Viral Vector Production

Dielectric spectroscopy (DS), also known as capacitance measurement, has been used as a PAT tool for viral vector production processes (lentivirus, adenovirus, reovirus, influenza, baculovirus) with mammalian and insect cells. It has been viewed as an important advancement for viral process control for continuous virus production processes.

Numerous groups have explored this technology to monitor live cell density, identify critical events and control the process. The benefits of using capacitance technology in these applications are multifold. We summarize these benefits here, that have been supported by peer-reviewed published empirical evidence, references for which have also been provided above. Considering capacitance measurement correlates with the live cell density (or more accurately, viable biovolume) profile of the culture, it provides a complete, real time and detailed picture of all of the critical events, as described below. This leads to better process monitoring, automation and control and in turn, improved productivity.

Cells: Sf-9
Viral vector: Baculovirus
Publication using capacitance measurement:
Zeiser, A., Elias, C.B., Voyer, R., Jardin, B. and Kamen, A.A., 2000. On-line monitoring of physiological parameters of insect cell cultures during the growth and infection process. Biotechnology progress, 16(5), pp.803-808.

Cells: HEK-293
Viral vector: Lentiviral
Publication using capacitance measurement:
Ansorge, S., Lanthier, S., Transfiguracion, J., Henry, O. and Kamen, A., 2011. Monitoring lentiviral vector production kinetics using online permittivity measurements. Biochemical engineering journal, 54(1), pp.16-25.

Cells: HEK-293
Viral vector: AAV
Publication using capacitance measurement:
Lesch, H.P., Heikkilä, K.M., Lipponen, E.M., Valonen, P., Müller, A., Räsänen, E., Tuunanen, T., Hassinen, M.M., Parker, N., Karhinen, M. and Shaw, R., 2015. Process development of adenoviral vector production in fixed bed bioreactor: from bench to commercial scale. Human gene therapy, 26(8), pp.560-571.

Kamen, A. and Henry, O., 2004. Development and optimization of an adenovirus production process. The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications, 6(S1), pp.S184-S192.

Henry, O., Dormond, E., Perrier, M. and Kamen, A., 2004. Insights into adenoviral vector production kinetics in acoustic filter-based perfusion cultures. Biotechnology and bioengineering, 86(7), pp.765-774.

Cells: HEK293 and Sf-9
Viral vector: reovirus, influenza, lentivirus and baculovirus
Publication using capacitance measurement:
Petiot, E., Ansorge, S., Rosa-Calatrava, M. and Kamen, A., 2017. Critical phases of viral production processes monitored by capacitance. Journal of biotechnology, 242, pp.19-29.

Cells: HEK293
Viral vector: influenza and lentivirus
Publication using capacitance measurement:
Emma, P. and Kamen, A., 2013. Real-time monitoring of influenza virus production kinetics in HEK293 cell cultures. Biotechnology progress, 29(1), pp.275-284.

Cells: SF9
Viral vector: recombinant adeno associated vectors
Publication using capacitance measurement:
Negrete, A., Esteban, G. and Kotin, R.M., 2007. Process optimization of large-scale production of recombinant adeno-associated vectors using dielectric spectroscopy. Applied microbiology and biotechnology, 76(4), pp.761-772.

Cells: Proprietary Human Cell Line
Viral vector: Adenovirus
Publication using capacitance measurement:
Monica, T.J., Montgomery, T., Ayala, J.L., Schoofs, G.M., Whiteley, E.M., Roth, G., Garbutt, J.J., Harvey, S. and Castillo, F.J., 2000. Monitoring adenovirus infections with on-line and off-line methods. Biotechnology progress, 16(5), pp.866-871.

Cells: Vero
Viral vector: Measles virus
Publication using capacitance measurement:
Grein, T.A., Loewe, D., Dieken, H., Salzig, D., Weidner, T. and Czermak, P., 2018. High titer oncolytic measles virus production process by integration of dielectric spectroscopy as online monitoring system. Biotechnology and bioengineering, 115(5), pp.1186-1194.

