How capacitance measurement can improve viral vector and virus-based vaccine production

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How Capacitance Measurement Can Improve Viral Vector and Virus-Based Vaccine Production

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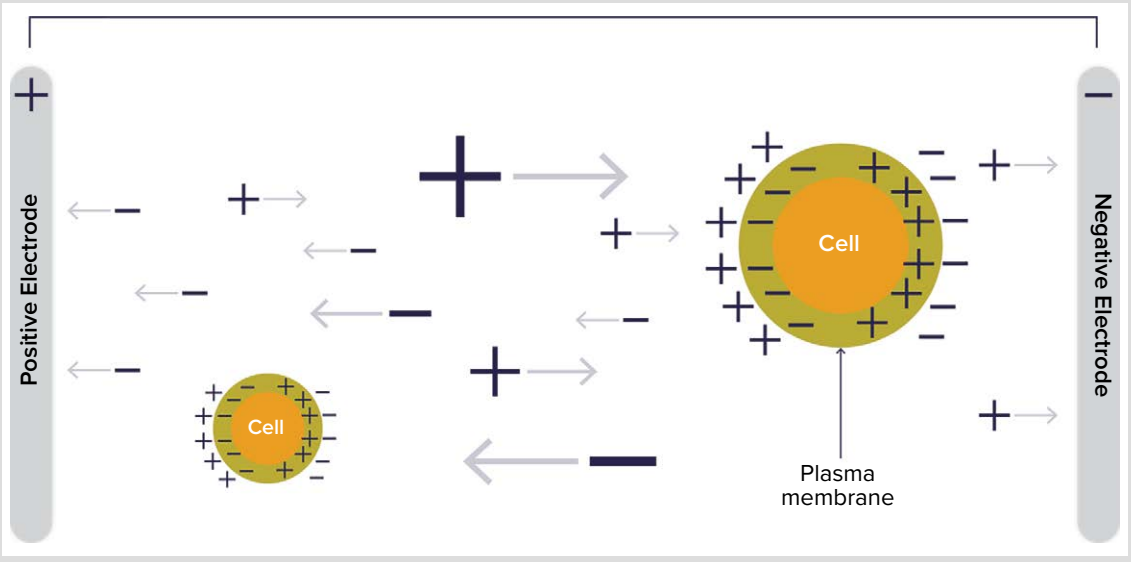
According to recent market analysis, the worldwide vaccine market generated approximately US$33 billion in revenue and was growing at a rate of around 5–7% annually in 2019 (**1**). Many vaccines cited in that report are used to prevent diseases caused by viruses such as influenza, measles, and human papillomavirus (HPV). In 2020, the coronavirus pandemic introduced and accelerated unprecedented development of a viral vaccine to prevent COVID-19, and the results are set to generate billions of dollars in revenue for the biopharmaceutical industry in 2021 and beyond. That is increasing development of viral vectors and virus-based vaccines. For example, emergency-approved COVID-19 vaccines from Oxford University with AstraZeneca and from Johnson & Johnson — as well as a promising candidate from CanSino Biologics — all are based on adenoassociated virus (AAV) viral-vector platforms (**2–4**).

However, manufacturing and scale-up of viral vector and virus-based vaccines can be fraught with difficulty (**5**) and complicated further if intensified cell culture is used for production (**6**). Complexities include being able to produce a reliable in-process fingerprint of cell density and size and finding the optimal times for culture infection and virus harvest. Determining those process parameters can help biomanufacturers maintain critical quality attributes (CQAs) of a vaccine while minimizing downstream process steps and associated costs and timelines. Incorrect harvest times can yield excessive amounts of cell debris that require more expensive clarification and purification. The upstream scale-up strategy of viral vectors can be challenging because processes that work at small scale might require optimization in shake flasks or minibioreactor systems and several engineering runs to optimize cell culture for production of viral vector or virus-based vaccines. That process can be costly in both time and resources, and the manufacturing scale can be expensive in use of media, buffers, and reagents.

Therefore, it is crucial to find a method that will monitor cells not just for the culture density, but also for their size to determine when a culture has been infected successfully. Additionally, it is important to use a technology that serves as an in-line process analytical technology (PAT) that enables scientists to monitor and control a process without using at-line or off-line sampling. At-line sampling is not desirable for viral-infection monitoring of cells because it reduces bioreactor volumes. Thus, samples can be taken only at intervals of 12 to 24 hours, so analyses cannot produce a detailed process fingerprint or help to provide timely data feedback for control purposes. At- and off-line sampling techniques also are not ideal because they require scientists to remove samples from a bioreactor, which creates the potential risk for contaminating the bioreactor during either manual or automated liquid handling.

