

Compositions and methods for initiating a mucosal immune response

5

10

Field of the Invention

15 [0001] The present invention relates to compositions comprising a biological active material. The present invention also relates to pharmaceutical compositions, dosage forms and methods for initiating a mucosal immune response.

Background

20 [0002] The following discussion of the background art is intended to facilitate an understanding of the present invention only. The discussion is not an acknowledgement or admission that any of the material referred to is or was part of the common general knowledge as at the priority date of the application.

[0003] *The mucosal immune response and its role in immunity*

25 [0004] The first point of entry for many airborne viruses, including influenza and COVID-19, is the mucosal surface, comprising the mouth, throat, nasal cavity and lungs. Many viral infections are also mucosal in their pathology, infecting those same areas and causing numerous mild to fatal effects on the body, from blocked noses to lung function failure. Infections can also subsequently enter the body and become systemic, with a second array
30 of symptoms and pathologies. The mucosal system has an array of immunogenic and defensive mechanisms to deal with invading organisms, and these are somewhat different to those found within the body proper. It would seem inefficient, and perhaps even unnecessary, to generate antibodies against mucosal viruses by stimulating the systemic immunity cascade, since the attacking virus would need to become systemic, or otherwise
35 exposed to the systemic immune system, to be subject to defensive action by the body. This may be too late for vulnerable patients.

[0005] *Challenges delivering a biological active material to the mucosa and initiating a mucosal immune response*

[0006] The mucosa is designed for several functions, one being a first line of protection against invading airborne pathogens. The mucosal surfaces are protected by layers of sticky
5 mucous, comprising high MW polysaccharides, that can trap, bind and sweep away pathogens. This same process makes the presentation of a biological active designed to evoke an immune response through epithelial contact also difficult. Saliva, mucus and other viscous polysaccharidic secretions thwart sufficiently prolonged contact of the immunogen directly with the epithelial layers to initiate the immune response cascade. The inventors
10 have identified that what is needed is a protocol and dosage form that will minimize removal and maximize contact time with as much immunogenic material as possible.

[0007] *Challenges stabilising a biological active material in a dosage form*

[0008] Many respiratory pathogens (flu, COVID, RSV, whooping cough) are vaccinated against systemically, through IM injection. Many vaccines are prepared and stored in
15 solution, ready for injection, and many require refrigeration or freezing to allow for transport and storage. This is very inconvenient for both rich (necessitating a trip to a clinic) or poor countries (low levels of refrigeration and clinics). What is required is a stable, solid dosage form that can be stored and transported at ambient temperatures for convenient use orally, rather than via injection. There is a need for stabilized biomolecules and vaccines without
20 the need for refrigeration.

[0009] There is a need in the art for improved compositions and methods for delivering a biological active material to the mucosa and initiating a mucosal immune response. It is an objective of the invention to overcome one or more problems foreshadowed by the prior art.

Summary of the Invention

25 [0010] In a first aspect, the invention broadly resides in a.

[0011] In a first aspect, the invention broadly resides in a composition Preferably, said composition comprises a biologically active material Preferably, the composition is adapted to release the biologically active material to a mucosal surface of a patient in need thereof.

[0012] In a preferred embodiment, the composition is adapted to induce a biological or
30 immunological response at the epithelial layer of the mucosal system.

[0013] In another preferred embodiment, the composition is a solid dosage form.

[0014] Preferably, the composition comprises one or more agents selected from the group consisting of: an amorphous non-ionic matrix forming agent; an ionic water soluble

post-disintegration viscosity agent; a low MW ionic matrix agent; a second non-ionic matrix agent; and a non-ionic crystalline matrix forming agent.

5 [0015] Preferably, the composition comprises the following agents: an ionic water soluble post-disintegration viscosity agent; a low MW ionic matrix agent; a second non-ionic matrix agent; and a non-ionic crystalline matrix forming agent.

[0016] Preferably, the composition comprises one or more agents selected from the group consisting of: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a second water soluble post-disintegration viscosity agent; a low MW ionic matrix agent; a second non-ionic matrix agent; and a non-ionic
10 crystalline matrix forming agent.

[0017] Preferably, the composition comprises the following agents: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a second water soluble post-disintegration viscosity agent; a low MW ionic matrix agent; a second non-ionic matrix agent; and a non-ionic crystalline matrix forming agent.

15 [0018] Preferably, the composition comprises one or more agents selected from the group consisting of: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a second water soluble post-disintegration viscosity agent; an adjuvant; a low MW ionic matrix agent; a second non-ionic matrix agent; a non-ionic crystalline matrix forming agent; a second non-ionic crystalline matrix forming agent; and a further non-ionic matrix agent.

[0019] Preferably, the composition comprises the following agents: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a second water soluble post-disintegration viscosity agent; an adjuvant; a low MW ionic matrix agent; a second non-ionic matrix agent; a non-ionic crystalline matrix forming agent; a
25 second non-ionic crystalline matrix forming agent; and a further non-ionic matrix agent.

[0020] In another preferred embodiment, the composition is a rapidly disintegrating solid dosage form.

[0021] In another preferred embodiment, the biologically active material is present in the composition at a concentration selected from the group consisting of: between 1 nanogram and 100 milligrams; and between 0.01 nanogram and 1000 milligrams.
30

[0022] Preferably, the biologically active material is present in the composition at a concentration selected from the group consisting of: 1% (w/w); 2% (w/w); 3% (w/w); 4% (w/w); 5% (w/w); 6% (w/w); 7% (w/w); 8% (w/w); 9% (w/w); 10% (w/w); 11% (w/w); 12% (w/w); 13% (w/w); 14% (w/w); 15% (w/w); 16% (w/w); 17% (w/w); 18% (w/w); 19% (w/w);
35 20% (w/w); 21% (w/w); 22% (w/w); 23% (w/w); 24% (w/w); 25% (w/w); 26% (w/w); 27% (w/w); 28% (w/w); 29% (w/w); 30% (w/w); 31% (w/w); 32% (w/w); 33% (w/w); 34% (w/w); 35% (w/w); 36% (w/w); 37% (w/w); 38% (w/w); 39% (w/w); 40% (w/w); 41% (w/w); 42%

(w/w); 43% (w/w); 44% (w/w); 45% (w/w); 46% (w/w); 47% (w/w); 48% (w/w); 49% (w/w); 50% (w/w); and 2-95% (w/w); 3-65% (w/w); 4-55% (w/w); 5-45% (w/w); 6-35% (w/w); 7-25% (w/w); 8-15% (w/w); and within 2 decimal points of each figure.

[0023] Preferably, the biologically active material is selected from the group consisting of: haemagglutinins; vaccines; immunoglobulins; allergens; interferons; monoclonal immunoglobulins; and monoclonal antibodies.

[0024] Preferably, the biologically active molecules are components in vaccines for respiratory viruses which initially contact a mucosal surface prior to replication and spread of infection, including those selected from the group consisting of: influenza; COVID-19 (coronavirus disease 2019); and RSV (respiratory syncytial virus).

[0025] Preferably, the biologically active material is one of the family of biological molecules designed to elicit an immunogenic response at a mucosal surface, selected from the group consisting of: hemagglutinins; monoclonal antibodies; and viral fragments.

[0026] Preferably, the biologically active material is preferably selected from the group consisting of: hemagglutinins (HA).

[0027] Preferably, the HA is present at a level selected from the group consisting of: 0.1 to 1000µg; 1-100µg; 1-50µg; and 5-20µg.

[0028] Preferably, the biologically active material is selected from the group consisting of: from vitamins; pharmaceutical actives; small chemical entities; biologics; amino acids; proteins; peptides; polypeptides; nucleotides; nucleic acids; oligonucleotides; vaccines; monoclonal antibodies; nucleic acids; and a pharmaceutically acceptable salt; analog; homolog; derivative; solvate; hydrate; and polymorph thereof.

[0029] Preferably, the biologically active material is selected from the group consisting of: anti-obesity drugs; central nervous system stimulants; carotenoids; corticosteroids; elastase inhibitors; anti-fungals; oncology therapies; anti-emetics; analgesics; cardiovascular agents; anti-inflammatory agents; such as NSAIDs and COX-2 inhibitors; anthelmintics; anti-arrhythmic agents; antibiotics (including penicillins); anticoagulants; antidepressants; antidiabetic agents; antiepileptics; antihistamines; antihypertensive agents; antimuscarinic agents; antimycobacterial agents; antineoplastic agents; immunosuppressants; antithyroid agents; antiviral agents; anxiolytics; sedatives (hypnotics and neuroleptics); astringents; alpha-adrenergic receptor blocking agents; beta-adrenoceptor blocking agents; blood products and substitutes; cardiac inotropic agents; contrast media; cough suppressants (expectorants and mucolytics); cytokines; diagnostic agents; diagnostic imaging agents; diuretics; dopaminergics (anti-parkinsonian agents); haemostatics; immunological agents; lipid regulating agents; muscle relaxants; parasympathomimetics; parathyroid calcitonin and biphosphonates; prostaglandins; radio-pharmaceuticals; sex hormones (including steroids); anti-allergic agents; stimulants and anoretics; sympathomimetics; thyroid agents; vaccines;

vasodilators; xanthines; and a pharmaceutically acceptable salt, analog, homolog, derivative, solvate, hydrate, and polymorph thereof.

[0030] Preferably, the biologically active material is selected from the group consisting of: an anti-viral vaccine; anti-bacterial vaccine; and a cytokine.

5 [0031] Preferably, the biologically active material is selected from the group consisting of: vaccines that generate mucosal immunity against an antigen.

[0032] Preferably, the biologically active material is selected from the group consisting of: Vaccines that aim to generate mucosal immunity include: Oral Polio Vaccine (OPV): The oral polio vaccine helps induce a protective mucosal immune response against the poliovirus
10 in the digestive tract; Rotavirus Vaccine: Rotavirus vaccines aim to protect against severe diarrhea and gastroenteritis caused by rotaviruses, which primarily affect the gastrointestinal mucosa; Cholera Vaccine: Cholera vaccines target the mucosal immune response in the gut to protect against cholera, a bacterial infection that affects the intestinal mucosa; Influenza Vaccine: These vaccines aim to generate mucosal immunity against influenza viruses in the
15 respiratory tract; Intranasal COVID-19 Vaccines; Mucosal HSV-2 Vaccine; and Norovirus Vaccine.

[0033] Preferably, the biologically active material is selected from the group consisting of: FDA-approved influenza vaccines in the United States include Afluria, Fluarix, Flublok, Flucelvax, Flulaval, Fluzone (available in various formulations including standard-dose, high-
20 dose, and intradermal), FluMist Quadrivalent, Fluzone High-Dose (for individuals aged 65 and older), Flud (for individuals aged 65 and older), Flud Quadrivalent, and Flucelvax Quadrivalent.

[0034] Preferably, the biologically active material is selected from the group consisting of: Approved vaccines, by brand name, that are associated with the mucosal immune
25 response include: Vaccines: FluMist Quadrivalent: A live attenuated influenza vaccine (LAIV) administered intranasally to stimulate mucosal immunity against influenza; Rotarix: An oral vaccine used to protect against rotavirus, which primarily infects the gastrointestinal mucosa; RotaTaq: Another oral vaccine designed to prevent rotavirus infection; Oral Polio Vaccine (OPV): Used to induce a mucosal immune response against poliovirus in the digestive tract;
30 Cholera Vaccine (Vaxchora): Administered orally to generate mucosal immunity against *Vibrio cholerae*, the bacterium causing cholera; Shigella Vaccine: Several experimental Shigella vaccines are in development to combat Shigella infections, which affect the gastrointestinal mucosa; Adenovirus Type 4 and Type 7 Vaccine (Barr Labs): Used to protect military personnel from acute respiratory disease caused by adenovirus. The
35 adenovirus primarily affects respiratory mucosa; Adenovirus Vaccine (Ad26.COV2.S): One of the COVID-19 vaccines, Janssen's (Johnson & Johnson) Ad26.COV2.S, primarily targets the respiratory mucosa to induce immunity against the SARS-CoV-2 virus.

- [0035] Preferably, the biologically active material is selected from the group consisting of: biologics.
- [0036] Preferably, the biologically active material is selected from the group consisting of: Biologics that can influence the mucosal immune response include: Anti-TNF Biologics:
- 5 Tumor necrosis factor (TNF) is a proinflammatory cytokine that plays a role in various autoimmune diseases. Biologics like Adalimumab (Humira), Infliximab (Remicade), and Etanercept (Enbrel) target TNF and are used to treat conditions such as inflammatory bowel disease (IBD) and rheumatoid arthritis; Anti-IL Biologics: Interleukins (IL) are signalling molecules that regulate immune responses. Biologics like Ustekinumab (Stelara) and
- 10 Vedolizumab (Entyvio) target specific interleukins to treat conditions like Crohn's disease and psoriasis; Anti-IL-17 Biologics: Some biologics, like Secukinumab (Cosentyx), target interleukin-17 (IL-17) to treat conditions like psoriasis and ankylosing spondylitis, which can involve mucosal inflammation; Anti-IL-23 Biologics: Biologics like Guselkumab (Tremfya) target interleukin-23 (IL-23) and are used to treat conditions like plaque psoriasis and
- 15 psoriatic arthritis; Anti-CD Biologics: Monoclonal antibodies targeting cluster of differentiation (CD) markers on immune cells, such as Vedolizumab (Entyvio), can modulate the mucosal immune response in the gastrointestinal tract and treat inflammatory bowel diseases like Crohn's disease and ulcerative colitis; Biologics Targeting B Cells: Rituximab (Rituxan) is a biologic that depletes B cells, which are involved in autoimmune responses. It is used to
- 20 treat conditions like rheumatoid arthritis and certain types of vasculitis; Anti-Integrin Biologics: Natalizumab (Tysabri) is an integrin receptor antagonist that modulates immune cell migration and is used in the treatment of multiple sclerosis; Biologics for Allergic Asthma: Omalizumab (Xolair) is a biologic that targets IgE antibodies and is used in the treatment of allergic asthma.
- 25 [0037] Preferably, the biologically active material is selected from the group consisting of: Approved antibodies, by brand name, that are associated with the mucosal immune response include: Antibodies and Immunotherapies: Palivizumab (Synagis): An antibody used to provide passive immunity against respiratory syncytial virus (RSV) in high-risk infants. RSV primarily affects the respiratory mucosa; Casirivimab and Imdevimab (REGN-
- 30 COV2): A combination of monoclonal antibodies authorized for the treatment of COVID-19. They target the spike protein of the SARS-CoV-2 virus, which infects the respiratory mucosa; Tocilizumab (Actemra): Tocilizumab is an immunosuppressive drug used to treat conditions like cytokine release syndrome (CRS), which can be associated with severe respiratory symptoms in some cases.
- 35 [0038] Preferably, the biologically active material is selected from the group consisting of: H1N1 influenza vaccines include: Flublok Quadrivalent: Developed by Sanofi Pasteur, this vaccine was designed to protect against multiple influenza strains, including H1N1;

- Fluad Quadrivalent: An adjuvanted influenza vaccine from Seqirus that includes protection against H1N1; Vaxigrip Tetra: This is a quadrivalent inactivated influenza vaccine developed by Sanofi Pasteur. It targets several influenza strains, including H1N1; QIVc: An H1N1-specific quadrivalent inactivated vaccine candidate; Influvac Tetra: This is a quadrivalent inactivated influenza vaccine from Mylan that includes protection against H1N1; NanoFlu: Developed by Novavax, NanoFlu is designed to protect against several influenza strains, including H1N1.
- 5 [0039] Preferably, the biologically active material is selected from the group consisting of: H1N1 influenza vaccine; interferon alpha-2a; interferon alpha-2b; infliximab and rituximab.
- 10 [0040] Preferably, the biologically active material is one of the family of interferons or a pharmaceutically acceptable derivative, such as a pegylated derivative or a non-pegylated.
- [0041] Preferably, the biologically active material is preferably selected from the group consisting of: interferon.
- 15 [0042] Preferably, the biologically active material is preferably selected from the group consisting of: interferon type I; interferon type II; interferon type III.
- [0043] Preferably, the biologically active material is preferably selected from the group consisting of: interferon alpha; and interferon beta.
- [0044] Preferably, the biologically active material is preferably selected from the group consisting of: interferon alpha-2a; interferon alpha-2b or a pharmaceutically acceptable derivative, such as a pegylated derivative or a non-pegylated.
- 20 [0045] Preferably, the biologically active material is preferably selected from the group consisting of: interferon alpha (α), beta (β), and gamma (γ) and the two types: type I includes the alpha and beta forms and type II consists of gamma form.
- 25 [0046] Preferably, the interferon may be present in each dosage preferably selected from the group consisting of: 0.01 ng to 1000 μ g; 0.1ng to 100 μ g; 1ng to 10 μ g; 1 ng to 1 μ g; 1 ng to 100ng; 1 nanogram to 20ng.
- [0047] Preferably, the concentration of the interferon may be stated in International Units (IU), where 1ng interferon comprises 100IU.
- 30 [0048] Preferably, the interferons may be present from 1IU to 10,000,000IU, preferably 100IU to 1,000,000IU, more preferably 500IU to 10,000IU.
- [0049] Preferably, the interferons may be present from 1IU to 100,000,000IU, preferably 10IU to 1,000,000IU, more preferably 100IU to 10,000IU, most preferably 100IU to 2000IU.
- [0050] Preferably, the biologically active molecules are components in vaccines for respiratory viruses which initially contact a mucosal surface prior to replication and spread of infection, including those selected from the group consisting of: influenza; COVID-19 (coronavirus disease 2019); and RSV (respiratory syncytial virus).
- 35

- [0051] Preferably, the biologically active material is one of the family of biological molecules designed to elicit an immunogenic response at a mucosal surface, selected from the group consisting of: hemagglutinins; monoclonal antibodies; and viral fragments.
- 5 [0052] Preferably, the biologically active material is preferably selected from the group consisting of: hemagglutinins (HA).
- [0053] Preferably, the HA is present at a level selected from the group consisting of: 0.1 to 1000µg; 1-100µg; 1-50µg; and 5-20µg.
- [0054] Preferably, the biologically active material has a molecular weight selected from the group of ranges consisting of: greater than 1000Da; from 1MDa to 100000MDa; from 10 1kDa to 100000kDa; from 1kDa to 10000kDa; from 1kDa to 1000kDa; from 1kDa to 100kDa; from 1kDa to 50kDa; from 1kDa to 40kDa; from 1kDa to 30kDa; from 1kDa to 20kDa; from 1kDa to 10kDa; from 1kDa to 500kDa; from 2kDa to 400kDa; from 3kDa to 300kDa; from 4kDa to 200kDa; from 5kDa to 100kDa;
- 15 [0055] Preferably, the monoclonal antibodies (MAB) are biologically active against cancer cells.
- [0056] Preferably, the MAB initiates action against cancer cells through contact with the mucosal surface.
- [0057] Preferably, the composition comprises an amorphous non-ionic matrix forming agent.
- 20 [0058] Preferably, the amorphous non-ionic matrix forming agent is selected from the group consisting of: amylopectin; an agent selected from the amylopectin family; and amylogum CLS.
- [0059] Preferably, the amorphous non-ionic matrix forming agent is selected from the group consisting of: amylopectin.
- 25 [0060] Preferably, the amorphous non-ionic matrix forming agent is present at a concentration selected from the group consisting of: 5-20%; 8-10%; or 13-14%; 5%; 6%; 7%; 8%; 9%; 10%; 11%; 12%; 13%; 14%; 15%; 16%; 17%; 18%; 19%; 20% and within 2 decimal points of each figure (w/w%).
- [0061] Preferably, the amylopectin is not in the form of starch or modified starch.
- 30 [0062] Preferably, the amylopectin, which has a low viscosity even at elevated concentrations, essential for providing a scaffold for the monosaccharide particles and allowing non-friability and handleability, was found to be most useful at 10-20% w/w.
- [0063] Preferably, the composition comprises an ionic water soluble post-disintegration viscosity agent.
- 35 [0064] Preferably, the ionic water soluble post-disintegration viscosity agent is selected from the group consisting of: anionic substituted cellulose or starch derivatives, such as salts of carboxymethyl celluloses (CMC) or starch glycolates.

