expressed structural proteins, both in vivo within the cell where the structural proteins and recombinant proteins are expressed in forming VLPs, and outside of the cell after isolation and purification. However, the capturing of such VLPs in a mixture containing high suspended solids content and particles of varying sizes, typically in situations involving plant-derived materials, are technically very challenging. VLPs may be immunogenic when injected into animals to induce the production of anti-viral antibodies that can block infection. Thus, for example, mice vaccinated with virus-like particles produced with proteins from an H5N1 avian strain can be protected against challenge with lethal H5N1 viruses.

There remains a need in the industry for a method of capturing particles of interest with improved efficiency and yield. There is also a need for such a method to be scaleable for the commercial level to capture of particles of interest in a cost effective manner.

SUMMARY ~~OF THE INVENTION~~

The present disclosure~~invention~~ is based, at least in part, on the discovery that Expanded Bed Adsorption (EBA) chromatography is particularly efficient for the capture of a particle of interest from a mixture. In certain embodiments, the method can advantageously be used for the capture of a particle of interest in a one step binding and elution process that is scaleable to commercial levels.

Generally~~In a general aspect~~, there is provided a method for capturing a particle of interest from a mixture comprising the use of an expanded bed of adsorbent.

In a first aspect of the present disclosure, there is provided a method for capturing virus like particles of interest from a mixture comprising disrupted cells of a plant; wherein said method comprises the steps of: (i) providing an expanded bed of adsorbent comprising a resin material; (ii) equilibrating the resin material at a pH in the range of about pH 6.0 to 8.0; and (iii) loading the mixture onto the expanded bed of adsorbent to bind the virus like particles, wherein the degree of expansion of the expanded bed is in the range of from about 1 to 5; (iv) optionally washing the adsorbent; and (v) optionally eluting the virus like particles from the adsorbent.

In a second aspect of the present disclosure, there is provided a method for capturing virus like particles of interest from a mixture comprising tobacco plant material comprising the steps of: (i) providing an expanded bed of adsorbent comprising diethylaminoethyl cellulose dextran in a column, wherein the density of the adsorbent is between about 2.5 g/ml to 3.5 g/ml, and wherein the average adsorbent particle size is about 50 µm; (ii) equilibrating the resin material at a pH in the range of about pH 6.0 to 8.0; (iii) providing a mixture comprising tobacco plant material, wherein said mixture has a conductivity of between about 4 mS/cm to 6 mS/cm; (iv) loading the mixture onto the column at a flow rate of about 2.5 cm/min to bind the virus like particles and wherein the degree of expansion of the expanded bed in the column is about 2; (v) washing the loaded column using a buffer having a pH in the range of about pH 6.0 to 8.0; and (vi) eluting the bound particle of interest from the column in one or more fractions.

In a third aspect of the present disclosure, there is provided the use of an expanded bed of adsorbent for capturing a virus like particle of interest from a mixture, wherein the mixture is derived from a plant that is transiently expressing a protein, said protein being present in the virus like particle; the resin material is equilibrated to a pH in the range of about pH 6.0 to 8.0; the mixture is loaded onto the column to bind the virus like particles and the degree of expansion of the expanded bed in the column is in the range of from about 1 to 5.

In one embodiment, the method comprises the steps of: (a) providing an expanded bed of adsorbent; (b) contacting the mixture with the adsorbent such that the constituents of the mixture contact and interact with the expanded bed of adsorbent; (c) optionally washing the adsorbent; and (d) optionally eluting the particle of interest from the adsorbent.

In one embodiment or a combination of the above-mentioned embodiment(s), the particle of interest is a biological particle of interest.

In one embodiment or a combination of the above-mentioned embodiment(s), the particle of interest comprises at least one protein.

In one embodiment or a combination of the above-mentioned embodiment(s), the particle of interest comprises at least two proteins, at least one of which is a protein of interest.

In one embodiment or a combination of the above-mentioned embodiment(s), the particle of interest is a virus like particle.

~~In a first aspect of the invention, t~~There is also provided a method for capturing virus like particles of interest from a mixture comprising the use of an expanded bed of adsorbent; suitably wherein said method comprises the steps of: (a) providing an expanded bed of adsorbent;

(b) contacting the mixture with the adsorbent such that the virus like particles in the mixture bind the adsorbent; (c) optionally washing the adsorbent; and (d) optionally eluting the virus like particles from the adsorbent.

