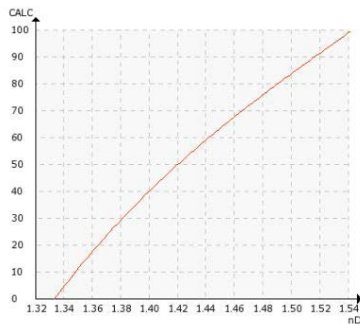


SUCROSE DENSITY

Typical end products

Viral vaccines e.g. seasonal influenza, meningitis, rabies, hepatitis B, polio, measles, mumps, rubella and other vaccines.

Chemical curve: R.I.per Brix at Ref. Temp. of 20 °C



Introduction

The viral vaccines are either produced by inoculating viruses into specific pathogen-free eggs or in an animal cell culture-based process. The allantoic fluid of these processes is harvested and purified by centrifugation and stabilised with buffer containing sucrose.

The continuous flow ultracentrifugation technique is typically used for producing purified and concentrated virus particles on a large scale. The internal subviral core of the virus is separated and fractionated on the basis of their sedimentation rate and the buoyant sucrose density.

Vaisala K-PATENTS® Pharma Refractometer PR-43-PC is used for accurate measurements of these sucrose densities.

Application

The ultracentrifuge contains six flow channels that process material flows during the operation. The different process steps are as follows:

1. The density gradient is loaded into the rotor. A sucrose gradient may consist of layers decreasing from 70 % sucrose to 20 % sucrose in 10 % increments.
2. As the rotor is gradually accelerated, the gradient reorients itself vertically along the outer rotor wall.
3. Sample fluid is pumped into the rotor on a continuous flow basis.
4. The sample particles sediment radially into the gradient of increasing density. They eventually band (iso-pycnally) in cylindrical zones where the gradient density equals a particle's buoyant density.
5. At the end of the run, the rotor is decelerated to rest. The gradient reorients itself to the original position without disturbing the particle bands.

The banded particles are now ready to be unloaded. Fractions are collected using a small peristaltic pump or air pressure according to the sucrose density gradients measured by the Process Refractometer.

Instrumentation and installation

The Pharma Refractometer PR-43-PC can be installed in the vaccines fractionation unit for in-line process control. The output is Ethernet or 4 to 20 mA signals proportional to sucrose solution density, concentration, Brix or other scale that has been selected for the instrument.

The measurement signal is used for reliable and timely determination of the product peak in the density gradient (0 to 60 % w/w sucrose), the subsequent collection of the virus rich fraction (Figure 1) and in diverting the virus rich fraction into the correct container.

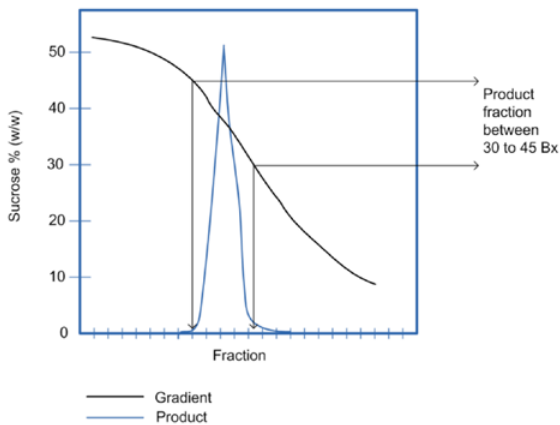




Figure 1. Collection of the virus rich fraction.

Another key area of the PR-43-PC application is in the research and development phase when the researchers need to develop a process understanding and to characterize the concentration (or density) of sucrose in each fraction. This information is valuable when formulating banding patterns and optimizing the target fraction. Once the operating procedure has been developed, the refractometer is required in full-scale production for determining the moment when to begin and to stop collecting the target fraction.

The typical system comprises of a Pharma Refractometer PR-43-PC unit and a Pharma Mini Flow Cell PMFC that allows the refractometer connection to the zone ultracentrifuge rotor unloading and fractionation phase. The standard Ethernet communication solution allows for simultaneous data logging and continuous monitoring of the measurement values and diagnostics by computer via an Ethernet connection.

Due to its unique digital sensing technology, the measurement by the refractometer is accurate and does not drift in the presence of bubbles or suspended particles. The refractometer is delivered factory calibrated and does not require re-calibration. Moreover, verification is easily performed using standard refractive index liquids.

Instrumentation	Description
	Pharma refractometer PR-43-PC for hygienic installations. The PR-43-PC is installed in the main processing line or vessel and no by-pass arrangements are required. Optional laboratory test cuvette (LTC) for off-line laboratory testing and validation.
	Sanitary Compact Refractometer PR-43-AC for hygienic installations in small pipe line sizes of 2.5 inch and smaller. The PR-43-AC refractometer is installed in the pipe bend. It is angle mounted on the outer corner of the pipe bend directly, or by a flow cell using a 3A Sanitary clamp, I-clamp or Varinline® connection.
User Interface	Selectable multichannel MI, compact CI or a web-based WI user interface options allow the user to select the most preferred way to access and use the refractometer measurement and diagnostics data.
Measurement range	Refractive Index (nD) 1.3200 – 1.5300, corresponding to 0-100 Brix.