- [0065] Preferably, the ionic water soluble post-disintegration viscosity agent is present at a concentration selected from the group consisting of: 0.5%-10%; 0.5%; 1%-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w).
- 5 [0066] Preferably, the ionic water soluble post-disintegration viscosity agent is present at a concentration 1.5-2.5% (w/w).
- [0067] Preferably, the ionic water soluble post-disintegration viscosity agent is present at a concentration 1.0-5.0% (w/w).
- [0068] Preferably, the optimum levels of alginic acid and CMC were discovered by experiment to be about 2.0% w/w solids content in the final wafer, in the ratio 1:1, with a
10 useful range of 1.5-2.5% w/w.
- [0069] Preferably, the absolute level of the two polymers used for this invention, alginic acid salt and carboxymethyl cellulose salt (CMC), is important to the performance of the wafer.
- [0070] Preferably, levels too low do not provide enough viscosity for the disintegrated
15 film to survive too long.
- [0071] Preferably, levels too high make the wafer too viscous to manufacture end dispense, and form a poorly-disintegrating wafer.
- [0072] Preferably, the composition comprises a second ionic water soluble post-disintegration viscosity agent.
- 20 [0073] Preferably, the second ionic water soluble post-disintegration viscosity agent is selected from the group consisting of: anionic substituted natural polysaccharides, such as salts of alginates or pectinate, including sodium alginate.
- [0074] Preferably, the second ionic water soluble post-disintegration viscosity agent is present at a concentration selected from the group consisting of: 0.5%; 1%-10%; 1%-10%;
25 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w%).
- [0075] Preferably, the second ionic water soluble post-disintegration viscosity agent is present at a concentration 1.5-2.5% (w/w).
- [0076] Preferably, the second ionic water soluble post-disintegration water soluble
30 viscosity agent is present at a concentration 1.0-5.0% (w/w).
- [0077] Preferably, the ionic water soluble post-disintegration viscosity agent and the second ionic water soluble post-disintegration viscosity agent are preferably present in the composition in the ratio 0.5-1.5, with a useful range of 1.5-2.5% w/w for each agent.
- [0078] Preferably, the total polymer concentration is at or within the range of 4-6%
35 (w/w), with each viscosity agent is at or within the range of 2-3%.
- [0079] Preferably, the composition comprises an adjuvant.
- [0080] Preferably, the composition comprises a plurality of adjuvants.

- [0081] Preferably, the adjuvant is selected from the group consisting of: squalene, aluminium salts, such as aluminium hydroxide, aluminium phosphate, and aluminium potassium sulfate and alpha-tocopherol.
- [0082] Preferably, the adjuvant is selected from the group consisting of squalene.
- 5 [0083] Preferably, the adjuvant is present at a concentration selected from the group consisting of: 0.1%-10%; 0.5%, 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w).
- [0084] Preferably, the adjuvant is present at a concentration 0.5-5% (w/w).
- [0085] Preferably, the adjuvant selected from the group consisting of: aluminium salts,
- 10 such as aluminium hydroxide, aluminium phosphate, and aluminium potassium sulfate.
- [0086] Preferably, the aluminium salt is present at a concentration selected from the group consisting of: 0.1%-10%; 0.5%, 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w).
- [0087] Preferably, the aluminium salt is present at a concentration 0.1-5% (w/w).
- 15 [0088] Preferably, the adjuvant selected from the group consisting of alpha-tocopherol.
- [0089] Preferably, the alpha-tocopherol is present at a concentration selected from the group consisting of: 0.01%-5%; and within 2 decimal points of each figure (w/w).
- [0090] Preferably, the alpha tocopherol is present at a concentration 0.01-0.5% (w/w).
- [0091] Preferably, the composition comprises a low MW ionic matrix agent.
- 20 [0092] Preferably, the low MW ionic matrix agent is selected from the group consisting of: amino acids.
- [0093] Preferably, the low MW ionic matrix agent is glycine.
- [0094] Preferably, the low MW ionic matrix agent is present at a concentration selected from the group consisting of: 1%-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and
- 25 within 2 decimal points of each figure (w/w).
- [0095] Preferably, the composition comprises a second non-ionic matrix agent.
- [0096] Preferably, the second non-ionic matrix agent is selected from the group consisting of: microcrystalline cellulose (MCC).
- [0097] Preferably, the second non-ionic matrix agent is present at a concentration
- 30 selected from the group consisting of: 1-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w%).
- [0098] Preferably, the composition comprises a non-ionic crystalline matrix forming agent.
- [0099] Preferably, the non-ionic crystalline matrix forming agent is selected from the
- 35 group consisting of: mannitol.
- [00100] Preferably, the non-ionic crystalline matrix forming agent is present at a concentration selected from the group consisting of: 20-50%; 5-50%; 5%; 6%; 7%; 8%; 9%;

- 10%; 11%; 12%; 13%; 14%; 15%; 16%; 17%; 18%; 19%; 20%; 21%; 22%; 23%; 24%; 25%; 26%; 27%; 28%; 29%; 30%; 31%; 32%; 33%; 34%; 35%; 36%; 37%; 38%; 39%; 40%; 41%; 42%; 43%; 44%; 45%; 46%; 47%; 48%; 49%; 50%; and within 2 decimal points of each figure (w/w).
- 5 [00101] Preferably, monosaccharides, preferably lactose and mannitol, were useful for the preparation of an ionically neutral, highly water-soluble matrix interpenetrated with a network of pores for rapid disintegration and dissolution.
- [00102] Preferably,, the combined concentration was found to be preferably most useful at 65-80% w/w.
- 10 [00103] Preferably, the composition comprises a second non-ionic crystalline matrix forming agent.
- [00104] Preferably, the second non-ionic crystalline matrix forming agent is selected from the group consisting of: lactose; and lactose monohydrate.
- [00105] Preferably, the second non-ionic matrix agent is present at a concentration
- 15 selected from the group consisting of: 5-50%; 5%; 6%; 7%; 8%; 9%; 10%; 11%; 12%; 13%; 14%; 15%; 16%; 17%; 18%; 19%; 20%; 21%; 22%; 23%; 24%; 25%; 26%; 27%; 28%; 29%; 30%; 31%; 32%; 33%; 34%; 35%; 36%; 37%; 38%; 39%; 40%; 41%; 42%; 43%; 44%; 45%; 46%; 47%; 48%; 49%; 50%; and within 2 decimal points of each figure (w/w%).
- [00106] Preferably, the composition comprises a further non-ionic matrix agent.
- 20 [00107] Preferably, the further non-ionic matrix agent is selected from the group consisting of: PEG1000-20,000, preferably PEG 1500.
- [00108] Preferably, the further non-ionic matrix agent is present at a concentration selected from the group consisting of: 1%-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w).
- 25 [00109] Preferably, the composition comprises has a pH selected from the group consisting of between 5 to 6; between 4 to 7.5; and between 7.0 – 7.5.
- [00110] Preferably, the composition comprises a pharmaceutically acceptable buffering reagent.
- [00111] Preferably, the buffering reagent is selected from the group consisting of: salts of
- 30 phosphates, carbonates, Tris; preferably combinations of phosphate salts; including sodium carbonate.
- [00112] Preferably, the buffering reagent is present at a concentration selected from the group consisting of: 0.1-0.5%; 0.1%; 0.2%; 0.3%; 0.4%; 0.5%; and within 2 decimal points of each figure (w/w).
- 35 [00113] Preferably, the wafers are buffered with a suitable buffer, most preferably a blend of mono- and di-alkaline salt phosphates, adjusted to afford a pH optimal for the stability of the chosen biological active, generally between 4 and 7.5.

- [00114] Preferably, the composition comprises a colouring agent.
- [00115] Preferably, the composition comprises a flavouring agent.
- [00116] Preferably, the composition comprises a stability agent.
- [00117] Preferably, the stability agent is selected from the group consisting of: metal
 5 chelators such as EDTA, pharmaceutically acceptable surfactant including polysorbate 80.
- [00118] Preferably, the composition comprises an anti-protease agent, such as such as antipeptidases or antitrypsin.
- [00119] Preferably, the composition disintegrates once placed in the oral cavity in a time period selected from the group consisting of: <10s; <20s; <30s; <40s; <50s; <60s; <70s;
 10 <80s; <90s; <100s; <110s; <120s (seconds).
- [00120] Preferably, the composition disintegrates once placed in the oral cavity in a time period selected from the group consisting of: <10s; <20s; <30s; <40s; <50s; <60s; <70s; <80s; <90s; <100s; <110s; <120s; <130s; <140s; <150s; <160s; <170s; <180s; <190s; <200s; <210s; <220s; <230s; <240s; <250s; <260s; <270s; <280s; <290s; <300s; <310s;
 15 <320s; <330s; <340s; <350s; <360s; <370s; <380s; <390s; <400s; <410s; <420s; <430s; <440s; <450s; <460s; <470s; <480s; <490s; <500s; <510s; <520s; <530s; <540s; <550s; <560s; <570s; <580s; <590s; <600s; <610s; <620s; <630s; <640s; <650s; <660s; <670s; <680s; <690s; <700s; <710s; <720s; <730s; <740s; <750s; <760s; <770s; <780s; <790s; <800s; <810s; <820s; <830s; <840s; <850s; <860s; <870s; <880s; <890s; <900s; <900s;
 20 <930s; <960s; <990s; <1020s; <1050s; <1080s; <1110s; <1140s; <1170s; <1200s; <1230s; <1260s; <1290s; <1320s; <1350s; <1380s; <1410s; <1440s; <1470s; <1500s; <1530s; <1560s; <1590s; <1620s; <1650s; <1680s; <1710s; <1740s; <1770s; <1800s; <1830s; <1860s; <1890s; <1920s; <1950s; <1980s; <2010s; <2040s; <2070s; <2100s; <2130s; <2160s; <2190s; <2220s; <2250s; <2280s; <2310s; <2340s; <2370s; <2400s; <2430s;
 25 <2460s; <2490s; <2520s; <2550s; <2580s; <2610s; <2640s; <2670s; <2700s; <2730s; <2760s; <2790s; <2820s; <2850s; <2880s; <2910s; <2940s; <2970s; <3000s; <3030s; <3060s; <3090s; <3120s; <3150s; <3180s; <3210s; <3240s; <3270s; <3300s; <3330s; <3360s; <3390s; <3420s; <3450s; <3480s; <3510s; <3540s; <3570s; <3600s; <3630s; <3660s; <3690s; <3720s; <3750s; <3780s; <3810s; <3840s; <3870s; <3900s; <3930s;
 30 <3960s; <3990s; <4020s; <4050s; <4080s; <4110s; <4140s; <4170s; <4200s; and <4230s (seconds).
- [00121] Preferably, the composition disintegrates in the oral cavity without leaving a residue of said dosage form in the oral cavity that is detectable by a subject, thereby avoiding the urge for the subject to swallow the composition.
- [00122] Preferably, the composition dissolves once placed in the oral cavity in a time period selected from the group consisting of: <10s; <20s; <30s; <40s; <50s; <60s; <70s; <80s; <90s; <100s; <110s; <120s; 180s, preferably 15-60s (seconds).

[00123] Preferably, the composition dissolves once placed in the oral cavity in a time period selected from the group consisting of: <10s; <20s; <30s; <40s; <50s; <60s; <70s; <80s; <90s; <100s; <110s; <120s; <130s; <140s; <150s; <160s; <170s; <180s; <190s; <200s; <210s; <220s; <230s; <240s; <250s; <260s; <270s; <280s; <290s; <300s; <310s; <320s; <330s; <340s; <350s; <360s; <370s; <380s; <390s; <400s; <410s; <420s; <430s; <440s; <450s; <460s; <470s; <480s; <490s; <500s; <510s; <520s; <530s; <540s; <550s; <560s; <570s; <580s; <590s; <600s; <610s; <620s; <630s; <640s; <650s; <660s; <670s; <680s; <690s; <700s; <710s; <720s; <730s; <740s; <750s; <760s; <770s; <780s; <790s; <800s; <810s; <820s; <830s; <840s; <850s; <860s; <870s; <880s; <890s; <900s; <900s; <930s; <960s; <990s; <1020s; <1050s; <1080s; <1110s; <1140s; <1170s; <1200s; <1230s; <1260s; <1290s; <1320s; <1350s; <1380s; <1410s; <1440s; <1470s; <1500s; <1530s; <1560s; <1590s; <1620s; <1650s; <1680s; <1710s; <1740s; <1770s; <1800s; <1830s; <1860s; <1890s; <1920s; <1950s; <1980s; <2010s; <2040s; <2070s; <2100s; <2130s; <2160s; <2190s; <2220s; <2250s; <2280s; <2310s; <2340s; <2370s; <2400s; <2430s; <2460s; <2490s; <2520s; <2550s; <2580s; <2610s; <2640s; <2670s; <2700s; <2730s; <2760s; <2790s; <2820s; <2850s; <2880s; <2910s; <2940s; <2970s; <3000s; <3030s; <3060s; <3090s; <3120s; <3150s; <3180s; <3210s; <3240s; <3270s; <3300s; <3330s; <3360s; <3390s; <3420s; <3450s; <3480s; <3510s; <3540s; <3570s; <3600s; <3630s; <3660s; <3690s; <3720s; <3750s; <3780s; <3810s; <3840s; <3870s; <3900s; <3930s; <3960s; <3990s; <4020s; <4050s; <4080s; <4110s; <4140s; <4170s; <4200s; and <4230s (seconds).

[00124] Preferably, the composition dissolves in the oral cavity without leaving a residue of said dosage form in the oral cavity that is detectable by a subject, thereby avoiding the urge for the subject to swallow the composition.

[00125] Preferably, the composition has a viscosity profile of: CMC and sodium alginate.

[00126] Preferably, the disintegrated bolus forms a "dome" with structure given by a judicious level of two main water-soluble polymeric chains.

[00127] Preferably, the polymers give the "dome" viscosity, keeping it in position and delaying drainage and swallowing, and also delay the ingress of saliva, the long chain mucopolysaccharides of which have difficulty in penetrating the "dome".

[00128] Preferably, the viscosity is not so great that bioactive are prevented from diffusing to the epithelial layer continuously (unlike solid film wafers which "melt" and trap the active in a highly viscous matrix with very little water present).

[00129] Preferably, the difference is engineered by the highly porous nature of the wafer, which rapidly absorbs extant rinse-water by capillary action to cause rapid disintegration and dissolution (wafer films have no pores, and so trap the water on the outside of the matrix).

- [00130] Preferably, by these features, the bioactive molecules are given a useful time of contact before being overwhelmed with excess saliva and inadvertent mouth movement (2-10 minutes).
- 5 [00131] Preferably, the composition has a solubility profile selected from the group consisting of: Preferably, the composition is greater than 96% water soluble.
- [00132] Preferably, the composition has a solubility profile generated by an agent selected from the group consisting of: amylopectin; mannitol; lactose; viscosity agents; and
- [00133] Preferably, the composition is greater than 96% water soluble.
- 10 [00134] Preferably, the composition has a non-ionisable profile selected from the group consisting of: >96% non-ionisable; >90% non-ionisable; and 65-75% non-ionisable.
- [00135] Preferably, the composition has a non-ionisable profile generated by an agent selected from the group consisting of: amylopectin, mannitol, lactose; and Preferably, the composition is >96% non-ionisable; >90% non-ionisable; 65-75% non-ionisable.
- 15 [00136] Preferably, water is removed from the combined agents by a freeze-drying process to a residual level of 0-10%, preferably 0-5%, most preferably 3-5%.
- [00137] Preferably, the composition has a porosity profile selected from the group consisting of: the proportion of water removed by lyophilization is 65-75%; and the proportion of water removed by lyophilization is >60%.
- [00138] Preferably, the composition has a porosity profile generated by an agent selected from the group consisting of: amylopectin; mannitol; lactose; and Preferably, the proportion of water removed by lyophilization is 65-75%; and the proportion of water removed by lyophilization is >60%.
- 20 [00139] Preferably, the composition comprises components as listed in any one of Compositions A to O.
- 25 [00140] Preferably, the composition comprises components as listed in any one of Compositions A to H.
- [00141] Preferably, the composition is selected from the group consisting of: Composition A; Composition B; Composition C; Composition D; Composition E; Composition F; Composition G; Composition H – Option A; Composition H – Option B; and Composition H – Option C; Composition I; Composition J; Composition K; Composition L; Composition M; Composition N; Composition O.
- 30 [00142] Preferably, the composition is selected from the group consisting of: Composition H – Option A; Composition H – Option B; and Composition H – Option C.
- [00143] Preferably, the composition is selected from the group consisting of: Composition I; Composition J; and Composition L.
- 35 [00144] Preferably, the composition is selected from the group consisting of: Composition I; Composition J; Composition K; Composition L.

