Cleaning Uniform Latex Particles by Continuous Diafiltration

Sub-micron uniform latex particles were rapidly processed to remove excess free protein.

Introduction

In vitro diagnostic tests using latex micro-particles often require that the particles be cleaned before and after an appropriate protein (e.g. albumin, antibodies, antigens or other ligands) is attached to the particle surface. After passive adsorption or covalent binding, excess free protein must be quantitatively removed to ensure reliable test results and optimum sensitivity. This application describes a fast and effective method to remove free protein from uniform latex particles by means of constant volume diafiltration.

Background

Instantaneous membrane rejection (or retention) is defined as:

$$\textbf{membrane rejection} = r = 1-(C_f/C_r)$$

where C_f is the concentration of a soluble component in the filtrate and C_r is the concentration in the retentate. When a membrane allows free passage of a solute, concentration on both sides of the membrane is the same and the rejection is zero. When the membrane completely retains a solute, concentration in the filtrate is zero and the membrane rejection is one or 100%. In this application we assume that the membrane rejection of the latex particles is 100% and the membrane rejection of free protein is close to zero. So, during constant volume diafiltration, the latex particle concentration remains constant while free protein permeates the membrane. Reduction of free protein concentration is given by the following equation:

$$\textbf{concentration} = Co\ e\ -v(1-r)$$

where v is the number of batch volumes of diafiltration, r is the rejection of the solute and Co is the starting concentration of free protein. When a protein's membrane rejection is zero, 63.2% passes into the filtrate for each wash volume of diafiltration.

Process Conditions

The initial batch size of 200 ml consisted of 20 ml of 10% 0.255 µm diameter polystyrene uniform latex particles (Seradyn lot 1057) with 2.5% bovine serum albumin (BSA) adsorbed in the presence of 50 mM tris buffer. A single 0.1 µm-rated MiniKros® module with 945 cm^2 of membrane surface (product no. M21M-100-01N) was installed on a MiniKros® Plus system (productno. SYMP-211-01N). The system was set up as illustrated in (Figure 1). The particle solution was recirculated at 10 liters per minute and the filtrate port was open throughout the run. Initial inlet pressures were 10 psig and remained constant. A buffer solution consisting of 50 mM tris buffer was added continuously to the processing reservoir at the same rate that filtrate was removed. By this technique, a constant 200 ml processing volume was maintained. 2500 ml of buffer solution (12.5 wash volumes) was added in 14 minutes.

Figure 1 MiniKros® Plus Dual Flow Path System

Results

Albumin concentration in the filtrate was measured by BCA reaction. The results indicate slight deviation from the ideal case where membrane rejection of the latex particles is one and membrane rejection of the protein is zero. Removal of free BSA was rapid and exceeded 99.99%. No latex particles were detected in the filtrate, and there was no measurable loss of adsorbed BSA. The filtrate rate remained relatively constant. It is expressed here as flowrate per unit of membrane surface, or L/m^2h.

Discussion

Constant volume diafiltration using MiniKros® and larger KrosFlo® filtration modules is a fast and effective alternative to multiple centrifugation/resuspension steps. Also useful for removing surfactant and water soluble monomers prior to coupling, this technique allows significant savings in time and labor. Spectrum disposable modules offer several advantages over filtration devices that are cleaned repeatedly. Modules are shipped surfactant-free and ready for use, and protein rejection is not affected by cleaning and multiple re-use.