) sedimentation-velocity analytical ultracentrifugation (SV-AUC) using the sedimentation profiles of a sample subjected to centrifugal forces to quantify the relative content of empty, partially filled and full capsids. For the quantification of the content ratio, every method is subject to a tradeoff between throughput and resolution.[5] With CDMS methodology still being considered experimental[6] among high-resolution methods, AUC remains the only viable method capable of quantifying partially filled capsids, despite requiring substantial sample amounts.[7]

One drawback of AUC is the current lack of availability of instrumentation that could be used within a good manufacturing practice (GMP) setting of a commercial quality control laboratory. Consistent and reproducible routine AUC analysis has been made possible by the Optima AUC (Beckman Coulter) analytical ultracentrifuge. Nevertheless, the fact that the instrument operation software does not comply to GMP standards, and that data analysis is performed with software that has not been documented and validated for GMP use, complicates its application in a GMP setting. The approach chosen to address that shortcoming is described in this article.

Background

The PPD® Laboratory services GMP Lab employs Optima AUC instruments controlled by the AUC Experimental Portal (on-board) software. Of the two available AUC modes, sedimentation velocity (SV-AUC) and sedimentation equilibrium (SE AUC), only the former is currently used for analysis. The following workflow applies to the data analysis:

- Setup, analysis run and data collection on the AUC onboard computer.
- The acquired sedimentation velocity raw data are transferred from the AUC onboard computer to a processing station for analysis.
- SEDFIT software (freely distributed by the NIH) is used to extract sedimentation coefficient data and create a continuous sedimentation coefficient distribution plot, referred to as c(s).

- The distribution plot is imported into GUSSI software (freely distributed by UT Southwestern Medical Center) which determines the relative concentration of the different species (area under the curve) and the sedimentation coefficients for each species observed.

Each of these steps was subjected to a risk analysis to determine required mitigation for GMP analyses.

GMP Implementation

1. Optima AUC Onboard Computer – Use within GMP through Mitigation Strategies

The PostgreSQL database for storing the raw data constitutes an open-source object-relational database management system and thus poses a potential risk of unauthorized users accessing it and obtaining data (lack of cybersecurity). This risk can easily be mitigated by not connecting the AUC onboard PC directly to the internet, but rather connecting it to a secondary computer used for Intermediate data saving.

The Optima AUC onboard computer also does not provide security against unauthorized/authorized users. The system provides three access levels (user, data management and service) that all lack attributability. By allowing analysts only access to the user level and requiring analyst user access be subjected to secondary witness documentation, any acquired GMP data becomes attributable to fulfill the respective ALCOA+ principle. A similar approach is taken for the Optima v.1.11 Service engineer account, used by vendors only, which enables vendors to change the date, time, system configuration, instrument settings, and delete raw data. In addition, the vendor pin cannot be changed by PPD Laboratory services. To mitigate this access security risk, our support services personnel act as a secondary witness and document and oversee all vendor visits.

To further limit access to the data acquired on the AUC onboard computer, the linked intermediate data saving PC is minimally configured for the purpose of creating and saving AUC methods and downloading data for transfer to our data backup system. Access to this capability is limited to a designated group of users within a security group, which is comprised of our approved AUC analysts. Data downloading is also witnessed by a second analyst. Since this requires modify and write access to the folder on the intermediate data saving PC, a five-minute schedule to sweep the compressed raw data into a secure back-up location was implemented to protect against data deletion or modification. Data processing is only allowed on a secondary processing PC to which the raw data have been downloaded from the secure backup location. Secondary witnessing is applied to this process as well. To protect against deletion of processed data, the directory contents are swept in 30-minute intervals to a secure backup location.

Any risk mitigation regarding data security as well as secondary witnessing is governed by our standard operation procedure governing the operation, calibration and maintenance of the Optima AUC.