In one embodiment ~~of this first aspect~~, the present disclosure~~invention~~ further relates to a method according to the preceding embodiment or a combination of the above-mentioned embodiment(s), wherein the virus like particles comprises a protein of an influenza virus, particularly a haemagglutinin; suitably a haemagglutinin subtype selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 or a combination of two or more thereof.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the adsorbent is contained in a column.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the mixture is or is derived from a plant.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the plant is a naturally occurring plant, a mutant plant, a non-naturally occurring plant or a transgenic plant.

In one embodiment or a combination of the above-mentioned embodiment(s), the mixture is or is derived from a leaf of a plant.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the mixture is produced by homogenising plant material prior to contact with the adsorbent. Thus, according to this embodiment, the mixture is homogenised plant material.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the plant is a plant cell – such as a plant cell grown in culture or outside of a plant such as in vitro grown plant cells or clumps of cells – such as carrot cells.

~~In one embodiment of the first aspect of the invention, t~~The present disclosure~~invention~~ provides a method according to any of the preceding embodiments or a combination of the preceding embodiment(s), wherein the mixture comprises disrupted cells of a plant; suitably wherein the plant is a tobacco plant infiltrated with nucleic acid molecules that express a protein transiently in the plant, said protein being present in the virus like particles; more suitably wherein the mixture is or is derived from aerial parts of the plant.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the adsorbent comprises a plurality of beads.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the beads are composite beads that comprise an inert core material.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the beads comprise, consist or consist essentially of a material selected from the group consiting of plastics, methacrylate, an anion exchanger, diethylaminoethyl, a cation exchanger, a polysaccharide, silica, poly(styrenedivinyl)benzene, polyacrylamide, ceramic and derivatives thereof or a combination of two or more thereof.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the beads comprise, consist or consist essentially of a polysaccharide; suitably cellulose, agarose, dextran, or derivatives thereof, or a combination of two or more of the foregoing.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the inert core material comprises, consists or consists essentially of a material selected from the group consisting of quartz, silica, Nd-Fe-B alloy, stainless steel, zirconium oxide, zirconia, metal silicates, metal borosilicates, ceramics including titanium diboride, titanium carbide, zirconium diboride, zirconium carbide, tungsten carbide, silicon carbide, aluminium nitride, silicon nitride, titanium nitride, yttrium oxide, silicon metal powder, and molybdenum disilicide; metal oxides and sulfides, including magnesium, aluminium, titanium, vanadium, chromium, zirconium, hafnium, manganese, iron, cobalt, nickel, copper and silver oxide; non-metal oxides; metal salts, including barium sulphate; metallic elements, including tungsten, zirconium, titanium, hafnium, vanadium, chromium, manganese, iron, cobalt, nickel, indium, copper, silver, gold, palladium, platinum, ruthenium, osmium, rhodium and iridium, and alloys of metallic elements, such as alloys formed between said metallic elements and derivatives thereof or a combination of two or more thereof.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the size of the beads is between about 5 µm and 500 µm or more.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the size of the beads that bind the particle of interest is between about 25 µm and 100 µm; suitably about 50 µm.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the size of the beads that bind material other than the