The main benefit of using a noninvasive in-line sampling technique is that it could produce a signal every few seconds, enabling scientists to produce detailed fingerprints of critical process parameters (CPPs) and key process indicators (KPIs) that can be used for automated feedback control. Additionally, if such a fingerprint is produced for a "golden batch," then it could be used to determine more rapidly when CPPs and KPIs begin to behave outside of

Figure 1: Live cells acting as capacitors under the influence of an electrical field



process specifications and thus could help operators troubleshoot a production run while it is in progress.

Below, I review experimental data showing how an in-line technology for capacitance measurement — also known as *dielectric spectroscopy* (DS) or *radiofrequency (RF) impedance spectroscopy* — can address many process-monitoring challenges associated with cell culture production of viral vectors and virus-based vaccines.

Capacitance for Monitoring Cells

Principle of Capacitance Measurement: A cell in suspension culture has a nonconducting, bilayered outer membrane that is impermeable to ions. The medium in which the cell grows is a suspension of ions, so under the influence of an electric field, the cells become polarized (Figure 1). If a cell is alive and its membrane intact, then it will act as a capacitor to store electrical energy.

As cell numbers and culture volumes increase, so does the number of polarized cell membranes, which in turn increases the capacitance. Thus, the capacitance of a cell suspension at one or more frequencies is directly proportional to the total membrane-bound volume of the cells. Because dead cells have leaky cell membranes, and solid particles and gas bubbles in the medium have no cell membranes, those cannot store electrical charge and will not contribute to capacitance in a cell suspension.

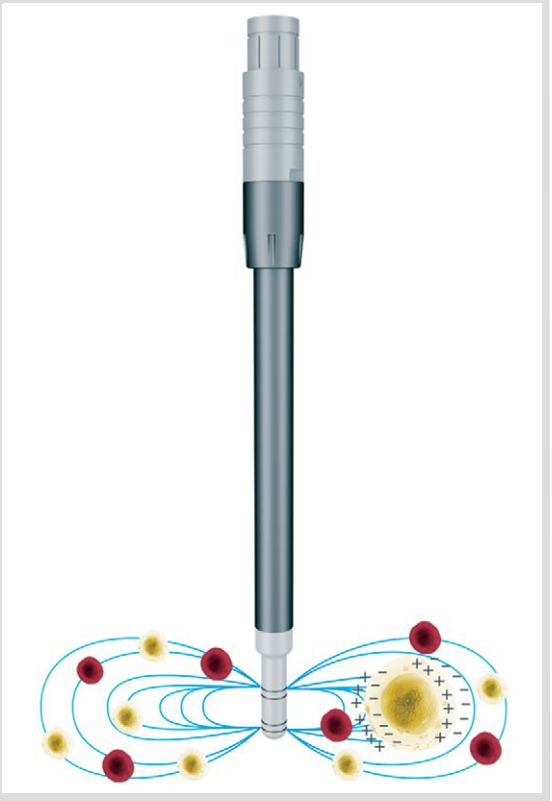
Capacitance in a cell culture can be created and measured using technology that was invented and patented in 1988 (**7**). This technology has been developed and commercialized by Aber Instruments (Aberystwyth, UK). The instrument consists of a probe with an inner and outer electrode along with associated electronics to enable data acquisition (Figure 2). The outer electrode creates a field of approximately 30–40 mm in the RF spectrum (between 50 kHz and 20 MHz). A sensor measuring voltage in pico-Farads (pF)/cm can be integrated into a bioreactor control unit to capture real-time data continuously for analysis.

CAPACITANCE measurement has been used extensively in biopharmaceutical development since the 1990s.

Capacitance measurement has been used extensively in biopharmaceutical development since the 1990s. It has become a standard technology for monitoring cell cultures in research and process development laboratories through to manufacturing-scale facilities at major biopharmaceutical companies and contract development and manufacturing organizations (CDMOs) worldwide.

The technology comes in reusable sizes and types that can be used with systems ranging from small glass bioreactors to larger stainless-steel vessels. Sensors are available for use in pilot- and manufacturing-scale stainless-steel bioreactors from all major life-science suppliers. Additionally, BioPAT ViaMass single-use sensors are integrated fully into Sartorius Flexsafe bags for single use in STR stirred-

Figure 2: Aber electrodes creating an electrical field and measuring capacitance of live cells



tank vessels (50–2,000 L) and rocking motion bioreactors up to 200 L. Those systems have been available through a Sartorius collaboration with Aber since 2013 (**8**).

