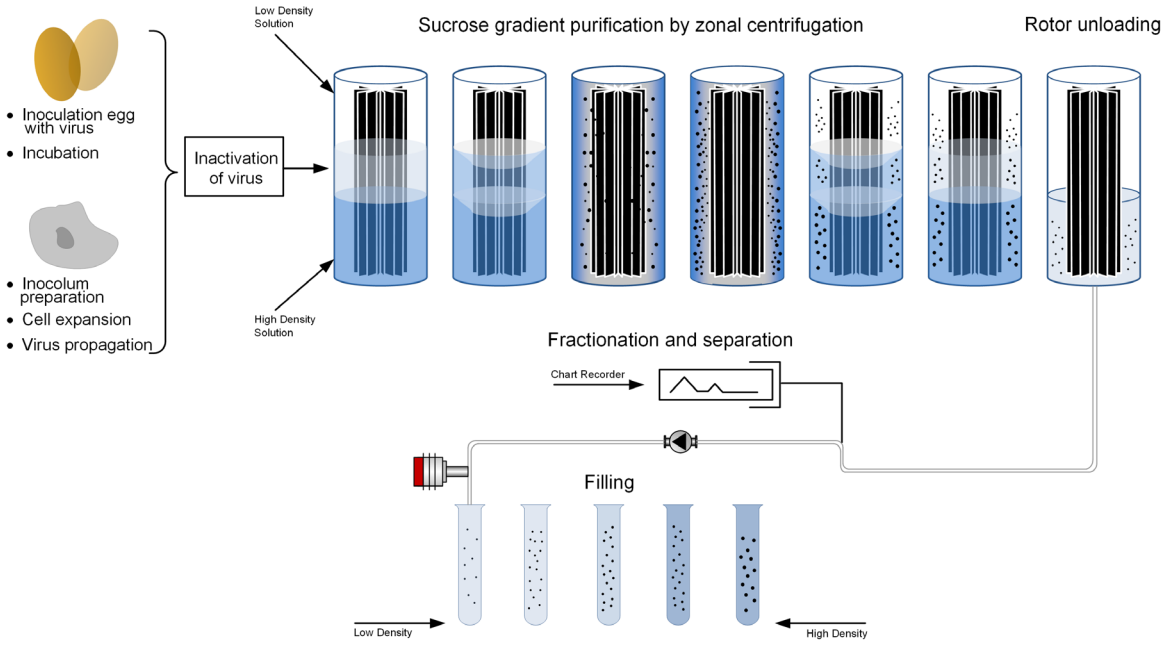
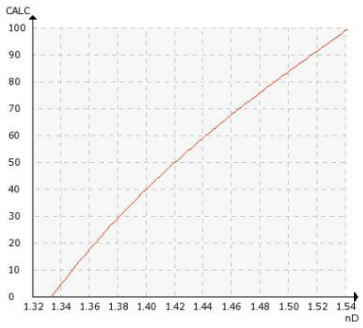


SUCROSE SOLUTION

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

Viral vaccines are used globally to protect humans against infections. Vaccines are made by first growing a virus in an egg-based or cell-based process. The virus is then inactivated or attenuated, concentrated and purified, before it is finally blended with other components for making the final vaccine formulation.

Purification is one of the most important steps in vaccine manufacturing, as it removes impurities originating from host cells or culture media and ensures the safety and efficiency of the final product. Common downstream processes for purification of the virus include density gradient ultracentrifugation, size exclusion chromatography (SEC) and ion-exchange chromatography. The choice of purification technology depends on the type of virus to be purified and the source of the starting material. For example, the separation of influenza viruses in large scales is performed using continuous flow centrifugation of the fluid with a sucrose density gradient.

Application

Sucrose density gradient has traditionally been used for the production of influenza vaccines, but it can also be used for the purification of other viruses such as Hepatitis B and Rabies. In sucrose gradient centrifugation the separation of the virus and possible impurities may be *rate-zonal* (based on particle size differences), *isopycnic* (based on the particle density differences), or a combination of both.