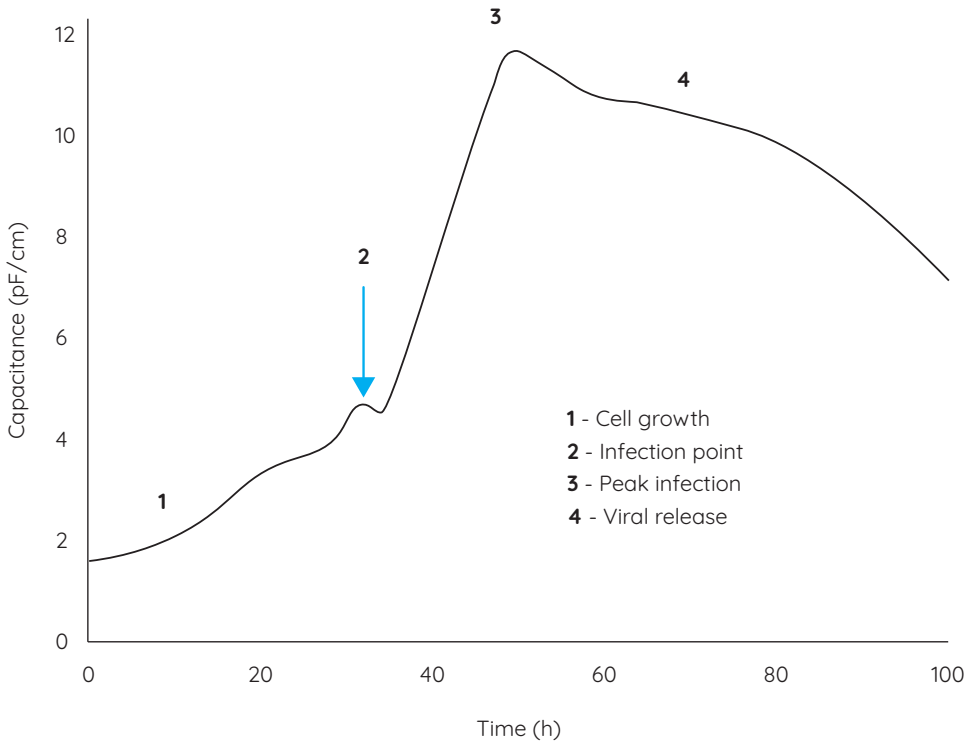
Monitoring growth of mammalian & insect cells

Capacitance measurement has been used to monitor the growth of mammalian (e.g. HEK293, Vero) or insect cells (Sf-9) in culture to get a real time fingerprint of the process. It is also common to grow mammalian cells on microcarriers, prior to infection. This technology is only sensitive to the presence of live cells and does not measure dead cells or microcarriers, hence tracking cell growth successfully.

Identification of successful infection and peak infection point

Several studies have shown that measurement of the capacitance of the cell culture was a consistent indicator of successful cell infection due to the increase in cell volume following viral DNA replication. Capacitance functions as a real time key performance indicator to confirm successful infection.

In addition, capacitance clearly identifies peak infection point. There is evidence indicating that the optimum harvest time is dependent on the peak infection point. Real time and reliable knowledge of peak infection point leads to improved process efficiency.



Identification of cell lysis and viral protein release

Following peak infection point, the viral particles lyse the cells and are released into the suspension. Capacitance measurement is able to track this cell death reliably and in real time, since the technology only measures live cells with intact membranes.

Identification of optimum harvest time

The optimal time of harvest can drift significantly in identical bioreactors, even with the same biologicals, and running with the same parameters. The harvest time is crucial for the final yield and quality of the recombinant product, particularly for proteins requiring posttranslational modification. The information on the viable cell concentration obtained from the capacitance profile can be used to determine optimum harvest time and hence contributes towards improved consistency and productivity.

Viral titer consistency and improving productivity

The viral titer decreases dramatically after the optimal time of harvest. Capacitance can be used to determine different times of harvest for each run. Therefore, using capacitance, consistent virus titers can be guaranteed even when the process changes. In a particular study, compared to an uncontrolled virus production process, the integration of DS increased the maximum virus concentration by more than three orders of magnitude. Integration of DS allows for enough virus production to provide one dose for one patient in one bioreactor with a working volume of 500 ml, compared to the 20 L required before.

Utilizing additional parameters to identify critical events

Several studies have utilized the entire capacitance frequency spectrum to estimate additional parameters such as critical frequency (fc). These frequency scanning parameters have been utilized to successfully correlate with critical time points during the viral production process including infection, protein synthesis, viral assembly, budding and release using frequency scanning. For instance, a synchronous peak in critical frequency appears during viral release, this is more pronounced in larger cell volumes. Critical frequency is also a critical parameter to monitor the initiation of cell death in viral production processes and can be used as a tool for

For further technical information please contact:
support@aberinstruments.com
or alternatively contact sales@aberinstruments.com