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MONITORING BY RAMAN SPECTROSCOPY OF RABIES VIRUS-LIKE PARTICLES PRODUCTION IN BIOREACTOR

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ABSTRACT

This study aimed to establish chemometric models using Raman spectroscopy data for biochemical monitoring of rabies Virus-Like Particles (VLP) production based on baculovirus/insect cell system. The models were developed using samples from Schott culture flasks. The following modeling techniques were assessed: Partial Least Squares (PLS) and Artificial Neural Networks (ANN). The applicability of the models was evaluated using experimental data from assays carried out in a benchtop bioreactor. The choice of spectral filtering has a major impact on the prediction accuracy of chemometric models. The optimal filtering approach should be individually optimized for each biochemical parameter. This study showed that Artificial Neural Network (ANN) models were more effective than Partial Least Squares (PLS) for biochemical monitoring of glucose and glutamine in Sf9 cells cultures in Schott and bioreactor for rabies VLP production. Both techniques were not very suitable for modeling viable cell concentration and viability.

Keywords: artificial neural network; bioprocess monitoring; partial least squares; rabies vaccines.

1 INTRODUCTION

The biopharmaceutical industry faces challenges in controlling critical parameters in bioprocesses due to the complexity of chemical, physical, and biological variables. Process Analytical Technology (PAT) enables real-time monitoring of cell culture processes using sensors or spectroscopic probes, making process monitoring more efficient and cost-effective from early development to large-scale production[1]. PAT is essential for production control, ensuring product quality and increasing yield. Spectroscopy, particularly UV/Vis, NIR, IR, and Raman spectroscopy, is widely used for non-invasive, rapid chemical analyses in bioprocesses.

Raman spectroscopy, a key tool in PAT, is valued for its specificity, compatibility with aqueous systems, and flexibility, making it ideal for monitoring and controlling upstream bioprocess stages[2]. It provides critical data on nutrients, metabolites, cell density, and viability in bioreactors, as well as downstream parameters like glycosylation and product concentration. Chemometric modeling of Raman data allows for the quantification of specific analytes and correlation of spectral changes with process parameters[3]. Techniques like Partial Least Squares (PLS)[4], Principal Components Regression (PCR), and Artificial Neural Networks (ANN)[5] are used for these analyses, providing high prediction and calibration accuracy.

The study addresses rabies virus (RABV), causing over 60,000 deaths annually[6]. Current vaccines are expensive and inaccessible in developing countries. Virus-like particles (VLPs), mimicking viral structures without genetic material, offer a promising alternative. Baculovirus systems in insect cells produce VLPs with high yield and correct protein conformation, though they also risk baculovirus contamination.[7]

Raman spectroscopy has proven useful in monitoring VLP production, including predicting glucose concentrations and cell density in 293F cell lines for HIV vaccines[8] and monitoring rabies VLPs from insect cell-baculovirus systems. This study aimed to develop chemometric models using Raman data for monitoring rabies VLP production, employing techniques like PLS and ANN. It assessed the impact of spectral filtering and validated the models with experimental data from a benchtop bioreactor.

2 MATERIAL & METHODS

As a host for baculovirus propagation and VLP production, Sf9 cells in suspension (ATCC 1711) cultured in SF900III serum-free medium (Thermo Fischer Scientific, USA) were used. For cell transfection assays, Sf9 cells were grown in a monolayer on the surface of a 25 cm^2 culture flask (Corning Inc.™, Corning, NY, USA). Starting with thawed cells, three passages were performed to obtain the inoculum for the bioreactor, with cell density initially at 0.5–1 × 10^6 cells/ml in 100 mL shake flasks (Schott AG™, Germany).

Four batch experiments were carried out in a 2 L Bioflo 110 bioreactor (New Brunswick Scientific, Edison, NJ) at 28 °C, with a marine propeller impeller at 80 rpm, with a working volume of 1 L[9]. The composition of the inlet gas mixture was changed, as the dissolved oxygen was controlled at 30% saturation. The gas flow was 200 mL/min. The Bioflo 110 was connected to a computer

with homemade software implemented in the LabVIEW programming language software (National Instruments, Texas, USA), capable of recording, in real-time, the variables pH, temperature, stirring speed, and dissolved oxygen tension (DOT, percentage air saturation). The bioreaction time in all experiments was around 120 h. Samples for cell density and viability, metabolism monitoring, recombinant baculovirus titration, as well as immunochemical characterization of virus-like particles were taken up to three times a day. Viable cell density (Xv) and Cell viability (CV) were determined using a Neubauer counting chamber with phosphate-buffered saline (PBS). Cell viability was quantified by trypan blue exclusion assay. Glucose and glutamine concentrations were measured using a YSI 2950D-3 Biochemistry Analyzer (YSI Life Sciences, Yellow Springs, OH, USA).

Offline Raman spectra were captured with a stainless-steel immersion probe (12.7 mm in diameter) with a sapphire window and used one of the four channels of the multichannel RXN2 Raman spectrometer (Kaiser Optical Systems Inc., KOSI, Ann Arbor, MI, USA) equipped with a 785 nm laser source (around 200 mW of sample power).[10]