The concentration and purification of the virus takes place in a centrifuge (e.g a continuous flow ultracentrifuge). First the density gradient, consisting of sucrose solutions, is loaded into the rotor. The concentration range of the density gradient is selected so that it covers the particle of interest, in this case the virus. In the production of influenza vaccines sucrose solutions between 0 and 60% are typically used.

Next the rotor is accelerated. This rotation forms a density gradient in the form of bands in which the sucrose concentration goes from 0 to 60%. The

sample fluid containing the virus is then loaded into the rotor.

The viral particles in the sample move along the density gradient and separate out to form bands according to their sedimentation rates or differences in density. For instance, in isopycnic centrifugation the particles move to a position where their density is equal to that of the solution (buoyant density). This operation is known as *isopycnic banding* as the result is a solution with stable bands.

At the end of the run, the rotor is deacelerated to rest and the bands are ready to be unloaded. The separation of different fractions can easily be done by measuring changes in refractive index or Brix with a process refractometer. The refractometer provides real-time readings that help to identify and collect the rich-virus fraction(s).

After the virus is purified, the manufacturing process continues with formulation, quality testing, filling and distribution.

Instrumentation and installation

The Vaisala K-PATENTS® Pharma Refractometer PR-43-PC has been designed to meet the requirements and needs in biopharmaceuticals processing. In vaccines production, the accurate and repeatable measurement by the refractometer helps to isolate reliably the virus fraction in the purification step, ensuring a safe and high-quality vaccine product, while increasing yield and productivity.

The Vaisala pharma refractometer is installed directly in the unloading line from the vaccine fractionation unit for in-line process control. The output is Ethernet or 4 to 20 mA signals that can be easily configured to provide values of sucrose solution density, concentration, Brix or any other scale preferred by the manufacturer.

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. For instance, the output signal of the refractometer can be used as a relay to control the pneumatic actuator of diverting valve, or can be connected to the PLC for unmanned operation to pump product into one barrel and waste to another.

The Vaisala pharma refractometer is also a valuable PAT tool during research and development phase. The scalability feature of the refractometer helps researchers collect sufficient real-time data during

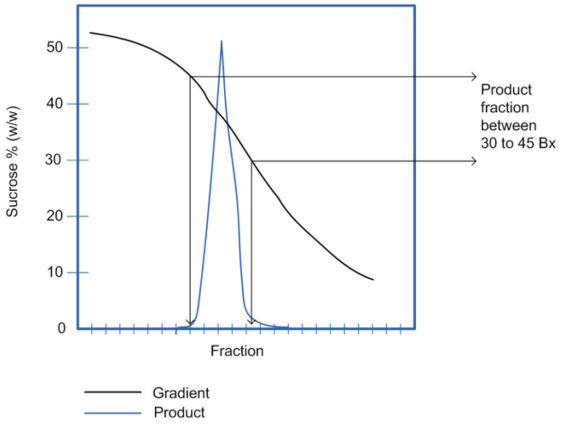


Figure 1. Collection of the virus rich fraction.

early development in the laboratory to gain process understanding. Refractive index measurements are useful to characterize the concentration (or density) of sucrose in each fraction as well as the concentration of the virus sample itself. The information helps to find banding patterns and optimizing the target fraction. Once purification protocols has been developed, the same pharma refractometer can be used in full-scale production for determining the moment when to begin and to stop collecting the target fraction.

The typical system comprises the Vaisala Pharma Refractometer PR-43-PC with a Pharma Mini Flow Cell PMFC that allows the refractometer connection to centrifuge rotor unloading and fractionation phase. The Pharma Mini Flow Cell is ideal for ensuring reliability of the measurement even at small volumes. 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.

When many instruments are required, the Multichannel User Interface (MI) connects up to 4 refractometers, thus reducing the investment cost. The MI provides user authentication, electronic records, data logging, event log and audit trail, all in compliance with pharmaceutical requirements.

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.

Ref. B211944EN-B © Vaisala 2020