- [00145] Preferably, the mucosal surface is selected from the group consisting of: oral; buccal; rectal; vaginal; oculi; otic; nasal.
- [00146] Preferably, the mucosal surface is selected from the group consisting of: oral; buccal; nasal.
- 5 [00147] Preferably, the composition is stable for a time period selected from the group consisting of: 1 week; 2 weeks; 3 weeks; 4 weeks; 5 weeks; 6 weeks; 7 weeks; 8 weeks; 9 weeks; 10 weeks; 11 weeks; 12 weeks; 13 weeks; 14 weeks; 15 weeks; 16 weeks; 17 weeks; 18 weeks; 19 weeks; 20 weeks; 21 weeks; 22 weeks; 23 weeks; 24 weeks; 25 weeks; 26 weeks; 27 weeks; 28 weeks; 29 weeks; 30 weeks; 31 weeks; 32 weeks; 33 weeks; 34 weeks; 35 weeks; 36 weeks; 37 weeks; 38 weeks; 39 weeks; 40 weeks; 41 weeks; 42 weeks; 43 weeks; 44 weeks; 45 weeks; 46 weeks; 47 weeks; 48 weeks; 49 weeks; 50 weeks; 51 weeks; 52 weeks; 1 month; 2 months; 3 months; 4 months; 5 months; 6 months; 7 months; 8 months; 9 months; 10 months; 11 months; 12 months; 13 months; 14 months; 15 months; 16 months; 17 months; 18 months; 19 months; 20 months; 21 months; 22 months; 23 months; 24 months; 25 months; 26 months; 27 months; 28 months; 29 months; 30 months; 31 months; 32 months; 33 months; 34 months; 35 months; 36 months; 37 months; 38 months; 39 months; 40 months; 41 months; 42 months; 43 months; 44 months; 45 months; 46 months; 47 months; 48 months; 49 months; 50 months; 51 months; 52 months; 53 months; 54 months; 55 months; 56 months; 57 months; 58 months; 59 months; 60 months; 1 month to 12 months; 12 months to 24 months; 12 months to 36 months; 12 months to 48 months; 1 year to 2 years; 1 year to 3 years; and 1 year to 5 years.
- 15 [00148] Preferably, the composition is stable as demonstrated by a potency assay run against a reference biological active material.
- [00149] In another aspect, the invention is a pharmaceutical composition comprising the composition of an aspect of the invention together with a pharmaceutical excipient or carrier.
- 25 [00150] In another aspect, the invention is a dosage form comprising the composition of an aspect of the invention.
- [00151] Preferably, the dosage form comprises the biologically active material at a dose selected from the group consisting of: between 0.001 to 100mg; between 2 and 50mg; between 5 and 40mg; between 10 and 30mg; and between 20 and 25mg.
- 30 [00152] Preferably, the dosage form comprises the biologically active material at a dose selected from the group consisting of: between 1 nanogram to 100mg; 1 nanogram to 10mg; 1 nanogram to 1mg; 1 nanogram to 100µg; 1 nanogram to 1µg.
- [00153] Preferably, the dosage form comprises the biologically active material at a dose selected from the group consisting of: between 0.1 ng to 1000 µg; 1 nanogram to 10µg; 1 nanogram to 1µg; 1 nanogram to 100ng; 1 nanogram to 10ng.
- 35 [00154] Preferably, the dosage form comprises the composition of HA.

- [00155] Preferably, the dosage form for influenza vaccines comprises the biologically active material at a dose selected from the group consisting of: from 0.1 to 1000µg; 1-100µg; 1-50µg, preferably 5-20µg.
- 5 [00156] Preferably, the dosage form for COVID-19 vaccines comprises the biologically active material at a dose selected from the group consisting of: from 0.1µg to 100mg; 10µg-10mg; 100µg to 5mg, preferably 200µg to 2mg.
- [00157] Preferably, the dosage form comprises the biologically active material at a dose selected from the group consisting of: from 0.1 to 1000µg; 1-100µg; 1-50µg; and 5-20µg.
- 10 [00158] Preferably, the biologically active material is selected from the group consisting of: interferon alpha-1a (or alpha 1 beta); interferon alpha-2b (or alpha 2 beta); ;interferon beta-1a; or any other interferon or combination, including pharmaceutically acceptable pegylates and non-pegylates.
- [00159] In another aspect, the invention is a vaccine comprising the composition of an aspect of the invention.
- 15 [00160] Preferably, the vaccine develops a biological response to treat or prevent disease or disorder selected from the group consisting of: H1N1; RSV; COVID-19; and any mucosal viral or bacterial infection targeting the respiratory tract.
- [00161] In another aspect, the invention is a method of treating or preventing or delaying a disease or disorder, said method comprising administering to a patient in need thereof a
- 20 therapeutically effective amount of the dosage form of an aspect of the invention.
- [00162] Preferably, the disease or disorder is selected from the group consisting of: influenza, RSV, whooping cough (pertussis) or COVID-19.
- [00163] Preferably, the disease or disorder is selected from the group consisting of:
- 25 Infectious Diseases: (such as Bacterial infections, viral infections, fungal infections, parasitic infections, sexually transmitted infections (STIs), tropical diseases, zoonotic diseases); Cardiovascular Diseases: (such as Coronary artery disease, hypertension (high blood pressure), heart failure, arrhythmias, stroke, vascular diseases); Respiratory Diseases: (such as Asthma, chronic obstructive pulmonary disease (COPD), pneumonia, tuberculosis, lung cancer); Neurological Diseases: (such as Alzheimer's disease, Parkinson's disease, multiple
- 30 sclerosis, epilepsy, stroke, autoinflammatory diseases); Gastrointestinal Diseases: (such as Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophageal reflux disease (GERD), cirrhosis, colorectal cancer); Endocrine and Metabolic Diseases: (such as Diabetes mellitus, thyroid disorders, obesity, Cushing's syndrome, Addison's disease, hormonal disorders); Autoimmune Diseases: (such as Rheumatoid arthritis, systemic lupus
- 35 erythematosus, multiple sclerosis, type 1 diabetes, psoriasis, autoimmune skin diseases, autoimmune thyroid diseases, autoimmune gastrointestinal diseases); Cancer: (such as Breast cancer, lung cancer, prostate cancer, leukemia, skin cancer); Genetic Disorders:

- (such as Down syndrome, cystic fibrosis, Huntington's disease, hemophilia, sickle cell anemia, rare genetic syndromes); Musculoskeletal Diseases: (such as Osteoarthritis, rheumatoid arthritis, osteoporosis, muscular dystrophy, fibromyalgia, rheumatic diseases); Renal (Kidney) Diseases: (such as Chronic kidney disease, acute kidney injury, polycystic kidney disease, nephrotic syndrome, kidney stones); Skin Diseases: (such as Eczema, psoriasis, acne, skin cancer, vitiligo); Hematological Diseases: (such as Anemia, thrombocytopenia, hemophilia, leukemia, lymphoma); Psychiatric Disorders: (such as Depression, anxiety disorders, schizophrenia, bipolar disorder, obsessive-compulsive disorder (OCD)); Immunodeficiency Disorders: (such as HIV/AIDS, primary immunodeficiency diseases, severe combined immunodeficiency (SCID)); Eye and Ear Disorders: (such as Glaucoma, cataracts, macular degeneration, deafness, and hearing disorders); Reproductive and Gynecological Diseases: (such as Polycystic ovary syndrome (PCOS), endometriosis, infertility, ovarian cancer, testicular cancer, reproductive system diseases); Oral and Dental Diseases: (such as Tooth decay (cavities), gum disease, oral cancer, temporomandibular joint disorder (TMJ)); Allergic Diseases: (such as Allergic rhinitis (hay fever), food allergies, eczema, asthma); Rare and Orphan Diseases: (such as Huntington's disease, amyotrophic lateral sclerosis (ALS), Pompe disease, Niemann-Pick disease, Prader-Willi syndrome); Nutritional Diseases: (such as Malnutrition, obesity, anorexia nervosa, bulimia nervosa, vitamin deficiencies); Rheumatic Diseases: (such as Spondyloarthropathies, juvenile idiopathic arthritis, polymyalgia rheumatica, giant cell arteritis); Vascular Diseases: (such as Atherosclerosis, varicose veins, deep vein thrombosis (DVT), peripheral artery disease (PAD)); Liver Diseases: (such as Hepatitis (A, B, C, etc.), non-alcoholic fatty liver disease (NAFLD), primary biliary cholangitis, Wilson's disease); Urinary Tract Diseases: (such as Urinary tract infections (UTIs), bladder disorders, interstitial cystitis, urolithiasis (kidney stones)); Sleep Disorders: (such as Insomnia, sleep apnea, narcolepsy, restless legs syndrome, circadian rhythm disorders).
- [00164] Preferably, the disease or disorder is selected from the group consisting of: infectious diseases: (bacterial infections, viral infections, fungal infections, parasitic infections); cardiovascular diseases: (coronary artery disease, hypertension (high blood pressure), heart failure, arrhythmias); neurological disorders: (alzheimer's disease, parkinson's disease, epilepsy, multiple sclerosis); respiratory diseases: (asthma, chronic obstructive pulmonary disease (COPD), pneumonia, tuberculosis); gastrointestinal disorders: (irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophageal reflux disease (GERD), liver cirrhosis); endocrine disorders: (diabetes mellitus, thyroid disorders, cushing's syndrome, addison's disease); autoimmune diseases: (rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, crohn's disease); musculoskeletal disorders: (osteoarthritis, rheumatoid arthritis, osteoporosis, muscular dystrophy); cancer:

(breast cancer, lung cancer, colon cancer, leukemia); mental health disorders: (depression, anxiety disorders, bipolar disorder, schizophrenia); genetic disorders: (down syndrome, cystic fibrosis, huntington's disease, hemophilia); skin disorders: (eczema, psoriasis, acne, melanoma); Immunodeficiency Disorders: (HIV/AIDS, primary immunodeficiency, severe combined immunodeficiency (SCID); Renal (kidney) diseases: (chronic kidney disease, kidney stones, polycystic kidney disease, glomerulonephritis); ophthalmic (eye) disorders: (glaucoma, cataracts, macular degeneration, retinitis pigmentosa); haematological disorders: (anemia, hemophilia, thrombocytopenia, sickle cell disease); Allergies: (hay fever (allergic rhinitis), food allergies, allergic asthma, anaphylaxis); reproductive health disorders: (infertility, polycystic ovary syndrome (PCOS), erectile dysfunction, endometriosis); metabolic disorders: (phenylketonuria (PKU), gaucher disease, tay-sachs disease, wilson's disease); rare and orphan diseases: (huntington's disease, amyotrophic lateral sclerosis (ALS), pompe disease, prader-willi syndrome).

[00165] Preferably, the disease or disorder is selected from the group consisting of: infectious diseases: (such as bacterial infections, viral infections, fungal infections, parasitic infections, sexually transmitted infections (STIs), tropical diseases, zoonotic diseases); autoimmune Diseases: (such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type 1 diabetes, psoriasis, autoimmune skin diseases, autoimmune thyroid diseases, autoimmune gastrointestinal diseases); and Cancer: (such as breast cancer, lung cancer, prostate cancer, leukemia, skin cancer).

[00166] Preferably, the disease or disorder is a viral infection selected from the group consisting of: Flaviviridae (Dengue fever; Zika virus infection; Yellow fever); Herpesviridae (Herpes simplex virus (HSV) infection; Varicella-zoster virus (VZV) infection (Chickenpox and Shingles); Epstein-Barr virus (EBV) infection (Mononucleosis); Cytomegalovirus (CMV) infection; Human herpesvirus 6 (HHV-6) infection); Retroviridae (Human immunodeficiency virus (HIV) infection (AIDS)); Orthomyxoviridae (Influenza (Flu)); Paramyxoviridae (Measles (Rubeola); Mumps); Poxviridae (Smallpox (Variola)); Coronaviridae (COVID-19 (SARS-CoV-2)); Papillomaviridae (Human papillomavirus (HPV) infection (Causes various types of warts, and some strains are associated with cervical cancer)); Picornaviridae (Common cold (Rhinovirus)); Hepadnaviridae (Hepatitis B); Caliciviridae (Norovirus infection (Viral gastroenteritis)); Togaviridae (Rubella (German Measles)); Adenoviridae: (Respiratory adenovirus infections); Bunyaviridae (Hantavirus infection); Rhabdoviridae (Rabies).

[00167] Preferably, the disease or disorder is a bacterial infection selected from the group consisting of: Streptococcus pyogenes (Group A Streptococcus) - Causes strep throat, skin infections, and more; Staphylococcus aureus - Responsible for various skin and soft tissue infections; Escherichia coli (E. coli) - Can lead to urinary tract infections, food poisoning, and other illnesses; Salmonella - Causes foodborne infections; Mycobacterium

- tuberculosis - Responsible for tuberculosis (TB); *Neisseria gonorrhoeae* - Causes gonorrhea; *Chlamydia trachomatis* - Responsible for chlamydia infections; *Helicobacter pylori* - Linked to peptic ulcers and gastritis; *Clostridium difficile* - Can cause antibiotic-associated diarrhea and colitis; *Streptococcus pneumoniae* - A common cause of pneumonia and other respiratory infections.
- 5 [00168] Preferably, the disease or disorder is treated by initiating a mucosal immune.
- [00169] Preferably, the disease or disorder is treated by initiating a mucosal immune is selected from the group consisting of: Respiratory infections such as the common cold, influenza, and pneumonia, as well as gastrointestinal infections like those caused by
- 10 rotavirus, norovirus, and foodborne bacteria (e.g., *Salmonella*, *E. coli*); *Helicobacter pylori* infections, which can lead to gastritis and peptic ulcers, involve the stomach's mucosal lining; Sexually transmitted infections like gonorrhea and chlamydia primarily affect the genital and urinary mucosal surfaces; Additionally, conditions like inflammatory bowel diseases, allergic rhinitis, asthma, sinusitis, otitis media, and other mucosal inflammations
- 15 require treatment strategies that target or modulate the mucosal immune response.
- [00170] Preferably, the dosage form is administered to deliver a therapeutic effective amount of the biological active material.
- [00171] Preferably, the therapeutically effective amount is administered to the subject utilising a dosing regimen selected from the group consisting of: twice hourly; hourly; once
- 20 every six hours; once every 8 hours; once every 12 hours; once daily; twice weekly; once weekly; once every 2 weeks; once every 6 weeks; once a month; every 2 months; every 3 months; once every 6 months; once yearly; and once.
- [00172] Preferably, the dosage form delivers a therapeutic effective amount of the biologically active material.
- 25 [00173] Preferably, the therapeutically effective amount is an amount of biologically active material selected from the group consisting of: between 0.001 to 100mg/kg; between 2 and 50mg/kg; between 5 and 40mg/kg; between 10 and 30mg/kg; between 20 and 25mg/kg; and 20mg/kg/day.
- [00174] Preferably, the therapeutically effective amount is an amount of biologically
- 30 active material selected from the group consisting of: between 0.01 nanogram to 100mg; 1 nanogram to 10mg; 1 nanogram to 1mg; 1 nanogram to 100µg; 1 nanogram to 1µg per dosage event.
- [00175] Preferably, the therapeutically effective amount is an amount of biologically
- 35 active material selected from the group consisting of: between 1 nanogram to 100mg; 1 nanogram to 10mg; 1 nanogram to 1mg; 1 nanogram to 100µg; 1 nanogram to 1µg per day.

- [00176] Preferably, the therapeutically effective amount is an amount of biologically active material selected from the group consisting of: between 0.1 ng to 1000 µg; 1 nanogram to 10µg; 1 nanogram to 1µg; 1 nanogram to 100ng; 1 nanogram to 10ng per day.
- 5 [00177] Preferably, the therapeutically effective amount is administered to the subject using a method selected from the group consisting of: orally; sublingually; buccally; rectally; vaginally; mucosally; by the ocular route; by the otic route; nasally; and by inhalation.
- [00178] Preferably, the therapeutically effective amount is administered to the subject using a method selected from the group consisting of: beneath the tongue or buccally.
- [00179] Preferably, the biologically active material initiates a mucosal immune response
10 against the antigen.
- [00180] Preferably, the biologically active material does not enter the circulation induces an immune cascade via surface contact and surface function.
- [00181] Preferably, the biologically active material induces an immune cascade via surface contact and surface function.
- 15 [00182] Preferably, the biologically active material does not enter the circulation system.
- [00183] Preferably, no detectable levels of the biologically active material enters the circulation system.
- [00184] Preferably, the detectable levels of the biologically active material that enters
20 the circulation system is selected from the group consisting of: less than 0.001%; less than 0.01%; less than 0.1%; less than 2%; less than 3%; less than 4%; less than 5%; less than 6%; less than 7%; less than 8%; less than 9%; less than 10%; less than 11%; less than 12%; less than 13%; less than 14%; less than 15%; less than 16%; less than 17%; less than 18%; less than 19%; less than 20%; less than 21%; less than 22%; less than 23%;
25 less than 24%; less than 25%; less than 26%; less than 27%; less than 28%; less than 29%; less than 30%; less than 31%; less than 32%; less than 33%; less than 34%; less than 35%; less than 36%; less than 37%; less than 38%; less than 39%; less than 40%; less than 41%; less than 42%; less than 43%; less than 44%; less than 45%; less than 46%; less than 47%; less than 48%; less than 49%; less than 50%; less than 51%; less than 52%; less than 53%; less than 54%; less than 55%; less than 56%; less than 57%;
30 less than 58%; less than 59%; less than 60%; less than 61%; less than 62%; less than 63%; less than 64%; less than 65%; less than 66%; less than 67%; less than 68%; less than 69%; less than 70%; less than 71%; less than 72%; less than 73%; less than 74%; and less than 75% (w/w%).
- 35 [00185] Preferably, said biologically active material is not absorbed by diffusion directly into the systemic circulation.

