NON-WOVEN ELECTROSTATIC MEDIA FOR CHROMATOGRAPHIC SEPARATION OF BIOLOGICAL PARTICLES

by

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Introduction - The filtration and separation of biological particles has been estimated as 40% of the cost in the manufacture of pharmaceuticals. Liquid chromatography that is the principal method for separating biological particles uses a permanent phase of ultrafine adsorbent particles such as porous silica or resin spheres with particle sizes down to about 1 micron. Speed and resolution are two competing performance factors in conventional chromatography using porous media. One feature is often achieved by sacrificing the other. Conventional wisdom focuses on surface area as the defining element for "dynamic capacity" when determining product throughput employing conventional chromatographic media.

Nano alumina media – An electropositive fibrous media has been developed for water filtration that is capable of retaining sub-micron particles at high flow rates. The active component in the filter media is a nano alumina fiber, only 2 nm in diameter and about 250-300 nm long that are attached to a microglass fiber. Figure 1 shows the nanofibers that appear as fuzz on the microglass. The nanoalumina is aluminum oxide monohydrate (AlOOH), also known as the pseudoboehmite.

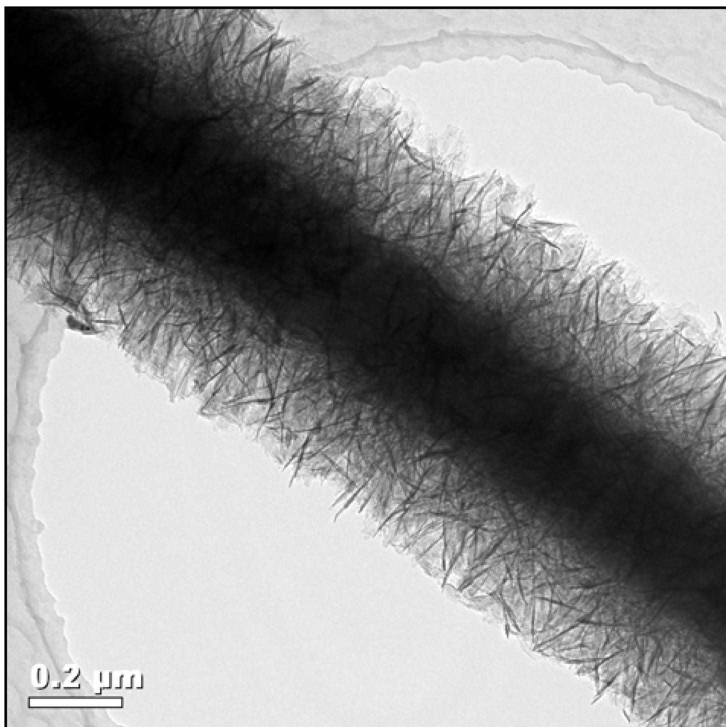

Figure 1 – Nano alumina uniformly distributed on a microglass fiber

The nano alumina as well as NanoCeram composite have isoelectric points at pH 11. Once attached to the microglass and then incorporated into a non-woven structure, the media is highly electropositive, with zeta potential of +50 mV at pH 7.

The nano alumina filter media is also highly effective in filtering out particles such as nucleic acids, proteins, cell debris and endotoxins, as well as larger particles such as bacteria.

Use as a chromatographic media – We have a work in process development program to exploit the use of nano alumina media for chromatographic separation of macromolecules. The advantages of such a media are:

1. The charged sites of the nano alumina are exposed to the moving phase. Particles do not have to traverse through pores that are fractions of a micron as in the case of porous silica. This increases the dynamic response for adsorption of particles.

2. Particles as large as hundreds of nanometers may be separable. Many capsided viruses are about 100 nm or so. They would be too large to separate by conventional HPLC adsorbent spheres.

3. The nano alumina is spatially constrained onto the microglass. No special packing requirements are envisioned.

4. Separations can occur at or near ambient pressure.

5. Separations have been performed at much higher flux as compared to packed columns or membrane chromatography devices.

6. The media is produced by low-cost paper-making manufacture. Approximately two tons of filter media have already been produced.

7. Salt would have little effect on separations. Proteins could be separated under salt free conditions

We elected to demonstrate feasibility of chromatographic use by separating two coliphages, MS2 (ATCC 15597-B1) and Alpha 3 (ATCC 13706-B2) that have identical particle size (27 nm). Both are uncapsided. And we also chose to use conventional culture to assay the viruses in eluted fractions. With live viruses, we would be assured that the particle was not affected in transit through the separation process.