Modeling of Spectral Data Partial least squares (PLS) and artificial neural networks (ANN) were used to develop predictive models from offline spectral data. Spectral filtering and Principal Component Analysis (PCA) were applied to improve prediction accuracy. Partial Least Squares (PLS) models were generated using SIMCA 17 software (Sartorius, Umeå, Sweden). Data were split for calibration and validation. Model selection was based on the lowest Root Mean Square Error from the cross-validation (RMSEcv) (Equation 1), where $\hat{y}_i$ is the estimated value by the regression model, y_i is the reference value and n is the number of samples in the calibration set. Artificial Neural Network (ANN) models used a multilayer perceptron architecture with one hidden layer. Various activation functions were tested. The best models were selected based on predictive capacity and analyzed using ANOVA and Tukey's test. The best model for each biochemical parameter was determined by contrast of the PLS and ANN absolute errors through a one-tailed T-test for two mean group comparison with 95% significance level ($\alpha = 0.05$). The best PLS and ANN models from Schott flask experiments were used to predict biochemical parameters in bioreactor samples. Predicted and observed values were correlated, and corrections were applied to assess prediction quality.

$$\mathrm{RMSEcv} = \sqrt{\sum_{i=1}^{n} \frac{(\hat{y}_i - y_i)^2}{n}} \quad (1)$$

3 RESULTS & DISCUSSION

Non-infected and infected Sf9 cells were grown in SF900 III culture medium in Schott flasks and in a bioreactor. Raman spectra were taken from samples, in parallel with standard sample analysis. The spectra were pre-processed and used for modelling the cultivation through PLS and ANN techniques.

For the Partial Least Squares regression (PLS) technique, the best models were selected based on the lowest RMSEcv values using SIMCA software. Previous studies on CHO cell culture using PLS and spectroscopy showed RMSEcv results comparable to the current study[11]. For example, glucose had an RMSEcv of 0.89 g/L in bioreactors of various scales, closely matching the results from the in-line sample database.

The number of principal components (PC) generated by PCA varied. Depending on the spectral range and pre-processing approach, the number of components sometimes exceeded 100. The results of the Artificial Neural Network (ANN) technique showed significant differences for almost all parameters except glucose, as indicated by ANOVA and Tukey's test.

Table 1 compares the Absolute Error for the best models obtained using PLS and ANN techniques for each parameter. The t-test revealed a statistically significant difference for parameters Xv, CV, with lower errors for the ANN technique. The errors for the best ANN models for Xv and Gln are similar to or lower than those reported in previous studies on CHO cell lines, indicating that ANN models generally perform better for most parameters in experiments using Schott flasks.

Table 1 Comparison between the absolute errors of the predictions generated by the best models using PLS and ANN regression techniques for different parameters in the bioreactor.

Parameter	PLS (Partial Least Squares regression)	ANN (Artificial Neural Network)
Xv (× 10^6 cells/mL)	1.50	1.02
CV (%)	70	46
Gluc (g/L)	1.24	1.30
Gln (g/L)	0.20	0.20

To evaluate the predictive power of PLS and ANN regression techniques for scaling up from Schott flasks to bioreactors, simulations compared the predictions of these two platforms for each parameter. The best models from Schott flasks were used for the simulations (Figure 1). Except for XV in PLS, all parameters exhibited lower mean absolute error (MAE) for Schott flask predictions. Parameters with the lowest errors, showed statistically significant differences in MAE. Comparing MAE from Table 1 with previous studies on CHO cells, errors for Xv, CV and Gln were similar, particularly for ANN models. However, for Glc, neither regression technique efficiently predicted scaling from Schott flasks to bioreactors. Previous studies confirmed that Schott flasks and bioreactors are equivalent under specific conditions for recombinant baculovirus production and rabies VLPs, although hydrodynamic and chemical differences affect viral stability, making Schott flasks suitable for parameter optimization in such experiments.

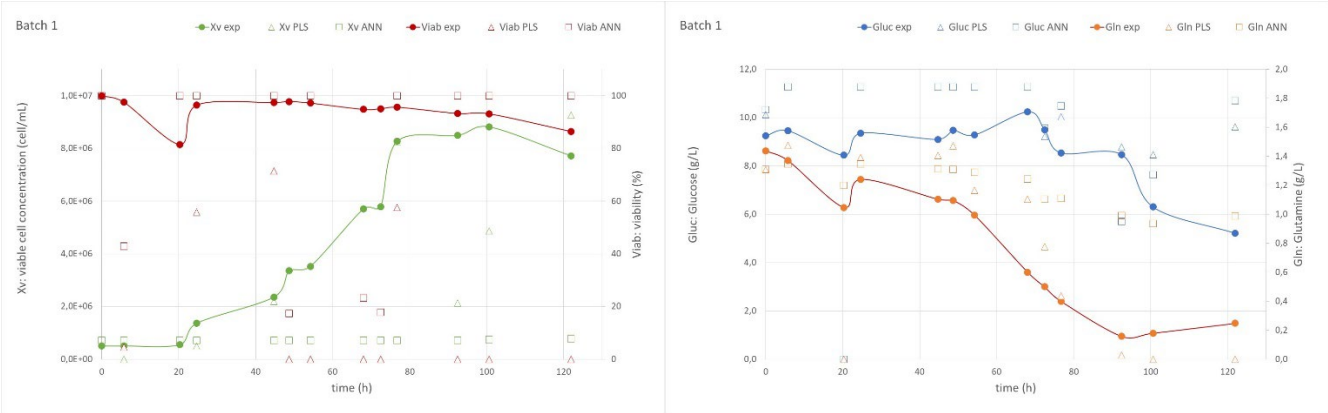

Figure 1 Experimental (exp) and simulated values by Partial Least Squares regression (PLS) and Artificial Neural Network (ANN) of viable cell concentration (Xv), viability (Viab), Glucose (Gluc) and Glutamine (Gln) concentration of run Batch 1.

4 CONCLUSION

This study showed that Artificial Neural Network (ANN) models were more effective than Partial Least Squares (PLS) for biochemical monitoring of glucose and glutamine in Sf9 cells cultures in Schott and bioreactor for rabies VLP production. Both techniques were not very suitable for modeling viable cell concentration and viability.

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