- [00186] Preferably, said method comprises one or more phases selected from the group consisting of: PHASE 1: Sublingual Administration: Post administration of sublingual vaccine/biologic onto the sublingual space (0 min); PHASE 2: Adhesion Immunogenic Precursor Phase: Time 1 to 20 mins (from antigens/vaccines/biologics touching the SL mucosa that is after wafer disintegration); adhesion/adsorption/binding of vaccine/biologic to the lamina propria mucosal cells (more specifically to the M cells or Microfold cells, Langerhans cells or LCs or dendritic cells or DCs) then within 15 to 20 min, reactions with the immune cells (immunogenic precursor). Mucosal M cells and mucosal Langerhans cells are specialized cells found in the mucosal surfaces of the body. They have distinct functions and characteristics. Following the attachment of vaccines/biologics by adhesion/adsorption to the apical membrane of the M cells allow the active transportation of biologics (e.g. vaccine/biologic particles) across the epithelial cell layer where interactions with immune cells can take place. M cells have the unique ability to take up biologics (e.g. vaccines, interferons, biologics) via endocytosis, phagocytosis, or transcytosis. These biologics are delivered to antigen-presenting cells, such as dendritic cells, and B lymphocytes triggering the adaptive immunity (immune potentiators). Langerhans cells are a type of dendritic cell and play a vital role in biologics presentation and initiation of immune responses. They capture vaccines/biologics, process them, and present them to T cells in lymph nodes, which is essential for the initiation of adaptive immune responses. In summary, mucosal M cells primarily function in transporting antigens across the mucosal barrier to initiate immune responses in mucosal-associated lymphoid tissues, while mucosal Langerhans cells function in antigen presentation and immune activation in both the skin and mucosal surfaces. Hence, sublingual vaccines/biologics usually do not reach the bloodstream but are captured by these antigen-presenting M cells, LCs cells and DCs cells, in the epithelia mucosa. This biologic uptake is then process in the cells and present their epitopes to T cells and B cells for the induce effective adaptive immunity (memory immunity cells) which can then be distributed to other parts of the body via lymphatic drainage; PHASE 3: Immune Sentinel Activation Phase: Time 20 mins to 2 hours: antigen/vaccine/biologic attached to the induced immune sentinel activation by LCs and dendritic cells released cytokines and chemokines within 30mins to 2 hours and attracts more immune cells to the site of SL mucosa to fight invasion and infection; and PHASE 4: Fingerprints of Specific Infections Professional Antigen-Presenting T, B and NK cells to Fight Specific Infections: Time by day 3: antigen specific B and T effector cells start to disseminate to other lymph nodes triggering humoral immune responses expected in 2 weeks.
- [00187] Preferably, the dosage form is administered to deliver a therapeutic effective amount of the interferon or vaccine.

- [00188] Preferably, the dosage form is administered to deliver a therapeutic effective amount of the interferon.
- [00189] In another aspect, the invention is a method of vaccination against a disease or disorder, said method comprising administering to a patient in need thereof a therapeutically effective amount of the dosage form of an aspect of the invention.
- [00190] In another aspect, the invention is a method of initiating a mucosal immune response against an antigen, said method comprising administering to a patient in need thereof a therapeutically effective amount of the dosage form of an aspect of the invention to a mucosal surface, Preferably, the biologically active material contains an amino acid sequence which is at least partially homologous to a sequence on the antigen.
- [00191] In another aspect, the invention is a method of generating IgA immunity against an antigen, said method comprising administering to a patient in need thereof a therapeutically effective amount of the dosage form of an aspect of the invention to a mucosal surface, Preferably, the biologically active material contains an amino acid sequence which is at least partially homologous to a sequence on the antigen.
- [00192] In another aspect, the invention is the use of the composition of an aspect of the invention in the manufacture of a medicament for the treatment or prevention of a disease or disorder.
- [00193] In another aspect, the invention is a process of preparing the composition of an aspect of the invention, said process comprising the steps of: (1) combining at least one matrix forming agent with a biological active material to form a mixture; and (2) freeze drying the mixture to form the solid dosage form.
- [00194] Preferably, the process further comprises the steps of: (a) Adjusted CMC and alginate to 1.5% (refer to 3x B220506 and 4x B220511); (b) SG taken from lab value for B220506; (c) Phosphate buffer with PS80 and vaccine until homogenous; (d) Measure out initial buffer charge then add to the CMC/alginate mixture and stir until homogenous; and (e) The rest of the excipients can then be added.
- [00195] In another aspect, the invention is a kit comprising the dosage form of an aspect of the invention together with instructions for its use.
- [00196] Preferably, the kit is directed to treating of preventing a disease or disorder selected from the group consisting of: H1N1; RSV; whooping cough (pertussis) and COVID-19
- [00197] A useful preventative measure against mucosal origin/mucosal pathology viruses would be to stimulate a powerful mucosal immunogenic response through an oral or mucosal contact mechanism. This would see the receptive surfaces primed to rise against airborne viruses and immediately they trigger a response at the mucosal surface, rather than allowing the virus to progress further down the infection cascade. The inventors have

designed an oral vaccine and immunogenic biologics matrix for the express purpose of this aim, by delivering appropriate antigens and bioactive molecules to the mucosal surface, not systemically as in injectable vaccines, and allowing a suitable contact time, by virtue of the matrix components.

5 [00198] The inventors provide the following non-limiting features of the invention

- a. Excess saliva is rinsed from the mouth before application.
- b. Once in place in the SL cavity, the wafer rapidly disintegrates.
- c. The disintegrated wafer is now coating the epithelial layer over several square cm, and the bioactive molecules in contact with the cell surfaces can begin an immunogenic cascade (see description below).
- 10 d. Because of the unique wafer matrix, the disintegrated bolus forms a "dome" with structure given by a judicious level of two main water-soluble polymeric chains.
- e. The polymers give the "dome" viscosity, keeping it in position and delaying drainage and swallowing, and also delay the ingress of saliva, the long chain mucopolysaccharides of which have difficulty in penetrating the "dome".
- 15 f. The viscosity is not so great that bioactive are prevented from diffusing to the epithelial layer continuously (unlike solid film wafers which "melt" and trap the active in a highly viscous matrix with very little water present).
- g. This difference is engineered by the highly porous nature of the wafer, which rapidly absorbs extant rinse-water by capillary action to cause rapid disintegration and dissolution. Wafer films have no pores, and so trap the water on the outside of the matrix.
- 20 h. By these features, the bioactive molecules are given a useful time of contact before being overwhelmed with excess saliva and inadvertent mouth movement (2-10 minutes).
- 25

[00199] The inventors provide the following non-limiting features of the first aspect of the invention, the composition.

- a. The absolute level of the two polymers used for this invention, alginic acid salt and carboxymethyl cellulose salt (CMC), is important to the performance of the wafer.
- 30 b. Levels too low do not provide enough viscosity for the disintegrated film to survive too long.

- c. Levels too high make the wafer too viscous to manufacture and dispense, and form a poorly-disintegrating wafer.
- d. The optimum levels of alginic acid and CMC were discovered by experiment to be about 2.0% w/w solids content in the final wafer, in the ratio 1:1, with a useful range of 1.5-2.5% w/w.
- e. Monosaccharides, preferably lactose and mannitol, were useful for the preparation of an ionically neutral, highly water-soluble matrix interpenetrated with a network of pores for rapid disintegration and dissolution. The combined concentration was found to be preferably most useful at 65-80% w/w.
- f. The matrix polymer amylopectin, which has a low viscosity even at elevated concentrations, essential for providing a scaffold for the monosaccharide particles and allowing non-friability and handleability, was found to be most useful at 10-20% w/w.
- g. The wafers are buffered with a suitable buffer, most preferably a blend of mono- and di-alkaline salt phosphates, adjusted to afford a pH optimal for the stability of the chosen biological active, generally between 4 and 7.5.

[00200] Further features of the present invention are more fully described in the following description of several non-limiting embodiments thereof. This description is included solely for the purposes of exemplifying the present invention. It should not be understood as a restriction on the broad summary, disclosure or description of the invention as set out above.

20 Brief Description of the Drawings

[00201] Below is a brief description of each of the figures and drawings.

[00202] Figure 1 shows Human IFN-alpha Product wafer 1 (Composition L) and Human IFN-alpha standard titration curves and associated GraphPad statistics. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00203] Figure 2 shows the EC50 values for Human IFN-alpha Product wafer 1 (Composition L) and Human IFN-alpha standard in (ng/ml).

[00204] Figure 3 shows Human IFN-alpha Product wafer-2 and Human IFN-alpha standard titration curves and associated GraphPad statistics. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50%

protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00205] Figure 4 shows the EC50 values for Human IFN-alpha Product Wafer 2 (Composition K) and Human IFN-alpha standard in (ng/ml).

- 5 [00206] Figure 5 shows the API and Human IFN-alpha standard titration curves and associated GraphPad statistics. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

- 10 [00207] Figure 6 shows the EC50 values for API IFN-alpha sample and Human IFN-alpha standard in (ng/ml).

- 15 [00208] Figure 7 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00209] Figure 8 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and Human IFN-alpha standard.

- 20 [00210] Figure 9 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 1 (Composition L) stored at RT for 2 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00211] Figure 10 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 1 (Composition L) stored at room temperature for 2 weeks and Human IFN-alpha standard.

- 25 [00212] Figure 11 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product Wafer 2 (Composition K) stored at 4°C for 2 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

- 30 [00213] Figure 12 shows the EC50 values in ng/ml for Human IFN-alpha product Wafer 2 (Composition K) stored at 4°C and Human IFN-alpha standard.

[00214] Figure 13 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product Wafer 2 (Composition K) stored at RT for 2 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00215] Figure 14 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 2 (Composition K) stored at room temperature for 2 weeks and Human IFN-alpha standard.

[00216] Figure 15 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00217] Figure 16 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and Human IFN-alpha standard.

[00218] Figure 17 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 1 (Composition L) stored at RT for 6 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00219] Figure 18 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 1 (Composition L) stored at room temperature for 6 weeks and Human IFN-alpha standard.

[00220] Figure 19 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 2 (Composition K) stored at 4°C for 6 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00221] Figure 20 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and Human IFN-alpha standard.

[00222] Figure 21 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 2 (Composition K) stored at RT for 6 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

- [00223] Figure 22 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 2 (Composition K) stored at room temperature for 6 weeks and Human IFN-alpha standard.
- [00224] Figure 23 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.
- [00225] Figure 24 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and Human IFN-alpha standard.
- [00226] Figure 25 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 1 (Composition L) stored at RT for 12 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.
- [00227] Figure 26 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 1 (Composition L) stored at room temperature for 12 weeks and Human IFN-alpha standard.
- [00228] Figure 27 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 2 (Composition K) stored at 4°C for 12 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.
- [00229] Figure 28 shows the tEC50 values in ng/ml for Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and Human IFN-alpha standard.
- [00230] Figure 29 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 2 (Composition K) stored at RT for 12 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.
- [00231] Figure 30 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 2 (Composition K) stored at room temperature for 12 weeks and Human IFN-alpha standard.
- [00232] Figure 31 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 2 (Composition K) stored at 4°C for 26 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal

response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

[00233] Figure 32 shows EC50 values in ng/ml for Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and Human IFN-alpha standard.

- 5 [00234] Figure 33 shows the titration curves and associated GraphPad statistics for Human IFN-alpha product wafer 2 (Composition K) stored at RT for 26 weeks and Human IFN-alpha standard. The EC50 values represent dilutions at which the half-maximal response was reported. The U/ml which gives 50% protection is calculated by dividing the previously determined U/ml of the standard by the dilution EC50 for this plate.

- 10 [00235] Figure 34 shows the EC50 values in ng/ml for Human IFN-alpha product wafer 2 (Composition K) stored at room temperature for 26 weeks and Human IFN-alpha standard.

[00236] Figure 35 shows no statistical difference for the EIA samples.

Detailed Description of the Invention

- 15 [00237] For convenience, the following sections generally outline the various meanings of the terms used herein. Following this discussion, general aspects regarding compositions, use of medicaments and methods of the invention are discussed, followed by specific examples demonstrating the properties of various embodiments of the invention and how they can be employed.

- 20 [00238] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. The invention includes all such variations and modifications. The invention also includes all of the steps, features, formulations and compounds referred to or indicated in the specification, individually or collectively and any and all combinations or any two or more of the steps or
25 features.

- [00239] Each document, reference, patent application or patent cited in this text is expressly incorporated herein in their entirety by reference, which means that it should be read and considered by the reader as part of this text. That the document, reference, patent application or patent cited in this text is not repeated in this text is merely for reasons of
30 conciseness. None of the cited material or the information contained in that material should, however, be understood to be common general knowledge.

[00240] Manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference

herein, are hereby incorporated herein by reference, and can be employed in the practice of the invention.

[00241] The invention described herein may include one or more ranges of values (e.g., size, concentration etc.). A range of values will be understood to include all values within the range, including the values defining the range, and values adjacent to the range that lead to the same or substantially the same outcome as the values immediately adjacent to that value which defines the boundary to the range.

[00242] The present invention is not to be limited in scope by any of the specific embodiments described herein. These embodiments are intended for the purpose of exemplification only. Functionally equivalent products, formulations and methods are clearly within the scope of the invention as described herein.

1. DEFINITIONS –

[00243] The meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

[00244] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages can mean $\pm 1\%$.

[00245] The invention described herein may include one or more range of values (e.g., size, concentration etc.). A range of values will be understood to include all values within the range, including the values defining the range, and values adjacent to the range which lead to the same or substantially the same outcome as the values immediately adjacent to that value which defines the boundary to the range. For example, a person skilled in the field will understand that a 10% variation in upper or lower limits of a range can be totally appropriate and is encompassed by the invention. More particularly, the variation in upper or lower limits of a range will be 5% or as is commonly recognised in the art, whichever is greater.

[00246] In this application, the use of the singular also includes the plural unless specifically stated otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and

components that comprise more than one subunit unless specifically stated otherwise. Also, the use of the term "portion" can include part of a moiety or the entire moiety.

[00247] Throughout this specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[00248] "Therapeutically effective amount" as used herein with respect to methods of treatment and in particular drug dosage, shall mean that dosage that provides the specific pharmacological response for which the drug is administered in a significant number of subjects in need of such treatment. It is emphasized that "therapeutically effective amount," administered to a particular subject in a particular instance will not always be effective in treating the diseases described herein, even though such dosage is deemed a "therapeutically effective amount" by those skilled in the art. It is to be further understood that drug dosages are, in particular instances, measured as oral dosages, or with reference to drug levels as measured in blood. Amounts effective for such a use will depend on: the desired therapeutic effect; the potency of the biologically active material; the desired duration of treatment; the stage and severity of the disease being treated; the weight and general state of health of the patient; and the judgment of the prescribing physician. Treatment dosages need to be titrated to optimize safety and efficacy. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the indication for which the active agent is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titrate the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 $\mu\text{g/kg}$ to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 $\mu\text{g/kg}$ up to about 100 mg/kg; or 1 $\mu\text{g/kg}$ up to about 100 mg/kg; or 5 $\mu\text{g/kg}$ up to about 100 mg/kg.

[00249] The frequency of dosing will depend upon the pharmacokinetic parameters of the active agent and the formulation used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed

by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

5 [00250] As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible.

[00251] As used herein the term "subject" generally includes mammals such as: humans; farm animals such as sheep, goats, pigs, cows, horses, llamas; companion animals such as dogs and cats; primates; birds, such as chickens, geese and ducks; fish; and reptiles. The subject is preferably human. In one embodiment, the human subject is an infant, child, or
10 adolescent. In one embodiment, the human subject is a paediatric patient and aged 21 or younger at the time of their diagnosis or treatment.

[00252] Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout. Unless otherwise defined, all other scientific and technical terms used herein have the same meaning as commonly understood
15 to one of ordinary skill in the art to which the invention belongs.

[00253] Features of the invention will now be discussed with reference to the following non-limiting description and examples.

2. EMBODIMENTS

Composition

20 [00254] The composition of the present invention comprises at least one matrix forming agent. In the freeze-dried systems of the prior art, gelatin is the most commonly used carrier or structure forming agent due to its wall-forming ability. Gelatin is an ionic water soluble polymer, and as such, when mixed with active pharmaceutical ingredients in water; the
25 increasing viscosity of the solution over time may cause a decreasing solubility of poorly soluble drugs in the mixture, and lead to a suspension of the drug in gelatin matrix. This can cause phase separation to occur; and the drug in amorphous or crystalline forms may not be homogeneously dispersed in the matrix, which will eventually affect the dissolution and absorption of the final product.

30 [00255] Applicant has found that other polymer materials suitable for forming a matrix may be selected for specific application in the field of drug delivery, especially for site-specific drug delivery system such as in the oral cavity. Matrix forming agents of the present invention may be selected from the group consisting of: non-mammalian gelatin, dextrin, soy protein, wheat protein, psyllium seed protein, acacia gum, guar gum, agar gum, xanthin

gum, polysaccharides; alginates; sodium carboxymethylcellulose; carrageenans; dextrans; pectins; sugars; amino acids; starch; modified starches; carboxymethylcellulose; hydroxypropylmethylcellulose; hydroxypropyl cellulose and methyl cellulose inorganic salts; synthetic polymers; amylopectin, polypeptide/protein or poly-saccharide complexes.

- 5 Examples of at least one matrix forming agent that are carbohydrates include mannitol, dextrose, lactose, galactose, sorbitol and trehalose and cyclodextrin. Examples of matrix forming agents that are inorganic salts may be selected from the group consisting of: sodium phosphate, sodium chloride and aluminium silicates. The at least one matrix forming agent may also be an amino acid. Examples of suitable amino acids include glycine, L-alanine, L-aspartic acid, L-glutamic acid, L-hydroxyproline, L-isoleucine, L-leucine and L-phenylalanine.
- 10

- [00256] In a highly preferred embodiment, at least one matrix forming agent is sodium carboxymethylcellulose. When at least one matrix forming agent is sodium carboxymethyl cellulose, the polymer is present in a concentration of from about 0.1% to about 19% by dry weight of the solid dosage form. In a preferred embodiment the sodium
- 15 carboxymethylcellulose is present in an amount of about 0.1% to about 15% by dry weight of the dosage form. In a highly preferred embodiment of the present invention, the sodium carboxymethyl cellulose is present in an amount of about 0.1 % to about 1.0% by dry weight of the solid dosage form. In another embodiment of the present invention, the dissolving dosage form comprises amylopectin as at least one matrix forming agent. Amylopectin is
- 20 capable of increasing the release of the biologically active material by promoting formulation disintegration. Amylopectin may be present in the dosage form at a concentration about 2% up to no greater than 20% by dry weight of the solid dosage form. In a highly preferred form of the present invention, amylopectin is present in an amount of about 2% to about 17% dry weight of the dosage form.