Monitoring Viral Production

Capacitance measurement is regarded as the most accurate on-line method to monitor live-cell density in mammalian cell cultures (**9**), but it also can be used to monitor viral vaccine production. When virus-infected cells are monitored with capacitance, they demonstrate a typical growth curve (Figure 3).

To begin with, capacitance measurements can be used to monitor the growth of the host cells in real time. That allows for automated identification of the optimum infection point. When cells are infected, their size increases initially, which is shown by a sharp increase in capacitance. When they reach maximum capacitance, they are at the peak of infection and thus their maximum size.

Once cells have achieved their maximum infection capacity, they lyse and die to release viruses, which is shown as a sharp decrease in capacitance because dead cells no longer can hold an electrical charge. Using capacitance therefore can help scientists to identify, optimize, and automate their optimum harvest time point.

Proof-of-Concept Case Studies

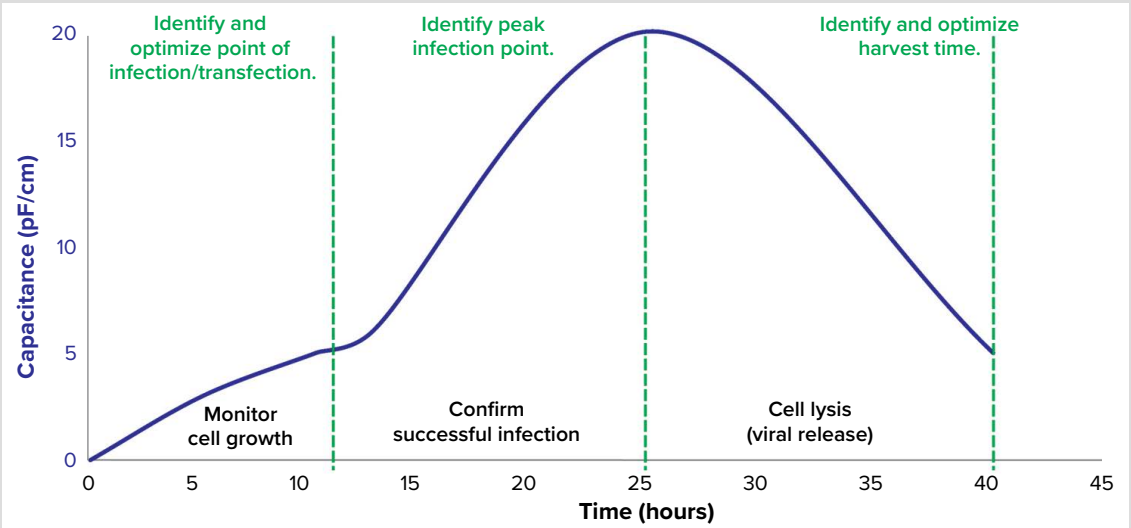
A number of published studies on production of baculovirus, AAV, and measles show how capacitance measurement and the real-time process fingerprint it generates can be used to monitor a number of different viral-vector and virus-vaccine processes.

Monitoring Cells Infected with Baculovirus Vectors: Scientists at the Biotechnology Research Institute in Canada used a capacitance sensor from Aber Instruments to monitor *Spodoptera frugiperda* (*Sf*9) insect cells at a viable cell density (VCD) of 3–50 × 10^6 cells/mL transfected with baculovirus at 0.001–100 multiplicity of infection (MOI) in batch and fed-batch conditions (**10**). On comparing capacitance data with off-line measurements of VCD and cell size, the researchers found that VCD and cell-volume profiles matched closely during the cell-growth phase and that capacitance measurement was a good indicator of baculoviral infection. It showed a plateau 18–24 hours post infection when viruses were being produced and a peak about 48 hours post infection that correlates to the start of cell lysis.

Those results indicate that capacitance measurements provide accurate information on the physiological status of cells. When combined with off-line measured parameters such as cell volume, such measurements could contribute to detailed process understanding for optimizing operations, providing for control of a vaccine produced using a baculovirus-based viral vector production process.

Tracking Cell Volume for AAV Production: Two studies on AAV viral vector production at the Biotechnology Research Institute in Canada used capacitance technology (**11, 12**). Scientists found that when their human embryonic kidney (HEK293) cells cultured in fed-batch, perfusion, or acoustic-filter–based perfusion culture were infected by AAV, cell

Figure 3: Typical real-time fingerprint of a viral vector and virus-based vaccine production process



volume measured by a Multisizer 4e Coulter counter (Beckman Coulter Life Sciences) increased in a similar pattern to that of capacitance measured with an Aber sensor. The peak diameter measurement increased by 50% (2 mm), which matched with the peak capacitance measurement.