2. Data Rationale for Use of SEDFIT and GUSSI in AUC Analyses as a GMP Application

AUC is a classical (Svedberg and Rinde 1924[8]), yet contemporary (Uchimaya et al. 2016[9]) technique for the characterization of macromolecular and colloidal particles in solution. As described in numerous recent reviews, book chapters and monographs (see, for instance, Uchimaya et al. 2016; Schuck et al. 2016;[10] Patel et al. 2016),[11] AUC permits the separation of the components of a solute sample in the centrifugal field, as well as the characterization of their individual solution properties and, even, their interactions. The basis of the sedimentation velocity (SV) experiments is mathematically captured by the Lamm equation (Lamm, 1929),[12] which describes the sedimentation and diffusion of particles in a sector-shaped cell. The relationship between the solvent and physical properties of sedimenting particles is defined by the Svedberg equation. Analysis of AUC data can be performed with or without the application of modeling-based approaches to extract particle properties. A review by Edwards et al. 2020[13] listed the following available free software for use in SV-AUC: DCDT+, SVEDBERG, SEDANAL, SEDFIT and UltraScan III.

Of the listed software options, only SEDANAL, SEDFIT and UltraScan III provide a numerical approach to fitting the Lamm equation to the AUC raw data. SEDANAL analysis, as well as the approximate (non-numerical) SVEDBERG analysis require user-selected species models, for which, in our opinion, the impact of potential user bias on the final analysis results is not known. Only SEDFIT[14] and UltraScan III[15] (freely distributed by University of Texas Health Science Center) provide a continuous distribution analysis of species with different sedimentation coefficients, which represents the data desired by our clients. This can be either a proportion of empty and (genetic material) filled AAV capsids and their mean sedimentation coefficients or in the case of protein aggregate analysis their relative percentage and approximate molecular weights.

UltraScan III is a sophisticated AUC analysis software due to its advanced data analysis and extensive modeling capabilities, while the UltraScan LIMS system provides web-based access to the database and supercomputing functionality. To avoid web-based data analysis for client confidentiality (or other) reasons, UltraScan-in-a-box provides the means to run the AUC data analysis locally.

SEDFIT on the other hand, while not validated to cGMP standards (including IQ/OQ/PQ), is a software tool for the biophysical analysis of macromolecular assembly which would be considered a reliable source as it has been widely used by academic groups for more than 20 years and gained popularity in the industry in the last five years or so.

While SEDFIT provides the sedimentation distribution analysis raw data, it is used in conjunction with GUSSI,[16] which can illustrate the output of SEDFIT and perform simple calculations from the sedimentation velocity data. This includes areas under the curve analysis for the determination of relative distributions, like percent empty capsules.[17]

Our evaluation of these two software (UltraScan and SEDFIT/GUSSI)

resulted in the decision that SEDFIT/GUSSI use was the preferred route to establish AUC as a GMP analysis at our laboratory. In September 2023 the NIH group leading the development of SEDFIT came up with a solution to address the lack of GMP compliance of their software, introducing a command line interface that permits automated configuration of SEDFIT, data loading and retrieval of results.[18] By that time, we had already established a pathway to surround SEDFIT with a GMP environment that did not require programming in a secondary software (e.g., MATLAB from MathWorks).

3. SEDFIT and GUSSI – Use within GMP

SEDFIT and GUSSI are both open source, non-commercial, and community-supported software packages (i.e., there is no vendor/company ownership).

When using software without vendor ownership, there may be a lack of accountability and responsibility for the software's performance and reliability. Partial mitigation of this risk was achieved by downloading SEDFIT and GUSSI software specifically from https://sedfitsedphat.github.io/ and https://www.utsouthwestern.edu/research/core-facilities/mbr/software/, respectively (current available versions). Apart from this, further risk mitigation was achieved through version control of this software that is installed on the processing PC, and this version control process will be described within our standard operating procedure for this system. Since both software have no login credentials, Microsoft Active Directory groups were created to limit the access. The risk associated with the lack of different access levels and role-based controls in both software was mitigated by using an event log with Microsoft Event Viewer for each software, which adds an entry every time a user opens each program on its respective event log, documenting users who accessed the software and when. Appropriate use of the software and settings employed is controlled through screenshots assembled in a Word document that is printed as a pdf to a data vault. This also allows 100% secondary review of the analysis and the raw data within our GMP lab.

Calculations in the SEDFIT software are highly complex and cannot be independently verified. The risk associated with the inability to independently verify SEDFIT software calculations cannot be fully mitigated but is justifiable as outlined below.