- 25 [00257] To achieve dissolution of drugs, low MW diluents may be added as at least one matrix forming material. Diluents include microcrystalline cellulose (e.g., Avicel PH 101® and Avicel PH 102®), lactose, starch and sorbitol. These diluents may be present in the dosage form either alone or as a mixture in different ratios, and may be about 1% to about 80%, preferably about 2% to about 50%, either individually or cumulatively. In one embodiment of
- 30 the present invention, the dissolving dosage form comprises microcrystalline cellulose as the at least one matrix forming agent. Microcrystalline cellulose may act as a filler and binder in the dosage form of the present invention. Microcrystalline cellulose has the ability to compact with minimum compression pressures, and results in a hard, stable and dissolving dosage form. Due to its large surface area and high internal porosity, microcrystalline
- 35 cellulose is able to absorb and retain large amounts of water, which is desirable in the dosage form of the invention. When the solid dosage form of the present invention

comprises microcrystalline cellulose, it is present in an amount of about 1% to about 10%, and preferably from about 1% to about 8% by dry weight of the dosage form. The effectiveness of the dissolving dosage form of the present invention relies on the drug dissolving in a small volume of fluid, such as in the oral cavity, prior to absorption into the systemic circulation. Therefore, the rate of dissolution of the dosage form is important. In a preferred embodiment of the present invention, the dosage form comprises a super-disintegrant as at least one matrix forming material.

[00258] In a highly preferred embodiment, the dissolving dosage form of the present invention comprises glycine. Glycine is an amino acid with excellent wetting properties and is suitable for the dissolving formulation. Low amounts of glycine may be used in the formulation of the present invention to control the dissolution rate of the dosage form. Furthermore, glycine may also be used as an anti-collapsing agent, which maintains the dosage form from shrinking either during the manufacture process or after packing. In one embodiment, the dosage form of the present invention comprises from about 0.5% to about 5% dry weight of the dosage form. According to another embodiment of the invention, the solid dosage form may include a matrix forming agent such as mannitol. Mannitol is a component that may aid in the crystalline structure and impart hardness of the dosage form. When mannitol is present in the dosage form, it occurs in a concentration of from about 5% to about 80%, and preferably from about 10% to about 60% by dry weight of the dosage form.

[00259] In addition, the dissolving dosage form of the present invention may include lubricants, such as polyethylene glycol (PEG) 1000, 1500, 2000, 4000 and 6000, sodium lauryl sulphate, fats or oils. One advantage of the use of these lubricants is to aid in the removal of the dosage form from the mould. These lubricants may be present in the dosage form either alone or as a mixture in different ratios, and may be between 0.05% to 5%, preferable between 0.1% and 2%, preferable about 1.5%, either individually or cumulatively. In one embodiment, the composition includes between 0.05% to 5% polyethylene glycol 1500, preferably between 0.1% and 2% by dry weight of the dosage form, or as mixtures of the various glycols. The invention extends, in another aspect thereof, to improve sublingual absorption of weak base compounds, the composition comprising a solid buffer reagent that affords to produce a saliva pH of 4-6 when dissolved in oral cavity. Increasing the pH of the solution of a weak base compound can increase the ratio of unionized to ionized, which will lead to enhanced sublingual absorption. The solid buffer reagent include sodium dihydrogen phosphate dihydrate, sodium hydrogen phosphate, sodium hydrogen carbonate and sodium carbonate, which may be present in the dosage form either alone or as a mixture in different ratios in a concentration of about 0.01% to about 10% by weight of the composition.

Preferably, the buffer reagent is sodium carbonate, which may be present in a concentration of about 0.01% to about 10% by weight of the composition, preferably between 0.1% to 1%, most preferably about 0.3%.

5 [00260] When mannitol is present in the dosage form, it occurs in a concentration of from about 5% to about 80%, and preferably from about 10% to about 60% by dry weight of the dosage form.

[00261] The composition may, in certain embodiments, include an absorption enhancer. The absorption enhancer may be a polysaccharide and may be positively charged. Preferably, the absorption enhancer is β -cyclodextrin or its derivatives. The β -cyclodextrin or
10 derivative may be present in a concentration of from about 0.01% to about 10% by dry weight of the dosage form, preferably between 0.2% to 2%, and most preferably about 1 %. The solid dosage form of the present invention may comprise flocculating agents to maintain disbursement of the biologically active material evenly dispersed in the matrix during the manufacture process. The flocculating agent may be gums. Preferably, the gum is xanthan
15 gum. The xanthan gum may be present in a concentration of about 0.01% to about 10% by dry weight of the composition, preferably from about 0.2% to 2%, and most preferably about 1%.

[00262] To aid dissolution of the biologically active material into the aqueous environment, a surfactant may be added to the solution as a wetting agent. Suitable
20 surfactants include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents may be used and include benzalkonium chloride or benzethonium chloride. The list of possible non-ionic detergents includes lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, Polysorbate 40, 60, 65 and 80, sucrose fatty acid
25 ester, methyl cellulose and carboxymethyl cellulose. These surfactants may be present in the dosage form either alone or as a mixture in different ratios. Additives which potentially enhance uptake of the compounds are fatty acids such as oleic acid, linoleic acid and linolenic acid.

[00263] In order to enhance the aesthetic and taste appeal of the dissolving dosage form
30 to the subject, the dosage form may also contain colouring agents, such as FD & C dyes Blue No. 2 and Red No. 40; flavoring agents, such as orange, mixed berry, cherry, peppermint, raspberry and caramel; and/or sweeteners such as aspartame, stevia, sucralose and saccharin.

[00264] The solid dosage form of the present invention is suitable for oral administration to a subject. As discussed above, the dosage form comprises biologically active material. The biologically active material is therefore delivered to the subject via the oral cavity mucosa and into the systemic blood system within a relatively short period of time. In a preferred embodiment, an effective plasma concentration of the biologically active material is reached within a period of no more than two hours, preferably within 30 minutes, and most preferably within 10 minutes.

[00265] Furthermore, an advantage of the present invention is that the solid dosage form completely dissolves within 2 seconds to 60 seconds, preferably 2 seconds to 30 seconds, and most preferably within 2 seconds to 10 seconds after administration of the dosage form. In a highly preferred embodiment of the present invention, there is no residue remaining of the dosage form of the present invention after administration that is detectable by the patient. As such, the subject has no urge to swallow the dosage form.

[00266] The subject receiving the dissolving dosage form of the present invention may be an animal or human being. When the subject is a human being, it may be an adult or a child, including elderly adults and infants. In particular, the subject is a subject that is unable to or has difficulties in swallowing.

[00267] The solid dosage form may comprise sodium carboxymethylcellulose as a formulation aide in low levels. When the amount of sodium carboxymethylcellulose is between about 0.1% and 15% by dry weight of the dosage form, the wafer releases the active agent, without leaving a residue in the oral cavity. In addition, the use of gelatin was avoided by the inventors, and therefore prevents the unwanted residue left in the oral cavity after administration. The addition of lactose and or mannitol was also found to be advantageous in the dosage formulation of the present invention.

[00268] Thus, in one embodiment, the present invention provides a solid dosage form adapted for the release of biologically active material in the oral cavity wherein the dosage form comprises: (i) biologically active material and (ii) at least one matrix forming agent, wherein the dosage form substantially dissolves in the oral cavity, wherein the dosage form comprises 0.1-0.3% sodium carbonate, 0.1-4% sodium carboxymethylcellulose, 0.1-10% PEG 1500, 1-4%% glycine, 1-10%% microcrystalline cellulose; 2-17% amylopectin, 10-30% lactose and 30-50% mannitol as a dry weight of the solid dosage form, and which does not result in substantial detectable levels of residue left over in the oral cavity of the patient.

Pharmaceutical Compositions

[00269] The present invention also provides a pharmaceutical composition comprising the composition of the invention together with a pharmaceutically acceptable carrier.

[00270] Therapeutic compositions are within the scope of the present invention. Preferably the compositions are combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition (which may be for human or animal use). Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. See, e.g., Remington's Pharmaceutical Sciences, 2th Ed. (1995, Mack Publishing Co., Easton, Pa.) which is herein incorporated by reference.

[00271] The pharmaceutical composition can contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, colour, isotonicity, odour, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulphite or sodium hydrogen-sulphite, Vitamin E, Vitamin E phosphate – lipid soluble vitamins, nano emulsions); buffers (such as borate, bicarbonate, tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin), fillers; monosaccharides, disaccharides; and other carbohydrates (such as glucose, mannose, or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); colouring, flavouring (natural and natural derived products) and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols – and artificial sweeteners (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapol); stability enhancing agents (sucrose

or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants.

5 [00272] The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the composition of the invention. The preferred form of the pharmaceutical composition depends on the intended mode of administration and therapeutic application.

10 [00273] The primary vehicle or carrier in a pharmaceutical composition is aqueous and non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution, possibly supplemented with other materials. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute thereof. In one
15 embodiment of the present invention, pharmaceutical compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents in the form of an aqueous solution and non-aqueous.

[00274] The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at
20 physiological pH or at a slightly lower pH, typically within a pH range of from about 5 (4.5) to about 8

[00275] Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations of the invention in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such
25 as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. Additional examples of sustained-sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, for example, films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate,
30 ethylene vinyl acetate or poly-D(-)-3-hydroxybutyric acid. Sustained-release compositions may also include liposomes, which can be prepared by any of several methods known in the art.

[00276] The pharmaceutical composition to be used for in vivo administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes.

In addition, the compositions generally are placed into a container having a sterile access port. Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution.

5 [00277] In yet a further preferred embodiment, the composition retains its effective biological activity for a period selected from the group consisting of; greater than 24 hours; greater than 36 hours; and greater than 48 hours. Preferably, the composition is stable for periods selected from the group consisting of: 6 months, 1 year and 2 years. In one example, the composition is stable at temperatures selected from the group consisting of: -4°C, 4°C, 18°C and 25°C.

10 [00278] As discussed above, the medicaments of the present invention may include one or more pharmaceutically acceptable carriers. The use of such media and agents for the manufacture of medicaments is well known in the art. Except insofar as any conventional media or agent is incompatible with the pharmaceutically acceptable material, use thereof in the manufacture of a pharmaceutical composition according to the invention is
15 contemplated. Pharmaceutical acceptable carriers according to the invention may include one or more of the following examples:

[00279] (1) surfactants and polymers, including, however not limited to polyethylene glycol (PEG), polyvinylpyrrolidone , polyvinylalcohol, crospovidone, polyvinylpyrrolidone-polyvinylacrylate copolymer, cellulose derivatives, hydroxypropylmethyl cellulose,
20 hydroxypropyl cellulose, carboxymethylethyl cellulose, hydroxypropylmethyl cellulose phthalate, polyacrylates and polymethacrylates, urea, sugars, polyols, and their polymers, emulsifiers, sugar gum, starch, organic acids and their salts, vinyl pyrrolidone and vinyl acetate; and/or

[00280] (2) binding agents such as various celluloses and cross-linked
25 polyvinylpyrrolidone, microcrystalline cellulose; and/or (3) filling agents such as lactose monohydrate, lactose anhydrous, microcrystalline cellulose and various starches; and/or

[00281] (4) lubricating agents such as agents that act on the increased ability of the dosage form to be ejected from the packaging cavity, and/or

[00282] (5) sweeteners such as any natural or artificial sweetener including sucrose,
30 xylitol, sodium saccharin, cyclamate, aspartame, and acesulfame K; and/or

[00283] (6) flavouring agents; and/or

[00284] (7) preservatives such as potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben,

alcohols such as ethyl or benzyl alcohol, phenolic chemicals such as phenol, or quarternary compounds such as benzalkonium chloride; and/or

[00285] (8) buffers; and/or

5 [00286] (9) diluents such as pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or mixtures of any of the foregoing; and/or

[00287] (10) wetting agents such as corn starch, potato starch, maize starch, and modified starches, croscarmellose sodium, crosspovidone, sodium starch glycolate, and mixtures thereof; and/or

10 [00288] (11) disintegrants; and/or

[00289] (12) effervescent agents such as effervescent couples such as an organic acid (e.g., citric, tartaric, malic, fumaric, adipic, succinic, and alginic acids and anhydrides and acid salts), or a carbonate (e.g. sodium carbonate, potassium carbonate, magnesium carbonate, sodium glycine carbonate, L-lysine carbonate, and arginine carbonate) or
15 bicarbonate (e.g. sodium bicarbonate or potassium bicarbonate); and/or

[00290] (13) other pharmaceutically acceptable excipients.

[00291] Medicaments of the invention suitable for use in animals and in particular in human beings typically must be sterile and stable under the conditions of manufacture and storage. The medicaments of the invention comprising biologically active material can be
20 formulated as a solid, a liposome, or other ordered structures suitable to high drug concentration adapted for oral delivery.

[00292] In another embodiment, the biologically active material may be combined into a medicament with another biologically active material, or even the same biologically active material.

25 [00293] Medicaments of the invention can be orally administered to a subject. Solid dosage forms for oral administration include wafers. Further, incorporating any of the normally employed excipients, such as those previously listed, and generally 0.1% to 95% of the biologically active material, and more preferably at a concentration of 0.1% to 75% will form a pharmaceutically acceptable non-toxic oral administration.

30 Dosage Form

[00294] Dosage forms are within the scope of the invention. In a preferred embodiment, the invention provides a dosage form comprising the composition as described in the first aspect of this invention.

[00295] In a further embodiment, the dosage form is a wafer.

- 5 [00296] Preferably, the dosage form is stored in a sealed and sterile container.

Method for treating, preventing or vaccination

[00297] The invention also provides a method of treating a disorder, said method comprising administering to a patient in need thereof a therapeutically effective amount of the dosage form of the invention.

- 10 [00298] In a further preferred embodiment, the dosage form is administered at an amount to at least partially treat the disorder.

[00299] In a further preferred embodiment, the therapeutically effective amount is an amount of biologically active material selected from the group consisting of: between 1 to 100mg/kg/day; between 2 and 50mg/kg/day; between 5 and 40mg/kg/day; between 10 and 30mg/kg/day; between 20 and 25mg/kg/day; and 20mg/kg/day. Preferably, the therapeutically effective amount is an amount of biologically active material vis selected from the group consisting of: 10mg/day; 15mg/day; 40mg/day; 400mg/day; 600mg/day; 800mg/day; 1280mg/day; 1500mg/day.

[00300] In a further preferred embodiment, therapeutically effective amount is an amount of biologically active material selected from the group consisting of: between 1 to 100mg/kg/day; between 2 and 50mg/kg/day; between 5 and 40mg/kg/day; between 10 and 30mg/kg/day; between 20 and 25mg/kg/day; and 20mg/kg/day. Preferably, the therapeutically effective amount is an amount of biologically active material vis selected from the group consisting of: 10mg/day; 15mg/day; 40mg/day; 400mg/day; 600mg/day; 800mg/day; 1280mg/day; 1500mg/day.

[00301] In a further preferred embodiment, T_{max} occurs between 1 and 4 hours.

[00302] In a further preferred embodiment, T_{1/2} occurs between 1.1 and 2.4 hours.

[00303] In a further preferred embodiment, the therapeutically effective amount is administered to the subject to treat the disorder.

- 30 [00304] Preferably the therapeutically effective amount is administered to the subject utilising a dosing regimen selected from the group consisting of: twice hourly; hourly; once every six hours; once every 8 hours; once every 12 hours; once daily; twice weekly; once

weekly; once every 2 weeks; once every 6 weeks; once a month; every 2 months; every 3 months; once every 6 months; and once yearly.

[00305] Preferably the therapeutically effective amount is administered to the subject using a method selected from the group consisting of: sublingually.

5 [00306] Preferably, the disorder is a:

[00307] A subject that can be treated with the invention will include humans as well as other mammals and animals.

[00308] In a further preferred embodiment, the method comprises administering to a patient in need thereof a therapeutically effective amount of the dosage form of the invention together with an additional active ingredient. In a preferred form, the additional active
10 ingredient is administered using a dosing regimen selected from the group consisting of: at the same time as administering the dosing form of the invention; before administering the dosing form of the invention; after administering the dosing form of the invention; concurrently with administering the dosing form of the invention; sequentially before
15 administering the dosing form of the invention; and sequentially after administering the dosing form of the invention.

[00309] Preferably, the additional active ingredient is selected from the group consisting of: a polypeptide; an antibody; a small chemical entity.

Use of a composition in the manufacture of a medicament

20 [00310] Uses are within the scope of this invention. The invention also provides a use of the composition of the first aspect of the invention in the manufacture of a medicament for the treatment of a disorder.

[00311] In one preferred embodiment, the invention is

Kit

25 [00312] The invention also provides a kit comprising the dosage form of one aspect of the invention together with instructions for its use.

Device

[00313] Devices are within the scope of the invention. In a preferred embodiment, the invention provides a device, wherein the device comprises: (1) the composition as described
30 in the first aspect of this invention; and (2) an applicator.

Method for stabilising

[00314] Methods for stabilizing the composition are within the scope of the invention.

[00315] In a further preferred embodiment, the said method protects the composition against degradation.

5 [00316] In yet a further preferred embodiment, the composition retains its effective biological activity for a period selected from the group consisting of; greater than 24 hours; greater than 36 hours; greater than 48 hours.

[00317] The addition of approved pharmaceutical excipients to stabilise the composition is preferred from a safety standpoint, as the simpler methodology is likely to produce a less variable outcome and the choice of excipient can be limited to those with Generally
10 Regarded as Safe (GRAS) status. Excipients for the stabilisation of protein solutions can be classified into four broad categories: salts, sugars, polymers or protein/amino acids, based on their chemical properties and mechanism of action. Salts (e.g., chlorides, nitrates) stabilise the tertiary structure of proteins by shielding charges through ionic interactions. Sugars (e.g., glycerol, sorbitol, fructose, trehalose) increase the surface tension and
15 viscosity of the solution to prevent protein aggregation. Similarly, polymers (e.g. polyethylene glycol, cellulose derivatives) stabilise the protein tertiary structure by increasing the viscosity of the solution to prevent protein aggregation and intra- and inter-molecular electrostatic interactions between amino acids in the protein. Proteins (e.g. human serum albumin) are able to stabilise the structure of other proteins through ionic, electrostatic and hydrophobic
20 interactions. Similarly, small amino acids with no net charge, such as alanine and glycine, stabilise proteins through the formation of weak electrostatic interactions.