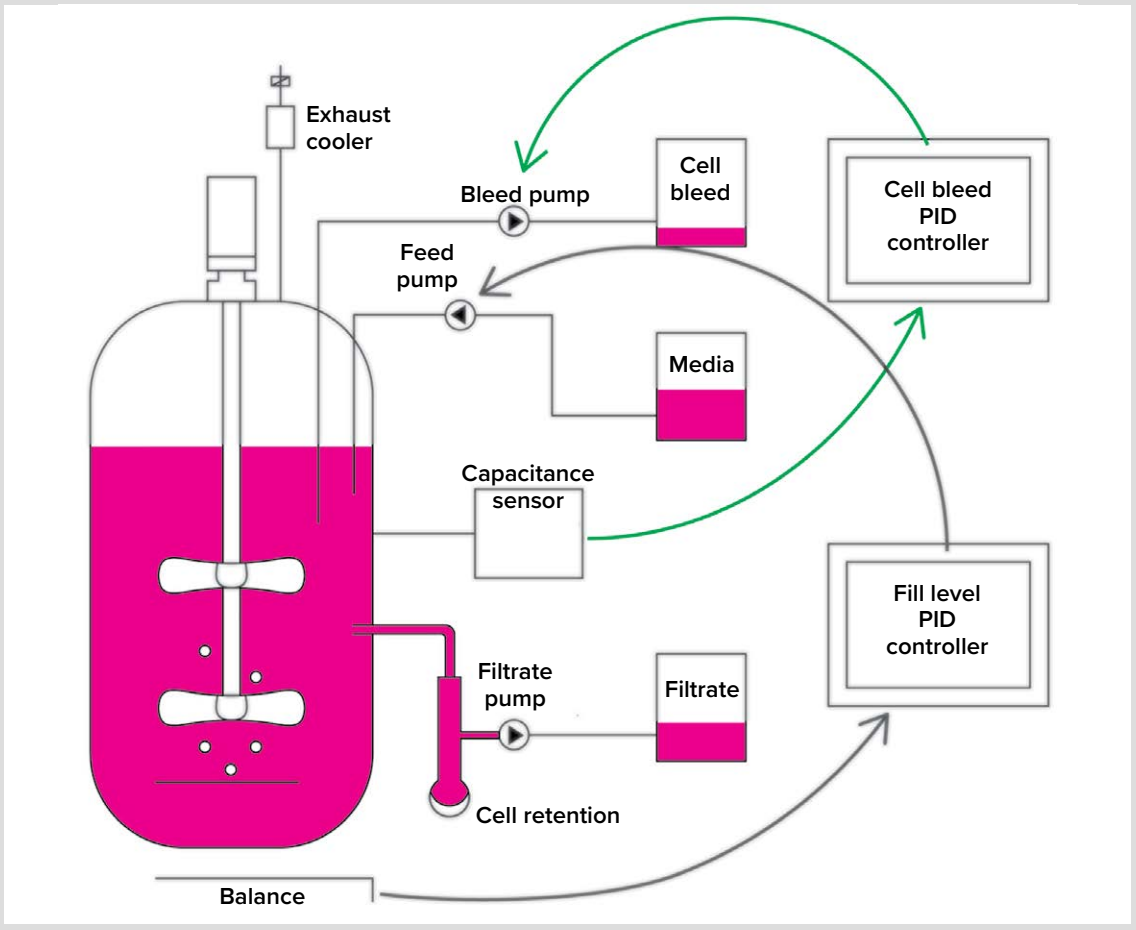
Those studies suggest that using capacitance to measure changes in cell volume can offer a rapid and accurate on-line monitoring method for predicting when HEK293 cells have been infected successfully by AAV and when such infection is at its peak. Knowing when peak infection has occurred also could help developers determine the optimum harvest point for maximum productivity of AAV viral vectors.

Monitoring Cell Volume and Adhesion to Microcarriers with Measles Virus: In a study at the University of Applied Sciences Mittelhessen in Germany, researchers integrated capacitance sensors into a stirred-tank bioreactor to characterize a measles-virus production process using adherent Vero cells cultured on microcarriers (**13**). The team used capacitance to monitor cell adhesion on the microcarriers. They used capacitance to determine adhesion time and observed that when Vero cells were spread fully across the microcarrier beads (as determined by microscopy), the capacitance correlated well with cell concentration measured off-line. Even with constant experimental parameters, the time it took for Vero cells to adhere to microcarriers varied from 3.8 to 7.3 hours after inoculation, thus making an in-line real-time measurement even more useful for determining adhesion time. In the same study, scientists also found that the optimal time for virus harvest correlated with the maximum capacitance signal from the sensor.