We tried two types of eluents. The first was a solution of 3% beef extract, 0.25% glycine and at a pH of 9.3. This solution had been developed earlier for extracting virus from electropositive media. Protocols are being developed by EPA and others for assay of norovirus, adenovirus, polio and bird flu using this or similar eluents. The second eluent was a 0.01 *M* sodium carbonate solution (pH= 10).

Experimental data to date – NC ("NanoCeram"®) nano alumina media discs, 25 mm in diameter, were loaded with a mixture of approximately equal quantities of MS2 and alpha 3 viruses at input concentrations from 100 to1000 phage particles/milliliter at a flowrate of 10 mL/min. Elutions were performed by passing 0.5-2.5 ml aliquots of beef extract solution through a stack of 1, 3, 6 and 12 layers of the same filter media at a flowrate of 5 mL/min in the same flow direction as the adsorption step. Results for 1 and 12 layers are shown in Figures 2 and 3.

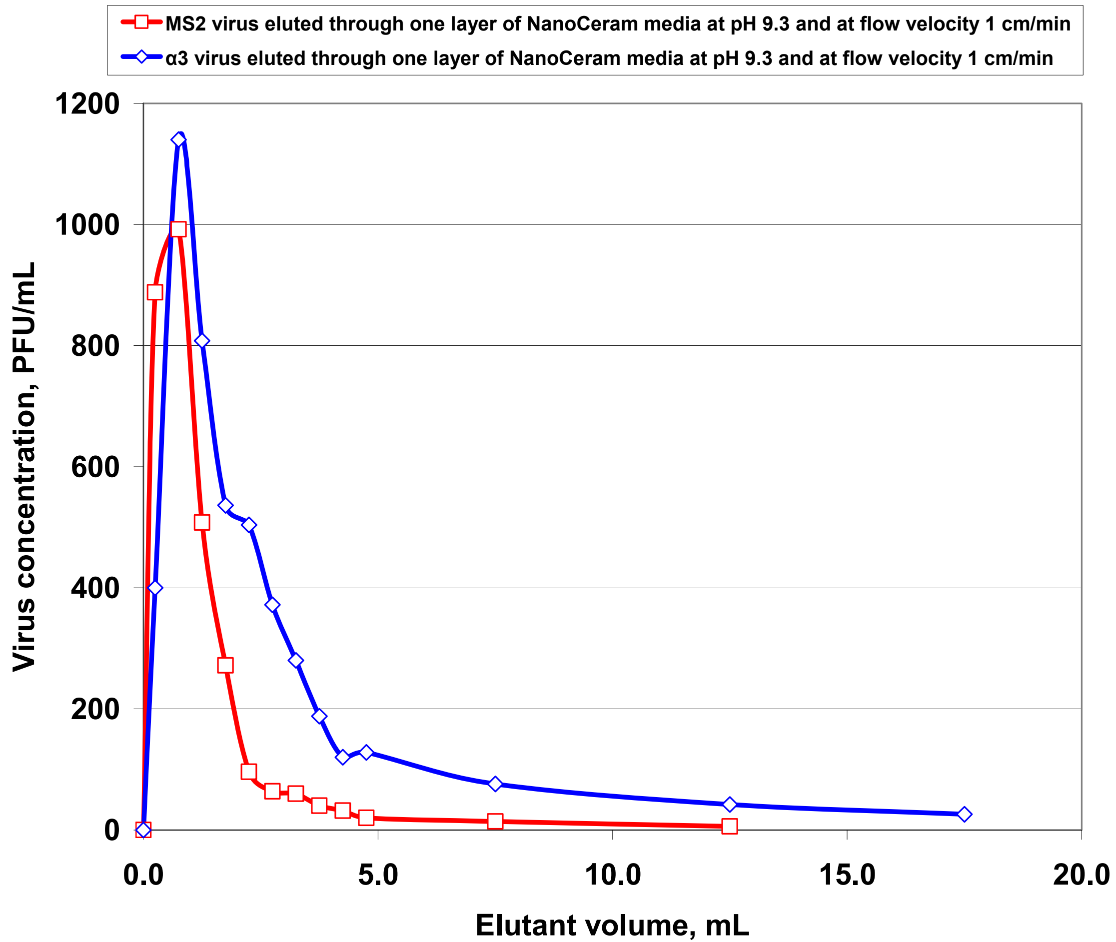

Figure 2 - Alpha 3 and MS2 viruses eluted through one NC disc

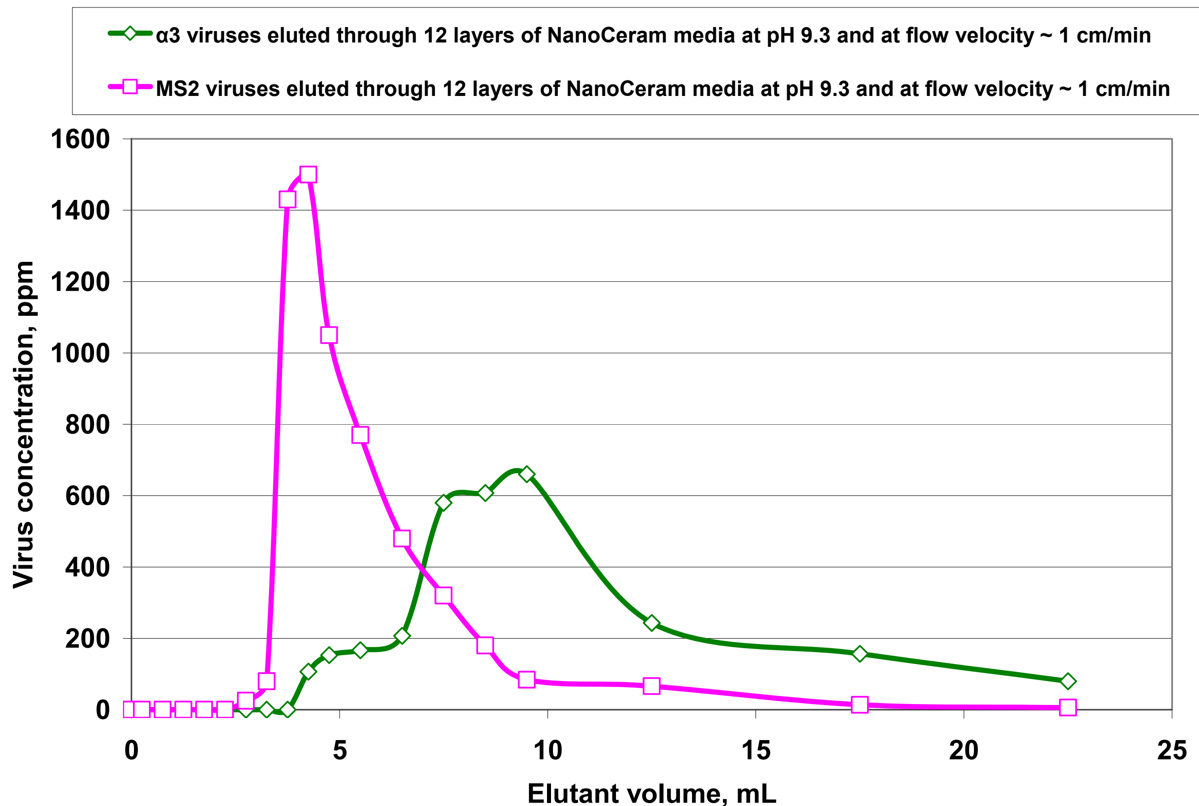

Figure 3 - Alpha 3 and MS2 viruses eluted through 12 layers of NC discs

Figures 2 and 3 show that the separation of the two viruses improves with increasing thickness of the media. Separation is effected at flow velocities of 1 cm/min and at less than 1 bar that are small fractions of that in HPLC separation. Further separation of viruses would occur if more than 12 layers were used.

Discussion and Conclusions - We have demonstrated feasibility of separation of peaks from viruses and presumably from other biological macromolecules. Separations were effected within minutes rather than tens of minutes or hours when using conventional approaches and at pressures less than 1 bar.

1. Further work is continuing using sodium carbonate as an eluent. That direction include the use of thicker layers (more discs) to further separate MS2 and Alpha 3.
2. Modifying the nano alumina structure by adding a third component such as nano silica. Media composites have been produced that embody 26 weight percent nano silica (10 nm). The resulting structure has an even higher zeta potential (+56 mV) than the basic nano alumina (+50 mV). Such variations of the surface of the nano alumina can alter the degree of attachment of different molecules, allowing alternative chromatographic process conditions.

3. Alteration of the pore size. Future versions can range in pore size from as low as about 0.5 to as high as tens of microns.

The nano alumina media can be used in the separation of virus, peptides, proteins, oligonucleotides and nano size particles such as virus and plasmid DNA as well as their separation from cell debris and contaminants that have low molecular weight.   Specific proteins, antigens or antibodies could be attached to the nano alumina to allow affinity chromatography.