[00318] As discussed above, the medicaments of the present invention may include one or more pharmaceutically acceptable carriers. The use of such media and agents for the manufacture of medicaments is well known in the art. Except insofar as any conventional
25 media or agent is incompatible with the pharmaceutically acceptable material, use thereof in the manufacture of a pharmaceutical composition according to the invention is contemplated. Pharmaceutical acceptable carriers according to the invention may include one or more of the following examples:

30 a. surfactants and polymers, including, however not limited to polyethylene glycol (PEG), polyvinylpyrrolidone, polyvinylalcohol, crospovidone, polyvinylpyrrolidone-polyvinylacrylate copolymer, cellulose derivatives, HPMC, hydroxypropyl cellulose, carboxymethylethyl cellulose, hydroxypropylmethyl cellulose phthalate, polyacrylates and polymethacrylates, urea, sugars, polyols, and their polymers,

emulsifiers, sugar gum, starch, organic acids and their salts, vinyl pyrrolidone and vinyl acetate; and/or

- b. binding agents such as various celluloses and cross-linked polyvinylpyrrolidone, microcrystalline cellulose; and/or (3) filling agents such as lactose monohydrate, lactose anhydrous, microcrystalline cellulose and various starches; and/or
- c. filling agents such as lactose monohydrate, lactose anhydrous, mannitol, microcrystalline cellulose and various starches; and/or
- d. lubricating agents such as agents that act on the increased ability of the dosage form to be ejected from the packaging cavity, and/or
- e. sweeteners such as any natural or artificial sweetener including sucrose, xylitol, sodium saccharin, cyclamate, aspartame, and acesulfame K; and/or
- f. flavouring agents; and/or
- g. preservatives such as potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic chemicals such as phenol, or quarternary compounds such as benzalkonium chloride; and/or
- h. buffers; and/or
- i. diluents such as pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or mixtures of any of the foregoing; and/or
- j. absorption enhancer such as glyceryl trinitrate; and/or
- k. other pharmaceutically acceptable excipients.

[00319] Medicaments of the invention suitable for use in animals and in particular in human beings typically must be sterile and stable under the conditions of manufacture and storage.

[00320] Methods to produce

[00321] According to a further aspect of the present invention, there is provided a method to produce the dosage form of the present invention comprising the steps of combining at least one matrix forming agent with a biological active material to form a mixture and then freeze drying the mixture to form the solid dosage form. In a preferred embodiment of the present invention, the mixture is measured (by weight or volume) into a preformed plastic or

aluminium blister mould (individual dose). The blister mould is placed into a freeze dryer for 24 hours and the resultant solid dosage form (wafer) is then sealed with aluminium or plastics foil to prevent moisture absorption.

5 [00322] In one embodiment of the present invention, the method may require that the pH of the mixture is adjusted to a pH within the range of between 3.0 and 8.0, preferably between 6.4 and 7.8. If required, the pH may be adjusted by using an acid, such as hydrochloric acid, phosphoric acid or citric acid; or a basic compound such as sodium hydroxide, sodium dihydrogen phosphate dehydrate, sodium hydrogen phosphate, sodium hydrogen carbonate and sodium carbonate.

10 [00323] In another embodiment, the method may include the step of using a solvent, such as water. If water is used as a solvent, it is preferable to be removed by freeze drying

[00324] The invention also provides a composition, methods and processes as described by the foregoing examples.

15 [00325] The present invention will now be described with reference to the following non-limiting Examples. The description of the Examples is in no way limiting on the preceding paragraphs of this specification, however, is provided for exemplification of the methods and compositions of the invention.

Examples

20 [00326] It will be apparent to persons skilled in the milling and pharmaceutical arts that numerous enhancements and modifications can be made to the above-described processes without departing from the basic inventive concepts. For example, in some applications the biologically active material may be pretreated and supplied to the process in the pretreated form. All such modifications and enhancements are considered to be within the scope of the present invention, the nature of which is to be determined from the foregoing description and
25 the appended claims. Furthermore, the following Examples are provided for illustrative purposes only, and are not intended to limit the scope of the processes or compositions of the invention.

A EXAMPLE 1 – COMPOSITIONS OF THE INVENTION

30 [00327] Compositions of the invention were prepared using standard methods of the art. Examples of compositions of the invention are presented below

[00328] COMPOSITION A

ITEM NO	RAW MATERIAL	WAFER CONTENTS (mg/wafer)
1	Biologically active material	Qs
2	Water (initial charge)	-
3	CMC	0.3
4	Mannitol	24.4
5	Lactose monohydrate	16.2
6	Amylogum CLS	8.1
7	Glycine	1.6
8	PEG 1500	0.8
9	MCC	1.6

[00329] COMPOSITION B

ITEM NO	RAW MATERIAL	WAFER CONTENTS (mg/wafer)
1	Biologically active material	Qs
2	Water (initial charge)	-
3	CMC	0.3
4	Mannitol	24.4
5	Lactose monohydrate	16.2
6	Amylogum CLS	8.1
7	Glycine	1.6
8	PEG 1500	0.8
9	MCC	1.6

[00330] COMPOSITION C

S.NO.	RAW MATERIAL	WAFER CONTENTS (mg/wafer)

1	Biologically active material	Qs
2	Water (initial charge)	-
3	CMC	0.3
4	Mannitol	24.4
5	Lactose monohydrate	16.2
6	Amylogum CLS	8.1
7	Glycine	1.6
8	PEG 1500	0.8
9	MCC	1.6

[00331] COMPOSITION D

ITEM NO	RAW MATERIAL	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	Biologically active material	Qs	qs
2	Stabilization Buffer pH 7-7.4 (initial charge)	5.74	5.77
3	CMC	2.16	2.2
4	Sodium alginate	2.16	2.2
5	Mannitol	43.75	44.0
6	Lactose monohydrate	29.33	29.5
7	Amylogum CLS	13.79	13.9
8	Glycine	0.48	0.5
9	PEG 1500	1.38	1.4
10	MCC	0.93	0.9
11	Stabilization Buffer pH 7-7.4 qs (rinse)	0.18	0.18

[00332] COMPOSITION E

ITEM NO.	RAW MATERIAL	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	Biologically active material	Qs	qs
2	Buffer (initial charge)		
3	CMC	1.9	1.5
4	Sodium alginate	1.9	1.5
5	Mannitol	45.8	35.5
6	Lactose monohydrate	14.0	10.9
7	Amylogum CLS	12.6	9.8
8	Glycine	5.6	4.3
9	PEG 1500	1.5	1.2
10	MCC	2.6	2.0
11	Buffer adjust qs	0.0	0.0

[00333] COMPOSITION F

ITEM NO.	RAW MATERIAL	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	Biologically active material	Qs	qs
2	Buffer (initial charge)		
3	CMC	3.2	2.5
4	Sodium alginate	3.2	2.5
5	Mannitol	44.7	35.5
6	Lactose monohydrate	13.7	10.9
7	Amylogum CLS	12.3	9.8
8	Glycine	5.5	4.3
9	PEG 1500	1.5	1.2
10	MCC	2.5	2.0
11	Buffer adjust qs	0.0	0.0

[00334] COMPOSITION G

ITEM NO.	RAW MATERIAL	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	Biologically active material	Qs	qs
2	Buffer (initial charge)		
3	CMC	3.7	3.0
4	Sodium alginate	3.7	3.0
5	Mannitol	44.1	35.3
6	Lactose monohydrate	13.5	10.8
7	Amylogum CLS	12.2	9.7
8	Glycine	5.4	4.3
9	PEG 1500	1.5	1.2
10	MCC	2.5	2.0
11	Buffer adjust qs		

[00335] COMPOSITION H

ITEM NO.	RAW MATERIAL	OPTION A	OPTION B	OPTION C
		SOLIDS CONT. (% w/w)	SOLIDS CONT. (% w/w)	SOLIDS CONT. (% w/w)
1	Biologically active material	Qs	qs	qs
2	Buffer (initial charge)			
3	CMC	1-5	1.5-4	1.9-3.7
4	Sodium alginate	1-5	1.5-4	1.9-3.7
5	Mannitol	40-50	42-46	43.75-45.8
6	Lactose monohydrate	10-50	10-30	13.5-29.33
7	Amylogum CLS	10-15	12-15	12.2-13.79

8	Glycine	0.2-10	0.3-6	0.48-5.6
9	PEG 1500	0.5-5	2-4	1.38-1.5
10	MCC	0.5-5	0.5-3	0.93-2.6
11	Buffer adjust qs			

[00336] COMPOSITION I: Interferon beta-1a 500 IU wafer V1

TRIAL FORMULATION - Version 1 (11/09/20)		BATCH SIZE: 1000 wafers	
S.NO.	RAW MATERIAL	BATCH QTY (grams)	WAFER CONTENTS (mg/wafer)
1	Interferon beta-1a (Rebif 44)	0.0208	0.0208
2	Water (initial charge)	164	-
3	CMC	0.33	0.3
4	Mannitol	24.36	24.4
5	Lactose monohydrate	16.24	16.2
6	Amylogum CLS	8.12	8.1
7	Glycine	1.63	1.6
8	PEG 1500	0.81	0.8
9	MCC	1.63	1.6
	TOTAL	218.04	53.1
	Theoretical number of wafers (at SG 1.09, bulk solids 24.37%, pH TBA)	1000 wafers	-

[00337] COMPOSITION J: Interferon Alpha 2-beta 2000 IU wafer

TRIAL FORMULATION - Version 1 (28/04/21)		BATCH SIZE: 1000 wafers	
S.NO.	RAW MATERIAL	BATCH QTY (grams)	WAFER CONTENTS (mg/wafer)
1	Interferon Alpha 2-beta (Prospec) 2000IU/wafer	0.0769	2000IU
2	Water (initial charge)	164.9	-

3	CMC	0.33	0.3
4	Mannitol	24.36	24.4
5	Lactose monohydrate	16.24	16.2
6	Amylogum CLS	8.12	8.1
7	Glycine	1.63	1.6
8	PEG 1500	0.81	0.8
9	MCC	1.63	1.6
	TOTAL	218.04	53.1
	Theoretical number of wafers (at SG 1.09, bulk solids 24.39%, pH TBA)	1000 wafers	-

[00338] COMPOSITION K: Interferon Alpha 2-beta 1000 IU wafer

TRIAL FORMULATION - Version 1 (28/04/21)		BATCH SIZE: 1000 wafers	
S.NO.	RAW MATERIAL	BATCH QTY (grams)	WAFER CONTENTS (mg/wafer)
1	Interferon Alpha 2-beta (Prospec) 1000IU/wafer	0.0385	1000IU
2	Water (initial charge)	164.9	-
3	CMC	0.33	0.3
4	Mannitol	24.36	24.4
5	Lactose monohydrate	16.24	16.2
6	Amylogum CLS	8.12	8.1
7	Glycine	1.63	1.6
8	PEG 1500	0.81	0.8
9	MCC	1.63	1.6
	TOTAL	218.04	53.1
	Theoretical number of wafers (at SG 1.09, bulk solids 24.38%, pH TBA)	1000 wafers	-

[00339] COMPOSITION L: Interferon alpha 2-beta (Biomart) 500 IU

TRIAL FORMULATION - Version 1 R1 (30/03/23)		BATCH SIZE: 600 (500 IU per wafer)		
ITEM NO.	RAW MATERIAL	BATCH QTY (grams)	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	Interferon Alpha 2-beta (Biomart) 500 IU/wafer (Solution 2 – add last)	3.000	0.15	0.11
2	Stabilization Buffer pH 7-7.4 (initial charge)	160.00	5.74	5.77
3	CMC	1.30	2.16	2.2
4	Sodium alginate	1.30	2.16	2.2
5	Mannitol	26.39	43.75	44.0
6	Lactose monohydrate	17.69	29.33	29.5
7	Amylogum CLS	8.32	13.79	13.9
8	Glycine	0.29	0.48	0.5
9	PEG 1500	0.83	1.38	1.4
10	MCC	0.56	0.93	0.9
11	Stabilization Buffer pH 7-7.4 qs (rinse)	5.02	0.18	0.18
	TOTAL	224.70	100.00	100.5
	Theoretical number of wafers (at SG 1.07, bulk solids 26.8%, pH 7-7.4)	600 wafers		-

[00340] COMPOSITION M: H1N1 1

TRIAL FORMULATION - Version 1 R1 (04/11/22)		BATCH SIZE: 500 wafers		
ITEM NO.	RAW MATERIAL	BATCH QTY (grams)	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	H1N1 vaccine solution concentrate (1402.7µg HA (haemagglutinin) per mL) - 15µg HA per wafer	5.35	13.9	10.7
2	Buffer (initial charge)	80.00		
3	CMC	0.75	1.9	1.5
4	Sodium alginate	0.75	1.9	1.5
5	Mannitol	17.67	45.8	35.5

6	Lactose monohydrate	5.42	14.0	10.9
7	Amylogum CLS	4.87	12.6	9.8
8	Glycine	2.17	5.6	4.3
9	PEG 1500	0.59	1.5	1.2
10	MCC	0.98	2.6	2.0
11	Buffer adjust qs	14.75	0.0	0.0
	TOTAL	133.31	100.0	77.4
	Theoretical number of wafers (at SG 1.07, bulk solids 28.9%, pH TBA)	498 wafers		-
Adjusted CMC and alginate to 1.5x (refer to 3x B220506 and 4x B220511). SG taken from lab value for B220506. Buffer copied from commercial packaging except for using 100µg PS80 instead of Octoxinol 9 <250µg per 0.5mL. Method of preparation: Add and dissolve buffer solids and PS80 into the phosphate buffer solution and stir until homogenous. Measure out initial buffer charge then add to the CMC/alginate mixture and stir until homogenous. The rest of the excipients can then be added (per usual process).				

[00341] COMPOSITION N: H1N1

TRIAL FORMULATION - Version 1 R1 (04/11/22)		BATCH SIZE: 500 wafers		
ITEM NO.	RAW MATERIAL	BATCH QTY (grams)	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	H1N1 vaccine solution concentrate (1402.7µg HA (haemagglutinin) per mL) - 15µg HA per wafer	5.35	13.5	10.7
2	Buffer (initial charge)	79.00		
3	CMC	1.25	3.2	2.5
4	Sodium alginate	1.25	3.2	2.5
5	Mannitol	17.67	44.7	35.5
6	Lactose monohydrate	5.42	13.7	10.9
7	Amylogum CLS	4.87	12.3	9.8
8	Glycine	2.17	5.5	4.3
9	PEG 1500	0.59	1.5	1.2
10	MCC	0.98	2.5	2.0

11	Buffer adjust qs	14.75	0.0	0.0
	TOTAL	133.31	100.0	79.4
	Theoretical number of wafers (at SG 1.07, bulk solids 29.7%, pH TBA)	498 wafers		-
Adjusted CMC and alginate to 2.5x (refer to 3x B220506 and 4x B220511). SG taken from lab value for B220506. Buffer copied from commercial packaging except for using 100µg PS80 instead of Octoxinol 9 <250µg per 0.5mL. Method of preparation: Add and dissolve buffer solids and PS80 into the phosphate buffer solution and stir until homogenous. Measure out initial buffer charge then add to the CMC/alginate mixture and stir until homogenous. The rest of the excipients can then be added (per usual process).				

[00342] COMPOSITION O: H1N1

TRIAL FORMULATION - Version 1 R1 (04/11/22)		BATCH SIZE: 500 wafers		
ITEM NO.	RAW MATERIAL	BATCH QTY (grams)	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	H1N1 vaccine solution concentrate (1402.7µg HA (haemagglutinin) per mL) - 15µg HA per wafer	5.35	13.4	10.7
2	Buffer (initial charge)	79.00		
3	CMC	1.50	3.7	3.0
4	Sodium alginate	1.50	3.7	3.0
5	Mannitol	17.67	44.1	35.3
6	Lactose monohydrate	5.42	13.5	10.8
7	Amylogum CLS	4.87	12.2	9.7
8	Glycine	2.17	5.4	4.3
9	PEG 1500	0.59	1.5	1.2
10	MCC	0.98	2.5	2.0
11	Buffer adjust qs	14.75		
	TOTAL	133.81	100.0	80.1
	Theoretical number of wafers (at SG 1.07bulk solids 29.9%, pH TBA)	500 wafers		-
Adjusted CMC and alginate to 3x (refer to 3x B220506 and 4x B220511). SG taken from lab value for B220506. Buffer copied from commercial packaging except for using 100µg PS80 instead of Octoxinol 9 <250µg per 0.5mL. Method of preparation: Add and dissolve buffer solids and PS80 into the phosphate buffer solution and stir until homogenous.				

Measure out initial buffer charge then add to the CMC/alginate mixture and stir until homogenous. The rest of the excipients can then be added (per usual process).

B EXAMPLE 2 – STABILITY OF COMPOSITIONS COMPRISING INTERFERON

B.1 AIM

[00343] To determine the stability of compositions of the invention.

5 B.2 MATERIALS AND METHODS

[00344] The materials and methods are presented in the further examples below.

B.3 RESULTS

[00345] A summary of the results of the stability studies of compositions I, L and K is presented in Tables 1, 2 and 3 below.

TABLE 1: IFN Beta 1-alpha 500IU activity in Waferix (COMPOSITION I)

Total IFN Titer Graph Pad (U/mL)									
U per mL	t=0	t=2W (4C)	t=2W (20C)	t=6W (4C)	t=6W (20C)	t=12W (4C)	t=12W (20C)	t=26W (4C)	t=26W (20C)
	550	560	390	470	460	590	570	440	380
	440	420	430	470	450	490	700	500	510
	430	580	620	720	390	600	670	410	410
	440	400	610	580	440	430	720	450	390
average	465	490	512.5	560	435	527.5	665	450	422.5
SD (U/mL)	57	93	120	119	31	82	67	37	60
U per wafer	93	98	102.5	112	87	105.5	133	90	84.5
% Recovery	18.6	19.6	20.5	22.4	17.4	21.1	26.6	18.0	16.9

10

TABLE 2: IFN Alpha 2-beta 500IU activity in Waferix (COMPOSITION L)

Total IFN Titer Graph Pad (U/mL)									
U per mL	t=0	t=2W (4C)	t=2W (20C)	t=6W (4C)	t=6W (20C)	t=12W (4C)	t=12W (20C)	t=26W (4C)	t=26W (20C)
	930	1100	1000	800	820	1800	1200	670	980
	960	970	810	660	790	1700	1500	780	830
	760	1300	990	880	920	1600	980	870	730
	960	960	1200	890	810	2000	1200	900	1400
average	902.5	1082.5	1000	807.5	835	1775	1220	805	985
SD (U/mL)	96	158	159	106	58	171	214	103	293
U per wafer	180.5	216.5	200.0	161.5	167	355	244	161	197
% Recovery	36.1	43.3	40.0	32.3	33.4	71.0	48.8	32.2	39.4

TABLE 3: IFN Alpha 2-beta 1000IU activity in WaterIX (COMPOSITION K)
Total IFN Titer Graph Pad (U/mL)

U per mL	t=0	t=2W (4C)	t=2W (20C)	t=6W (4C)	t=6W (20C)	t=12W (4C)	t=12W (20C)	t=26W (4C)	t=26W (20C)
	5400	5000	5500	6800	6700	7200	10000	6700	5400
	5800	4300	3700	6400	10000	6400	10000	4700	6800
	5900	7000	4000	2500	8100	5900	9400	9100	4800
	6400	4300	5500	4900	5600	6000	8700	6400	5700
average	5875	5150	4675	5150	7600	6375	9525	6725	5675
SD (U/mL)	411	1277	960	1947	1899	591	618	1812	838
U per water	1175	1030	935	1030	1520	1275	1905	1345	1135
% Recovery	117.5	103	93.5	103	152	127.5	190.5	134.5	113.5

B.4 CONCLUSION

[00346] The results of the stability studies of compositions I, L and K is 91%, 101.1% and 97% respectively after storing for 26 weeks

5

C EXAMPLE 3 – STABILITY OF COMPOSITIONS COMPRISING H1N1 INFLUENZA VACCINE

C.1 AIM

[00347] To determine the stability of compositions of the invention.