particle of interest, for example, plant materials, is between about 125 µm and 250 µm; suitably about 150 µm.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the method comprises a pre-capture step which comprises the use of an additional expanded bed of adsorbent. This first expanded bed of adsorbent comprises beads; suitably of a size range that is between about 125 µm and 250 µm; more suitably about 150 µm.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the adsorbent comprises a ligand.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the ligand comprises, consists or consists essentially of a ligand selected from the group consisting of (i) benzylamine or a derivative thereof; (ii) an alkylamine or a derivative thereof; (iii) an alkenylamine or a derivative thereof; (iv) an alkynylamine or a derivative thereof; (v) an alkoxyamine or a derivative therof; and (vi) an amine of mono- or bicyclic aromatic or heteroaromatic moities or a derivative thereof, or a combination of two or more thereof.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the ligand comprises, consists or consists essentially of an anion exchange resin.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the anion exchange resin comprises, consists or consists essentially of or is a diethylaminoethyl cellulose-based ion exchange resin; suitably a diethylaminoethyl cellulose dextran-based ion exchange resin.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the conductivity of the mixture prior to contact with the adsorbent is between about 1 mS/cm and 10 mS/cm; suitably 2 mS/cm to 3 mS/cm.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the pH of the mixture prior to contact with the adsorbent is between about pH 6.0 and pH 8.0.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the mixture is loaded onto the column at a linear flow rate in the range of between about 1 cm/min and 15 cm/min to bind the particle of interest.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the density of the adsorbent is in the range of between about 1 g/ml and 20 g/ml.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the degree of expansion of the expanded bed is in the range of from about 1 to 5.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, said method comprises a pre-capture step prior to contacting the mixture with the adsorbent.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the pre-capture step is selected from the group consisting of filtration, microfiltration, centrifugation, decantation and sedimentation or a combination of two or more thereof.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the pre-capture step comprises: (i) contacting the material with an ion exchange resin; or (ii) acid precipitating and filtering the material.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the material is contacted with the ion exchange resin in static binding or batch incubation mode.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the eluate or one or more fractions thereof obtained in step (d) is subjected to a further treatment step.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the further treatment step is selected from the group consisting of purification, filtration, microfiltration, centrifugation, decantation and sedimentation or a combination of two or more thereof.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the particle of interest comprises a protein of interest.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the protein of interest is an antigen.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect,~~ the protein of interest is haemagglutinin.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the haemagglutinin is a haemagglutinin subtype selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 or a combination of two or more thereof.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~including an embodiment of this first aspect~~, the expansion rate of the expanded bed during the elution step is between about 0.5 and 5.

~~In a second aspect, t~~There is also provided a method for capturing a particle of interest from a mixture comprising the steps of: (i) providing an expanded bed of adsorbent comprising a ligand in a column at a settled bed height of above 10 cm, wherein the density of the adsorbent is between about 1 g/ml and 20 g/ml, wherein the degree of expansion of the expanded bed is between about 1 and 5 and wherein the average adsorbent particle size is between about 5 µm and about 500 µm; (ii) equilibrating the resin material at a pH in the range of about pH 6.0 and 8.0; (iii) providing a mixture comprising the particle of interest, wherein said mixture has a conductivity of between about 1 mS/cm and 10 mS/cm; (iv) loading the mixture onto the column at a flow rate (for example, linear flow rate) in the range of between about 1 cm/min and 15 cm/min to bind the particle of interest; (v) washing the loaded column using a buffer having a pH in the range of about pH 6.0 and 8.0; and (vi) eluting the bound particle of interest from the column in one or more fractions.

In one embodiment ~~of this second aspect~~, the particle of interest is a virus like particle (VLP) or a ghost-like particle.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the mixture is or is derived from a plant.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the plant is a naturally occurring plant, a mutant plant, a non-naturally occurring plant or a transgenic plant.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the mixture is or is derived from a leaf of a plant.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the particle of interest comprises a protein of interest.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the protein of interest is an antigen.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the protein of interest is haemagglutinin.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the haemagglutinin is a haemagglutinin subtype selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 or a combination of two or more thereof.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the size of the beads that bind the particle of interest is between about 25 µm and 100 µm; suitably about 50 µm.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, the size of the beads that bind material other then the particle of interest, for example, plant materials, is between about 125 µm and 250 µm; suitably about 150 µm.

In one embodiment ~~of this second aspect~~, or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, a method is provided for capturing virus like particles of interest from a mixture comprising the steps of:

(i) providing an expanded bed of adsorbent comprising diethylaminoethyl cellulose dextran in a column, wherein the density of the adsorbent is between about 2.5 g/ml and 3.5 g/ml, and wherein the average adsorbent particle size is about 50 ;

(ii) equilibrating the resin material at a pH in the range of about pH 6.0 and 8.0;

(iii) providing a mixture comprising tobacco plant material, wherein said mixture has a conductivity of between about 2 mS/cm and 3 mS/cm;

(iv) loading the mixture onto the column at a flow rate of about 2.5 cm/min to bind the virus like particles and wherein the degree of expansion of the expanded bed in the column is about 2;

(v) washing the loaded column using a buffer having a pH in the range of about pH 6.0 and 8.0; and

(vi) eluting the bound particle of interest from the column in one or more fractions.

In one embodiment, the method of the preceding embodiment, or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, comprises a step (iii) comprising (a) providing whole tobacco plants that are transiently expressing a protein that is present in the virus like particles; (b) disrupting cells of the leaves and stems of the tobacco plants to produce the tobacco plant material; and (c) adjusting the pH and conductivity of the mixture by dilution with a buffer or deionized water.