In 16 separate bioreactor runs, they observed that the maximum measles-virus titer was achieved about 40 hours after maximum capacitance was reached. On comparing those results with data from another measles-virus production process that was monitored using off-line cell-volume measurements, the team found that using on-line capacitance measurement provided viral titers of $>10^{10}$ median tissue-culture infectious dose ($TCID_{50}$/mL), which was over three orders of magnitude higher than those achieved without using capacitance monitoring. These results indicate that using capacitance measurement can help predict when Vero cells for measles production have adhered fully to microcarriers and when to harvest virus for optimized titers.

What About Scalability? Although capacitance measurement systems are available to improve viral vectors and virus-based vaccine production at different scales and in different types of bioreactors, the technology must operate consistently across scales. To demonstrate scalability in a 2019 study, Biogen scientists used an Aber capacitance sensor to monitor Chinese hamster ovary (CHO) cultures in bench-scale (5 L), pilot-scale (200 L, 315 L), and manufacturing-scale (15,000 L) bioreactors (**14**). Results showed that the range of sensors provides consistent data across all bioreactor types and scales, with a coefficient of variance (R^2) close to 1.0. Capacitance data showed good correlation with those from off-line VCD measurements. The team also used

Figure 4: Feedback control of cell density using an on-line capacitance sensor; PID = proportional–integral–derivative



capacitance measurement as a KPI for process control in Biogen's current good manufacturing practice (CGMP) facility both to automate dilution of seed-train cultures during scale-up and as a method of predicting glucose demand. The authors noted that when using automated capacitance measurement, their inoculation strategy generated consistent results from six seed trains in GMP manufacturing.

Although that study did not involve a cell line used for viral production, the results do suggest that capacitance measurement of mammalian cells is scalable from bench to manufacturing scale and thus could reduce feed-strategy development timelines for production of viral vectors or viruses. Capacitance provides insights into culture performance that cannot be realized using traditional at-line or off-line cell counting methods.

Can Capacitance Measurement Help Maintain Intensified Cell Cultures? As discussed above, capacitance measurement can be used in monitoring perfusion cultures for AAV viral vector production (**12**). To optimize an intensified culture for viral production, the technology could be used in an automated, intensified cell culture setup to control cell bleeds, monitor feed addition, and detect the optimum point for harvesting (**15**).

To automate and control an intensified cell culture process, an on-line capacitance sensor from Aber Instruments is integrated into a single-use or stainless-steel bioreactor and controlled by a software-driven, automated control unit (Figure 4). As cells grow in the bioreactor, their capacitance increases. If their VCD measured by capacitance goes beyond a target set point, the control unit instructs a peristaltic pump to remove cells through a dip tube in the bioreactor to perform an automated cell bleed at a defined rate determined by that of cell growth. The control unit also instructs the feed pump to add media or feed to maintain VCD at a set point. Capacitance measurement could be applied in a control loop to prevent cell overgrowth in such an intensified mammalian cell culture for viral vector and virus-

based vaccine production. It would be possible only because the sensor is monitoring live cell volume in real time.

Real-Time Data for Rapid Results

Capacitance measurement offers many benefits for monitoring viral vectors and viruses in vaccine production. These include harvest-time optimization (because viral titer is linked closely to maximum capacitance), which can increase maximum virus concentration by over three orders of magnitude (**13**). In productivity terms, a biopharmaceutical company could produce one vaccine dose/patient in a single 500-mL bioreactor rather than a 20-L bioreactor while saving on media and reagent costs. That in turn would reduce filter sizes and buffer volumes in downstream purification processes.

The flexibility of capacitance measurement gives it the potential for use in viral-vector and virus production by suspension and adherent cells in batch, fed-batch, and continuous cultures. The technology can provide real-time measurements for use in controlling VCD in intensified cell culture processes. Additionally, capacitance measurement demonstrates consistency in results from sensor to sensor compared with off-line VCD measurements of mammalian cells across bioreactors scales. Thus, it could be used as a KPI for improving scale-up success, reducing the costs and timelines of viral vector and virus-based vaccine production — which is crucial, for example, in development of vaccines during a pandemic.

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