10

C.2 MATERIALS AND METHODS

[00348] The materials and methods are presented in the further examples below.

C.3 RESULTS

[00349] A summary of the results of the stability studies of compositions M, N and O is presented in Table 4 below.

TABLE 4: COMPOSITIONS M, N AND O

SRID Results			
Theoretical H1N1 HA potency (2019 CoA)	1403 ug/mL		
Seqirus potency (their storage sample)	890 ug/mL		
% of actual	63.44 %		
Theoretical HA per mL	30 ug		
Actual HA	19.03 ug		
HA recovery SRID	Wafer 1	Wafer 2	Wafer 3
HA ug	16.3	16	16.1
HA % of theory (X wafers)	85.7	84.1	84.6
Theoretical H1N1 HA potency (2019 CoA)	1403 ug/mL		
Seqirus potency (IX storage sample)	738 ug/mL		
% of actual	52.60 %		
Theoretical HA per mL	30 ug		
Actual HA	15.78 ug		
HA recovery SRID	Wafer 1	Wafer 2	Wafer 3
HA ug	16.3	16	16.1
HA % of theory (X wafers)	103.3	101.4	102.0
Eliza Results			
Theoretical H1N1 HA potency (2019 CoA)	1403 ug/mL		
Seqirus potency (their storage sample)	825 ug/mL		
% of actual	58.80 %		
Theoretical HA per mL	30 ug		
Actual HA	17.64 ug		
HA recovery SRID	Wafer 1	Wafer 2	Wafer 3
HA ug	12.7	11.4	12.1
HA % of theory (X wafers)	72.5	64.5	68.5

C.4 CONCLUSION

Testing of MPH lot 287971, used to formulate wafers, determined a drop in HA potency by SRID of 47% since the initial testing at batch release. This drop in potency would have impacted the target potency used to formulate wafers as the original SRID value for MPH drug substance was used. It is therefore reasonable to apply a correction to the mass balance of HA through wafer formulation which once applied suggests little or minimal loss of antigen when tested by SRID. Potency testing by EIA however suggests an average 24% loss of HA after wafer formulation. The reason for this discrepancy between EIA and SRID potency methods is not known for certain, however it may be due to the EIA's sensitivity to antigen presentation. Variables such as aggregation and formulation buffer matrix can impact accessibility and affinity of mAb binding thus affecting accuracy of measurement.

D EXAMPLE 4 – STABILITY OF COMPOSITIONS K AND L AT 0 WEEKS OF STORAGE

D.1 SUMMARY

[00350] IFN-alpha wafer samples from two different formulations (500IU/wafer and 1000IU/wafer, or Product Wafer-1 (Composition L) and Product Wafer-2 (Composition K), respectively) and an API sample (100 mcg) were tested in singlet alongside Human IFN-alpha reference material in a viral challenge assay using EMC virus on A549 cells. In preparing the samples, four product wafers were individually reconstituted in 200 µl of DMEM+10% FBS and tested as four individual samples. The API sample (100 mcg) was pre-diluted in DMEM+10% FBS and run as four individual samples. The assay plates were stained with crystal violet, a visual CPE was performed, and the dye was then solubilized, followed by absorbance reading at 570 nm. These data were then analyzed in GraphPad Prism using a sigmoidal fit (variable slope). The GraphPad Prism graphs and calculations are included for reference.

D.2 REFERENCE INFORMATION

[00351] Cytopathic Effect Assay Data Analysis

15 [00352] Visual Read

[00353] Samples are titrated in 96 well plates and protection are determined in comparison to virus (no IFN) and cell (no virus) controls. After maturation of the viral CPE the live cells are fixed and stained with a Crystal Violet solution. The wells are then examined visually to determine which dilution protects 50% of the cells from cytopathic effect. This dilution is calibrated to a standard interferon solution to obtain Units/ml of the sample.

[00354] *Endpoint Well:* The well which reflects the dilution at which 50% of the cells are protected from cytopathic effect of the virus.

25 [00355] *Raw IFN Titer:* The IFN titer at the endpoint well. The titer is derived from the standard interferon solution which has been calibrated to international standards.

[00356] GraphPad Analysis

[00357] GraphPad analysis provides graphic results of the CPE assay. After the Visual Read, the Crystal Violet is solubilized from the cells with methanol and the OD read on a plate reader at 570 nm. These OD values are then transformed to % protection by setting the cell control as 100% and the virus control as 0%. The data are then graphed and analyzed in GraphPad Prism using a variable slope sigmoidal curve fitting function. GraphPad analysis offers the following advantages:

- [00358] 1. It considers all the data points in determining the EC50
- [00359] 2. Examines the slope of different samples and provides a sliding scale for the EC50 value compared to the visual read.
- [00360] 3. Provides actual raw OD values for your in-house analysis if you so desire.
- 5 [00361] *Pre-Dilution*
- [00362] This value reflects any pre-dilution of the sample prior to testing in the CPE assay. The Total IFN Titer values for both Visual Read and GraphPad Analysis include any pre-dilution calculations.
- [00363] *Specific Activity*
- 10 [00364] Specific activity is calculated from the supplied sample concentration (mg/ml) and antiviral titer results (U/ml) from the GraphPad Analysis. Specific Activity = Titer (U/ml) / Concentration (mg/ml).
- [00365] *Visual Endpoint Well*
- [00366] The visual endpoint well for the eye read is the well (in 0.5 well increments)
- 15 representing approximately 50% cell viability and is determined by the amount of crystal violet staining.

D.3 RESULTS

- [00367] Please refer to Tables 5 and 6 and also Figures 1 to Figure 6 for the results.

[00368] **TABLE 5: Activity of Human IFN-alpha product wafers and API sample as a titer (U/ml) and specific activity (U/mg).**

Human IFN-alpha Activity as Titer (U/ml) and Specific Activity (U/mg)

Sample	Sample concentration (mg/ml)	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read U/ml	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
PRODUCT WAFER-1	9.50E-06	7.0	1419	1.4E+03	9.3E+02	9.8E+07
PRODUCT WAFER-1		7.0	1419	1.4E+03	9.6E+02	1.0E+08
PRODUCT WAFER-1		7.0	1419	1.4E+03	7.6E+02	8.0E+07
PRODUCT WAFER-1		7.0	1419	1.4E+03	9.6E+02	1.0E+08
PRODUCT WAFER-2	1.90E-05	9.0	5674	5.7E+03	5.4E+03	2.9E+08
PRODUCT WAFER-2		9.0	5674	5.7E+03	5.8E+03	3.1E+08
PRODUCT WAFER-2		9.0	5674	5.7E+03	5.9E+03	3.1E+08
PRODUCT WAFER-2		9.0	5674	5.7E+03	6.4E+03	3.4E+08
API	1.00E-01	9.0	2837	1.5E+07	1.6E+07	1.6E+08
API		9.0	2837	1.5E+07	1.4E+07	1.4E+08
API		9.0	2837	1.5E+07	1.9E+07	1.9E+08
API		9.0	2837	1.5E+07	1.3E+07	1.3E+08

[00369] Specific activity was calculated from supplied sample concentration (mg/ml) and GraphPad antiviral titer results(U/ml).

[00370] **TABLE 6: Activity of Human IFN-alpha product wafers and API sample and standard as EC50 in (ng/ml).**

Sample	EC50 (ng/ml)	Human IFN-alpha STD EC50 (ng/ml)
PRODUCT WAFER-1	1.1E-02	3.9E-03
PRODUCT WAFER-1	1.0E-02	
PRODUCT WAFER-1	1.3E-02	
PRODUCT WAFER-1	9.7E-03	
PRODUCT WAFER-2	4.4E-03	5.2E-03
PRODUCT WAFER-2	4.2E-03	
PRODUCT WAFER-2	4.6E-03	
PRODUCT WAFER-2	3.6E-03	
API	6.6E-03	4.6E-03
API	1.0E-02	
API	6.2E-03	
API	8.6E-03	

D.4 CONCLUSION

[00371] Recoveries of the IFN-alpha activity after formulation indicated that the 500IU recovery was approximately 40%, and the 1000IU wafer approximately 100%. Although the test results are variable at these low levels of IFN-alpha, replicate data indicate that IFN-alpha can successfully be incorporated into a wafer.

E EXAMPLE 5 – STABILITY OF COMPOSITIONS K AND L AT 2 WEEKS OF STORAGE

E.1 SUMMARY

5 [00372] Four IFN-alpha wafer samples from two different formulations (500IU/wafer and 1000IU/wafer, or Product Wafer-1 (Composition L) and Product Wafer-2 (Composition K), respectively) stored at ambient condition (4°C) and room temperature (RT) for 2 weeks were tested in singlet alongside Human IFN-alpha reference material in a viral challenge assay using EMC virus on A549 cells. In preparing the samples, the product wafers were
10 individually reconstituted in 200 µl of DMEM+10%FBS and tested as four individual samples. The GraphPad Prism graphs and calculations are included for reference.

[00373] The assay plates were stained with crystal violet, a visual CPE was performed, and the dye was then solubilized, followed by absorbance reading at 570 nm. These data were then analyzed in GraphPad Prism using a sigmoidal fit (variable slope).

15 E.2 REFERENCE INFORMATION

[00374] Cytopathic Effect Assay Data Analysis

[00375] Visual Read

[00376] Samples are titrated in 96 well plates and protection are determined in comparison to virus (no IFN) and cell (no virus) controls. After maturation of the viral CPE
20 the live cells are fixed and stained with a Crystal Violet solution. The wells are then examined visually to determine which dilution protects 50% of the cells from cytopathic effect. This dilution is calibrated to a standard interferon solution to obtain Units/ml of the sample.

[00377] *Endpoint Well:* The well which reflects the dilution at which 50% of the cells are
25 protected from cytopathic effect of the virus.

[00378] *Raw IFN Titer:* The IFN titer at the endpoint well. The titer is derived from the standard interferon solution which has been calibrated to international standards.

[00379] GraphPad Analysis

[00380] GraphPad analysis provides graphic results of the CPE assay. After the Visual
30 Read, the Crystal Violet is solubilized from the cells with methanol and the OD read on a plate reader at 570 nm. These OD values are then transformed to % protection by setting

the cell control as 100% and the virus control as 0%. The data are then graphed and analyzed in GraphPad Prism using a variable slope sigmoidal curve fitting function.

[00381] GraphPad analysis offers the following advantages:

[00382] 1. It considers all the data points in determining the EC50

5 [00383] 2. Examines the slope of different samples and provides a sliding scale for the EC50 value compared to the visual read.

[00384] 3. Provides actual raw OD values for your in-house analysis if you so desire.

[00385] *Pre-Dilution*

[00386] This value reflects any pre-dilution of the sample prior to testing in the CPE
10 assay. The Total IFN Titer values for both Visual Read and GraphPad Analysis include any pre-dilution calculations.

[00387] *Specific Activity*

[00388] Specific activity is calculated from the supplied sample concentration (mg/ml) and antiviral titer results (U/ml) from the GraphPad Analysis. Specific Activity = Titer (U/ml) /
15 Concentration (mg/ml).

[00389] *Visual Endpoint Well*

[00390] The visual endpoint well for the eye read is the well (in 0.5 well increments) representing approximately 50% cell viability and is determined by the amount of crystal violet staining.

20 **E.3 RESULTS**

[00391] Please refer to Tables 7 to 10 and also Figures 7 to Figure 14 for the results.

[00392] **TABLE 7: Activity of Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and room temperature for 2 weeks as a titer (U/ml) and specific activity (U/mg).**

Sample	Storage condition	Sample Concentration (mg/ml)	Sample Dilution	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read (U/ml)	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
Product wafer 1	4°C	9.5E-06	1	6.0	1003	1.0E+03	1.1E+03	1.2E+08
Product wafer 1			1	6.0	1003	1.0E+03	9.7E+02	1.0E+08
Product wafer 1			1	6.0	1003	1.0E+03	1.3E+03	1.3E+08
Product wafer 1			1	6.0	1003	1.0E+03	9.8E+02	1.0E+08
Product wafer 1	Room temperature for 2 weeks	9.5E-06	1	6.0	1003	1.0E+03	1.0E+03	1.1E+08
Product wafer 1			1	6.0	1003	1.0E+03	8.1E+02	8.5E+07
Product wafer 1			1	6.0	1003	1.0E+03	9.9E+02	1.0E+08
Product wafer 1			1	6.0	1003	1.0E+03	1.2E+03	1.2E+08

[00393] Specific activity calculated from supplied sample concentration (mg/ml) and GraphPad antiviral titer results(U/ml).

[00394] **TABLE 8: Activity of Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and room temperature for 2 weeks and standard as EC50 in ng/ml.**

Sample	Storage condition	EC50 (ng/ml)	Human IFN-alpha Standard EC50 (ng/ml)
Product wafer 1	4°C	1.3E-02	6.2E-03
Product wafer 1		1.5E-02	
Product wafer 1		1.2E-02	
Product wafer 1		1.6E-02	
Product wafer 1	Room temperature for 2 weeks	1.8E-02	7.6E-03
Product wafer 1		2.3E-02	
Product wafer 1		1.9E-02	
Product wafer 1		1.6E-02	

5

[00395] **TABLE 9: Activity of Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and room temperature for 2 weeks as a titer (U/ml) and specific activity (U/mg).**

Sample	Storage condition	Sample Concentration (mg/ml)	Sample Dilution	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read U/ml	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
Product wafer 2	4°C	1.9E-05	1	8.0	5874	5.7E+03	5.0E+03	2.5E+08
Product wafer 2			1	8.0	5874	5.7E+03	4.3E+03	2.3E+08
Product wafer 2			1	8.5	8025	8.0E+03	7.0E+03	3.7E+08
Product wafer 2			1	8.0	5874	5.7E+03	4.3E+03	2.2E+08
Product wafer 2	Room temperature for 2 weeks	1.9E-05	1	8.0	5874	5.7E+03	5.5E+03	2.9E+08
Product wafer 2			1	8.0	5874	5.7E+03	3.7E+03	1.9E+08
Product wafer 2			1	8.0	5874	5.7E+03	4.0E+03	2.1E+08
Product wafer 2			1	8.0	5874	5.7E+03	5.5E+03	2.9E+08

10 [00396] Specific activity calculated from supplied sample concentration (mg/ml) and GraphPad antiviral titer results(U/ml).

Sample	Storage condition	EC50 (ng/ml)	Human IFN-alpha Standard EC50 (ng/ml)
Product wafer 2	4°C	8.4E-03	6.6E-03
Product wafer 2		7.5E-03	
Product wafer 2		4.6E-03	
Product wafer 2		7.6E-03	
Product wafer 2	Room temperature for 2 weeks	6.9E-03	7.7E-03
Product wafer 2		1.0E-02	
Product wafer 2		9.4E-03	
Product wafer 2		6.9E-03	

[00397] **TABLE 10: Activity of Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and room temperature for 2 weeks and standard as EC50 in ng/ml.**

E.4 CONCLUSION

[00398] Recoveries of IFN-alpha activity in 500IU and 1000IU wafers after 2 weeks show no decline in activity.

5 F EXAMPLE 6 – STABILITY OF COMPOSITIONS K AND L AT 6 WEEKS OF STORAGE

F.1 SUMMARY

[00399] Four IFN-alpha wafer samples from two different formulations (500IU/wafer and 1000IU/wafer, or Product Wafer-1 (Composition L) and Product Wafer-2 (Composition K), respectively) stored at ambient condition (4°C) and room temperature (RT) for 6 weeks were tested in singlet alongside Human IFN-alpha reference material in a viral challenge assay using EMC virus on A549 cells. In preparing the samples, the product wafers were individually reconstituted in 200 µl of DMEM+10%FBS and tested as four individual samples. The GraphPad Prism graphs and calculations are included for reference.

15 [00400] The assay plates were stained with crystal violet, a visual CPE was performed, and the dye was then solubilized, followed by absorbance reading at 570 nm. These data were then analyzed in GraphPad Prism using a sigmoidal fit (variable slope). Please do not hesitate to contact us with further questions

F.2 REFERENCE INFORMATION

20 [00401] **Cytopathic Effect Assay Data Analysis**

[00402] *Visual Read*

[00403] Samples are titrated in 96 well plates and protection is determined in comparison to virus (no IFN) and cell (no virus) controls. After maturation of the viral CPE the live cells are fixed and stained with a Crystal Violet solution. The wells are then examined visually to determine which dilution protects 50% of the cells from cytopathic effect. This dilution is calibrated to a standard interferon solution to obtain Units/ml of the sample.

[00404] *Endpoint Well:* The well which reflects the dilution at which 50% of the cells are protected from cytopathic effect of the virus.

30 [00405] *Raw IFN Titer:* The IFN titer at the endpoint well. The titer is derived from the standard interferon solution which has been calibrated to international standards.

[00406] *GraphPad Analysis*

[00407] GraphPad analysis provides graphic results of the CPE assay. After the Visual Read, the Crystal Violet is solubilized from the cells with methanol and the OD read on a plate reader at 570 nm. These OD values are then transformed to % protection by setting the cell control as 100% and the virus control as 0%. The data are then graphed and analyzed in GraphPad Prism using a variable slope sigmoidal curve fitting function. GraphPad analysis offers the following advantages:

[00408] 1. It considers all the data points in determining the EC50

[00409] 2. Examines the slope of different samples and provides a sliding scale for the EC50 value compared to the visual read.

[00410] 3. Provides actual raw OD values for your in-house analysis if you so desire.

[00411] *Pre-Dilution*

[00412] This value reflects any pre-dilution of the sample prior to testing in the CPE assay. The Total IFN Titer values for both Visual Read and GraphPad Analysis include any pre-dilution calculations.

[00413] *Specific Activity*

[00414] Specific activity is calculated from the supplied sample concentration (mg/ml) and antiviral titer results (U/ml) from the GraphPad Analysis. Specific Activity = Titer (U/ml) / Concentration (mg/ml).