In one embodiment, the method of the preceding embodiment, or a combination of the above-mentioned embodiment(s) ~~of this second aspect~~, comprises prior to step (iv), a step comprising filtering the mixture of step (iii) and contacting the filtered mixture of step (iii) with

a first expanded bed of adsorbents comprising polyethylenimine, wherein the size of beads is about 150 µm, to remove undesired substances from the mixture.

~~In a third further aspect, t~~There is also provided an expanded bed of adsorbent comprising a particle of interest reversibly bound thereto.

In one embodiment ~~of this third aspect~~, the adsorbent comprises a plurality of beads.

In one embodiment or a combination of the above-mentioned embodiment(s) ~~of this third aspect~~, the size of the beads that bind the particle of interest is between about 25 µm and 100 µm; suitably about 50 µm.

~~In a fourth further aspect, t~~There is also provided the use of an expanded bed of adsorbent for capturing a particle of interest, particularly a virus like particle of interest from a mixture, more particularly a mixture which is derived from a plant that is transiently expressing a protein, said protein being present in the virus like particle.

In one embodiment ~~of this fourth aspect~~, the particle is a virus like particle (VLP) or a ghost-like particle.

There is also provided~~A fifth aspect relates to~~ an expanded bed of adsorbent comprising a particle of interest; suitably a virus like particle of interest reversibly bound thereto, particularly wherein the adsorbent is in expanded bed chromatography mode.

There is also provided the~~A sixth aspect relates to the~~ use of expanded bed chromatography for capturing a particle of interest; suitably a virus like particle; from a mixture.

The embodiment(s) and combinations of the embodiments described above ~~for the general and the first aspect of the invention~~ may ~~also~~ form embodiments ~~of~~ for all ~~at least the second, third, fourth, fifth and sixth~~ aspects of ~~this invention~~the present disclosure.

DEFINITIONS

The technical terms and expressions used within the scope of this application are generally to be given the meaning commonly applied to them in the pertinent art of plant and molecular biology. All of the following definitions apply to the complete content of this application. The word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. A single step may fulfill the functions of several features recited in the claims. The terms “essentially”, “about”, “approximately” and the like in connection with an attribute or a value particularly also define exactly the attribute or exactly the value, respectively. The term “about” in the context of a given numerate value or range refers to a value or range that is within 20 %, within 10 %, or within 5 % of the given value or range. The terms "parts per million" or "ppm" are used interchangeably herein to refer to a measure of purity of the particle of interest or protein of

(i) providing an expanded bed of adsorbent comprising diethylaminoethyl cellulose dextran in a column, wherein the density of the adsorbent is between about 2.5 g/ml to 3.5 g/ml, and wherein the average adsorbent particle size is about 50 µm;

(ii) equilibrating the resin material at a pH in the range of about pH 6.0 to 8.0;

(iii) providing a mixture comprising tobacco plant material, wherein said mixture has a conductivity of between about 4 mS/cm to 6 mS/cm;

(iv) loading the mixture onto the column at a flow rate of about 2.5 cm/min to bind the virus like particles and wherein the degree of expansion of the expanded bed in the column is about 2;

(v) washing the loaded column using a buffer having a pH in the range of about pH 6.0 to 8.0; and

(vi) eluting the bound particle of interest from the column in one or more fractions.

14. The method of claim 13, wherein step (iii) comprises (a) providing whole tobacco plants that are transiently expressing a protein that is present in the virus like particles; (b) disrupting cells of the leaves and stems of the tobacco plants to produce the tobacco plant material; and (c) adjusting the pH and conductivity of the mixture by dilution with a buffer or deionized water.

15. The method of claim 14, further comprising prior to step (iv), a step comprising filtering the mixture of step (iii) and contacting the filtered mixture of step (iii) with a first expaned bed of adsorbents comprising polyethylenimine, wherein the size of beads is about 150 µm, to remove undesired substances from the mixture.

~~16. An expanded bed of adsorbent comprising a virus like particle reversibly bound thereto resulting from the method of any one of claims 1-15.~~

16~~7~~. Use of an expanded bed of adsorbent for capturing a virus like particle of interest from a mixture, wherein the mixture is derived from a plant that is transiently expressing a protein, said protein being present in the virus like particle; the resin material is equilibrated to a pH in the range of about pH 6.0 to 8.0; the mixture is loaded onto the column to bind the virus like particles and the degree of expansion of the expanded bed in the column is in the range of from about 1 to 5.