GUSSI allows the sedimentation distribution plot data to be exported as a .dat file, which can be read into Excel to re-create the plots but Excel cannot reproduce the area under the curve results. The .dat file format could be converted into a .cdf file for import into Water's Empower software, which is able to confirm area under the curve calculations, but the conversion of .dat files to .cdf files would have to employ an uncontrolled open-source conversion program from the web, which is not a desirable pathway in a regulated environment. Therefore, the relative concentration of the different species (area under the curve) and the sedimentation coefficient for each species calculated by GUSSI can also not be independently verified, posing an additional quality risk.

We concluded that there is negligible risk associated with the use of the processing applications associated with the AUC implementation under GMP for which the calculations cannot be independently verified. The following literature research is provided in support of this assessment:

Garcia de la Torre, et al (2018)[19] published an article on the development of prediction software for the analysis analytical ultracentrifugation experiments in which the goodness of the prediction software was assessed by the results' proximity to the experimental results obtained using SEDFIT. While this assessment was performed using SEDFIT version 15.01b of 2015, we operate SEDFIT version 16.1-c of 2019, which can reasonably be considered an improved version. If there would be any doubt on the accuracy and precision of the SEDFIT results, it can be expected that a different analysis software would be employed for the prediction software assessment.

According to an article entitled "Reincarnation of the Analytical Ultracentrifuge: Emerging Opportunities for Nanomedicine,"[20] SEDFIT and recently intensively developed UltraScan are some of the most popular software for data analysis. While popularity is not a measure of precision and accuracy of a software, it is unlikely the scientific community would stick with a software that could lead to false results.

In a recent study performed in Japan,[21] SEDFIT and GUSSI were employed to determine empty and full capsid ratios of different AAV serotypes and compare results to those obtained based on A260 nm / A280 nm absorbance ratios. Such a study would be meaningless if the SEDFIT/GUSSI data could not be considered reliable.

In a commentary published by the NIH and the University of Texas Southwestern Medical Center[22] to supplement previous AUC protocols, the authors recommend the SEDFIT software for the analysis of AUC data.

With these appropriate controls in place at PPD Laboratory services, the FDA has allowed one of our clients to use the Optima AUC with SEDFIT and GUSSI to perform one of the GMP release tests for their commercialized drug product. Several other companies are evaluating AUC/SEDFIT use for AAV analyses, such as Genzyme,[23] AstraZeneca,[24] and Adverum (ACS Webinar on 20Sep2022). Genzyme Corporation filed a patent application for the use of AUC in the characterization of recombinant viral particles that included the use of SEDFIT.[25]

During the past year, our experience with the SEDFIT and GUSSI has been a fairly consistent end result for determining sedimentation coefficients of species and their continuous distribution plots (SEDFIT) as well as the final percent empty, partially filled and full capsids results determined by GUSSI, despite visual differences seen in the distribution plots. This supports the robustness of the software in determining the reportable results although the calculations cannot be independently confirmed.

Furthermore, Edwards et al.13 found that analysis using analogous UltraScan III or SEDFIT approaches results in nearly identical descriptions of the sedimenting systems. While we cannot verify calculations used in the processing applications (SEDFIT and GUSSI) associated with AUC, the consensus in the scientific community supports that the calculations are reliable.

Conclusion

While there is risk associated with using an instrument and associated software without proper audit trails and for which the calculations cannot be independently verified, the previous summary allows that risk to be considered low in lieu of the wide applicability of the entire AUC software suite in research and industry. IT departments have a set of tools that mitigate software limitations, and the four eyes principle (where two individuals are involved in the review and approval of critical processes or data) can be applied in instances where such tools are not available. To further ensure that instrument and software performance issues do not impact any GMP data, method-performance-specific controls can be established during method qualification and/or validation. The summary of all control data acquired can be used to establish a range for the reportable parameters that the control/reference standard has to meet as part of the method's system suitability. For example, in the analysis of AAV capsids, the percent empty and full capsid values for the control as well as the associated sedimentation coefficients must meet the method specific system suitability ranges for an analysis to be valid. This enables the detection of software-induced inaccuracies.

By the time this article is published, new developments may streamline GMP adaptations of AUC. For example, Alexander E. Yarawsky et al. recently published a paper under the guidance of Lake N. Paul that describes a new program, "BASIS," which allows for 21 CFR Part 11-compliant data handling and data analysis using the SEDFIT analysis software.[26] We are looking forward to learning about BASIS and other future developments in the rapidly evolving and exciting area of AUC.

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