[00415] *Visual Endpoint Well*

[00416] The visual endpoint well for the eye read is the well (in 0.5 well increments) representing approximately 50% cell viability and is determined by the amount of crystal violet staining

F.3 RESULTS

[00417] Please refer to Tables 13 to 16 and also Figures 15 to Figure 22 for the results.

[00418] **TABLE 13: Activity of Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and room temperature for 6 weeks as a titer (U/ml) and specific activity (U/mg).**

Sample	Storage condition	Sample Concentration (mg/ml)	Sample Dilution	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read U/ml	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
Product wafer 1	4°C	9.5E-06	1	6.0	1003	1.0E+03	8.0E+02	8.5E+07
Product wafer 1			1	6.0	1003	1.0E+03	6.6E+02	6.9E+07
Product wafer 1			1	6.0	1003	1.0E+03	8.3E+02	8.8E+07
Product wafer 1			1	6.0	1003	1.0E+03	8.4E+02	8.9E+07
Product wafer 1	Room temperature for 6 weeks	9.5E-06	1	6.0	1003	1.0E+03	8.2E+02	8.6E+07
Product wafer 1			1	6.0	1003	1.0E+03	7.5E+02	8.3E+07
Product wafer 1			1	6.0	1003	1.0E+03	9.2E+02	9.7E+07
Product wafer 1			1	6.0	1003	1.0E+03	8.1E+02	8.5E+07

[00419] Specific activity calculated from supplied sample concentration (mg/ml) and GraphPad antiviral titer results(U/ml).

[00420] **TABLE 14: Activity of Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and room temperature for 6 weeks and standard as EC50 in ng/ml.**

Sample	Storage condition	EC50 (ng/ml)	Human IFN-alpha Standard EC50 (ng/ml)
Product wafer 1	4°C	1.1E-02	3.8E-03
Product wafer 1		1.4E-02	
Product wafer 1		1.1E-02	
Product wafer 1		1.1E-02	
Product wafer 1	Room temperature for 6 weeks	1.0E-02	3.4E-03
Product wafer 1		1.1E-02	
Product wafer 1		9.1E-03	
Product wafer 1		1.0E-02	

[00421] **TABLE 15: Activity of Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and room temperature for 6 weeks as a titer (U/ml) and specific activity (U/mg).**

Sample	Storage condition	Sample Concentration (mg/ml)	Sample Dilution	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read U/ml	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
Product wafer 2	4°C	1.9E-05	1	8.0	5674	5.7E+03	6.8E+03	3.6E+08
Product wafer 2			1	8.0	5674	5.7E+03	6.4E+03	3.4E+08
Product wafer 2			1	7.5	4012	4.0E+03	2.5E+03	1.3E+08
Product wafer 2			1	8.0	5674	5.7E+03	4.9E+03	2.6E+08
Product wafer 2	Room temperature for 6 weeks	1.9E-05	1	8.5	8025	8.0E+03	6.7E+03	3.6E+08
Product wafer 2			1	9.0	11349	1.1E+04	1.0E+04	5.3E+08
Product wafer 2			1	9.0	11349	1.1E+04	8.1E+03	4.3E+08
Product wafer 2			1	8.5	8025	8.0E+03	5.6E+03	2.9E+08

10

[00422] Specific activity calculated from supplied sample concentration (mg/ml) and GraphPad antiviral titer results(U/ml).

[00423] **TABLE 16: Activity of Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and room temperature for 6 weeks and standard as EC50 in ng/ml.**

Sample	Storage condition	EC50 (ng/ml)	Human IFN-alpha Standard EC50 (ng/ml)
Product wafer 2	4°C	2.4E-03	3.4E-03
Product wafer 2		2.6E-03	
Product wafer 2		6.6E-03	
Product wafer 2		3.3E-03	
Product wafer 2	Room temperature for 6 weeks	4.0E-03	5.5E-03
Product wafer 2		2.6E-03	
Product wafer 2		3.3E-03	
Product wafer 2		4.8E-03	

F.4 CONCLUSION

[00424] Recoveries of IFN-alpha activity in 500IU and 1000IU wafers after 6 weeks show no decline in activity.

5

G EXAMPLE 7 – STABILITY OF COMPOSITIONS K AND L AT 12 WEEKS OF STORAGE

G.1 SUMMARY

[00425] Four IFN-alpha wafer samples from two different formulations (500IU/wafer and 1000IU/wafer, or Product Wafer-1 (Composition L) and Product Wafer-2 (Composition K), respectively) stored at ambient condition (4°C) and room temperature (RT) for 12 weeks were tested in singlet alongside Human IFN-alpha reference material in a viral challenge assay using EMC virus on A549 cells. In preparing the samples, the product wafers were individually reconstituted in 200 µl of DMEM+10% FBS and tested as four individual samples. The GraphPad Prism graphs and calculations are included for reference. The assay plates were stained with crystal violet, a visual CPE was performed, and the dye was then solubilized, followed by absorbance reading at 570 nm. These data were then analyzed in GraphPad Prism using a sigmoidal fit (variable slope)..

G.2 REFERENCE INFORMATION

[00426] Cytopathic Effect Assay Data Analysis

[00427] Visual Read

[00428] Samples are titrated in 96 well plates and protection is determined in comparison to virus (no IFN) and cell (no virus) controls. After maturation of the viral CPE the live cells are fixed and stained with a Crystal Violet solution. The wells are then examined visually to

determine which dilution protects 50% of the cells from cytopathic effect. This dilution is calibrated to a standard interferon solution to obtain Units/ml of the sample.

[00429] *Endpoint Well:* The well which reflects the dilution at which 50% of the cells are protected from cytopathic effect of the virus.

- 5 [00430] *Raw IFN Titer:* The IFN titer at the endpoint well. The titer is derived from the standard interferon solution which has been calibrated to international standards.

[00431] *GraphPad Analysis*

- 10 [00432] GraphPad analysis provides graphic results of the CPE assay. After the Visual Read, the Crystal Violet is solubilized from the cells with methanol and the OD read on a plate reader at 570 nm. These OD values are then transformed to % protection by setting the cell control as 100% and the virus control as 0%. The data are then graphed and analyzed in GraphPad Prism using a variable slope sigmoidal curve fitting function. GraphPad analysis offers the following advantages:

[00433] 1. It considers all the data points in determining the EC50

- 15 [00434] 2. Examines the slope of different samples and provides a sliding scale for the EC50 value compared to the visual read. 3. Provides actual raw OD values for your in-house analysis if you so desire.

[00435] *Pre-Dilution*

- 20 [00436] This value reflects any pre-dilution of the sample prior to testing in the CPE assay. The Total IFN Titer values for both Visual Read and GraphPad Analysis include any pre-dilution calculations.

[00437] *Specific Activity*

- 25 [00438] Specific activity is calculated from the supplied sample concentration (mg/ml) and antiviral titer results (U/ml) from the GraphPad Analysis. Specific Activity = Titer (U/ml) / Concentration (mg/ml).

[00439] *Visual Endpoint Well*

[00440] The visual endpoint well for the eye read is the well (in 0.5 well increments) representing approximately 50% cell viability and is determined by the amount of crystal violet staining.

G.3 RESULTS

[00441] Please refer to Tables 17 to 20 and also Figures 23 to 30 for the results.

[00442] **TABLE 17: Activity of Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and room temperature for 12 weeks as a titer (U/ml) and specific activity (U/mg).**

Sample	Storage condition	Sample Concentration (mg/ml)	Sample Dilution	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read U/ml	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
Product wafer 1	4°C	9.5E-06	1	6.0	1003	1.0E+03	1.6E+03	1.6E+08
Product wafer 1			1	6.0	1003	1.0E+03	1.7E+03	1.6E+08
Product wafer 1			1	6.5	1003	1.0E+03	1.8E+03	1.7E+08
Product wafer 1			1	6.0	1003	1.0E+03	2.0E+03	2.1E+08
Product wafer 1	Room temperature for 12 weeks	8.5E-06	1	6.0	1003	1.0E+03	1.2E+03	1.3E+08
Product wafer 1			1	6.0	1003	1.0E+03	1.5E+03	1.6E+08
Product wafer 1			1	6.5	1003	1.0E+03	5.8E+02	1.0E+08
Product wafer 1			1	6.0	1003	1.0E+03	1.2E+03	1.3E+08

[00443] Specific activity calculated from supplied sample concentration (mg/ml) and GraphPad antiviral titer results (U/ml).

[00444] **TABLE 18: Activity of Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and room temperature for 12 weeks and standard as EC50 in ng/ml.**

Sample	Storage condition	EC50 (ng/ml)	Human IFN-alpha Standard EC50 (ng/ml)
Product wafer 1	4°C	3.9E-03	2.9E-03
Product wafer 1		4.1E-03	
Product wafer 1		4.5E-03	
Product wafer 1		3.6E-03	
Product wafer 1	Room temperature for 12 weeks	5.0E-03	2.5E-03
Product wafer 1		4.1E-03	
Product wafer 1		6.2E-03	
Product wafer 1		4.9E-03	

[00445] **TABLE 19: Activity of Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and room temperature for 12 weeks as a titer (U/ml) and specific activity (U/mg).**

Sample	Storage condition	Sample Concentration (mg/ml)	Sample Dilution	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read U/ml	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
Product wafer 2	4°C	1.0E-05	1	8.0	5674	5.7E+03	7.2E+03	3.8E+08
Product wafer 2			1	8.0	5674	5.7E+03	6.4E+03	3.4E+08
Product wafer 2			1	8.0	5674	5.7E+03	5.9E+03	3.1E+08
Product wafer 2			1	8.0	5674	5.7E+03	6.8E+03	3.2E+08
Product wafer 2	Room temperature for 12 weeks	1.9E-06	1	9.0	11349	1.1E+04	1.8E+04	5.3E+08
Product wafer 2			1	9.0	11348	1.1E+04	1.6E+04	5.4E+08
Product wafer 2			1	8.0	5674	5.7E+03	9.4E+03	4.9E+08
Product wafer 2			1	8.0	5674	5.7E+03	8.7E+03	4.6E+08

[00446] Specific activity calculated from supplied sample concentration (mg/ml) and GraphPad antiviral titer results(U/ml).

[00447] **TABLE 20: Activity of Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and room temperature for 12 weeks and standard as EC50 in ng/ml.**

Sample	Storage condition	EC50 (ng/ml)	Human IFN-alpha Standard EC50 (ng/ml)
Product wafer 2	4°C	2.3E-03	3.4E-03
Product wafer 2		2.6E-03	
Product wafer 2		2.8E-03	
Product wafer 2		2.7E-03	
Product wafer 2	Room temperature for 12 weeks	2.4E-03	5.6E-03
Product wafer 2		2.4E-03	
Product wafer 2		2.6E-03	
Product wafer 2		2.8E-03	

G.4 CONCLUSION

[00448] Recoveries of IFN-alpha activity in 500IU and 1000IU wafers after 12 weeks show no decline in activity.

10

H EXAMPLE 8 – STABILITY OF COMPOSITIONS K AND L AT 26 WEEKS OF STORAGE

H.1 SUMMARY

[00449] Four IFN-alpha wafer samples from two different formulations (500IU/wafer and 1000IU/wafer, or Product Wafer-1 (Composition L) and Product Wafer-2 (Composition K), respectively) stored at ambient condition (4°C) and room temperature (RT) for 26 weeks were tested in singlet alongside Human IFN-alpha reference material in a viral challenge

15

- assay using EMC virus on A549 cells. In preparing the samples, the product wafers were individually reconstituted in 200 µl of DMEM+10% FBS and tested as four individual samples. The GraphPad Prism graphs and calculations are included for reference. The assay plates were stained with crystal violet, a visual CPE was performed, and the dye was then solubilized, followed by absorbance reading at 570 nm. These data were then analyzed in GraphPad Prism using a sigmoidal fit (variable slope).

H.2 REFERENCE INFORMATION

[00450] Cytopathic Effect Assay Data Analysis

[00451] Visual Read

- 10 [00452] Samples are titrated in 96 well plates and protection is determined in comparison to virus (no IFN) and cell (no virus) controls. After maturation of the viral CPE the live cells are fixed and stained with a Crystal Violet solution. The wells are then examined visually to determine which dilution protects 50% of the cells from cytopathic effect. This dilution is calibrated to a standard interferon solution to obtain Units/ml of the sample.
- 15 [00453] *Endpoint Well:* The well which reflects the dilution at which 50% of the cells are protected from cytopathic effect of the virus.

[00454] *Raw IFN Titer:* The IFN titer at the endpoint well. The titer is derived from the standard interferon solution which has been calibrated to international standards.

[00455] GraphPad Analysis

- 20 [00456] GraphPad analysis provides graphic results of the CPE assay. After the Visual Read, the Crystal Violet is solubilized from the cells with methanol and the OD read on a plate reader at 570 nm. These OD values are then transformed to % protection by setting the cell control as 100% and the virus control as 0%. The data are then graphed and analyzed in GraphPad Prism using a variable slope sigmoidal curve fitting function.
- 25 GraphPad analysis offers the following advantages:

[00457] 1. It considers all the data points in determining the EC50

[00458] 2. Examines the slope of different samples and provides a sliding scale for the EC50 value compared to the visual read.

[00459] 3. Provides actual raw OD values for your in-house analysis if you so desire.

- 30 [00460] *Pre-Dilution*

[00461] This value reflects any pre-dilution of the sample prior to testing in the CPE assay. The Total IFN Titer values for both Visual Read and GraphPad Analysis include any pre-dilution calculations.

[00462] *Specific Activity*

- 5 [00463] Specific activity is calculated from the supplied sample concentration (mg/ml) and antiviral titer results (U/ml) from the GraphPad Analysis. Specific Activity = Titer (U/ml) / Concentration (mg/ml).

[00464] *Visual Endpoint Well*

- 10 [00465] The visual endpoint well for the eye read is the well (in 0.5 well increments) representing approximately 50% cell viability and is determined by the amount of crystal violet staining.

H.3 RESULTS

[00466] Please refer to Tables 21 to 24 and also Figures 31 to 34 for the results.

- 15 [00467] **TABLE 21: Activity of Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and room temperature for 26 weeks as a titer (U/ml) and specific activity (U/mg).**

Human IFN-alpha Activity as Titer (U/ml) and Specific Activity (U/mg)								
Sample	Storage condition	Sample Concentration (mg/ml)	Sample Dilution	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read (U/ml)	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
Product wafer 1 (4°C)	4°C	9.50E-06	1	6.0	1419	1.4E+03	6.7E+02	7.1E+07
Product wafer 1 (4°C)			1	6.0	1419	1.4E+03	7.8E+02	8.2E+07
Product wafer 1 (4°C)			1	6.0	1419	1.4E+03	6.7E+02	9.1E+07
Product wafer 1 (4°C)			1	6.0	1419	1.4E+03	9.0E+02	9.5E+07
Product wafer 1 (RT)	Room temperature for 26 weeks	9.50E-06	1	6.0	1003	1.0E+03	9.8E+02	1.0E+08
Product wafer 1 (RT)			1	6.0	1003	1.0E+03	8.3E+02	8.8E+07
Product wafer 1 (RT)			1	6.0	1003	1.0E+03	7.3E+02	7.7E+07
Product wafer 1 (RT)			1	6.0	1003	1.0E+03	1.4E+03	1.4E+08

[00468] Specific activity calculated from supplied sample concentration (mg/ml) and GraphPad antiviral titer results(U/ml).

- 20 [00469] **TABLE 22: Activity of Human IFN-alpha product wafer 1 (Composition L) stored at 4°C and room temperature for 26 weeks and standard as EC50 in ng/ml.**

Sample	Storage condition	EC50 (ng/ml)	Human IFN-alpha Standard EC50 (ng/ml)
Product wafer 1	4°C	1.5E-03	7.8E-03
Product wafer 1		1.1E-03	
Product wafer 1		1.4E-03	
Product wafer 1		8.9E-04	
Product wafer 1	Room temperature for 26 weeks	2.0E-03	7.4E-03
Product wafer 1		2.1E-03	
Product wafer 1		2.3E-03	
Product wafer 1		1.4E-03	

[00470] TABLE 23: Activity of Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and room temperature for 26 weeks as a titer (U/ml) and specific activity (U/mg).

Sample	Storage condition	Sample Concentration (mg/ml)	Sample Dilution	Endpoint Well	Raw IFN Titer	Total IFN Titer Visual Read (U/ml)	Total IFN Titer Graph Pad (U/ml)	Specific Activity (U/mg)
Product wafer 2 (4°C)	4°C	1.80E-05	1	8.0	5674	5.7E+03	6.7E+03	3.5E+08
Product wafer 2 (4°C)			1	8.0	5674	5.7E+03	4.7E+03	2.6E+08
Product wafer 2 (4°C)			1	8.0	5674	5.7E+03	9.1E+03	4.8E+08
Product wafer 2 (4°C)			1	8.0	5674	5.7E+03	8.4E+03	3.4E+08
Product wafer 2 (RT)	Room temperature for 26 weeks	1.80E-05	1	7.0	2837	2.8E+03	5.4E+03	2.9E+08
Product wafer 2 (RT)			1	7.0	2837	2.8E+03	6.8E+03	3.6E+08
Product wafer 2 (RT)			1	7.0	2837	2.8E+03	4.8E+03	2.6E+08
Product wafer 2 (RT)			1	7.0	2837	2.8E+03	5.7E+03	3.0E+08

5

[00471] TABLE 24: Activity of Human IFN-alpha product wafer 2 (Composition K) stored at 4°C and room temperature for 26 weeks and standard as EC50 in ng/ml.

Sample	Storage condition	EC50 (ng/ml)	Human IFN-alpha Standard EC50 (ng/ml)
Product wafer 2	4°C	6.2E-04	5.0E-03
Product wafer 2		7.9E-04	
Product wafer 2		1.9E-04	
Product wafer 2		6.6E-04	
Product wafer 2	Room temperature for 26 weeks	1.0E-03	8.9E-03
Product wafer 2		4.0E-04	
Product wafer 2		6.4E-04	
Product wafer 2		4.9E-04	

H.4 CONCLUSION

- 10 [00472] Recoveries of IFN-alpha activity in 500IU and 1000IU wafers after 26 weeks show no decline in activity. The formulated wafers show that production and storage of IFN-alpha wafers at 4C and ambient are viable.

I EXAMPLE 8 – HAEMAGGLUTININ POTENCY DETERMINATION OF IX SYNRIX
SUBLINGUAL H1N1 VACCINE WAFERS

I.1 PURPOSE

5 [00473] Seqirus’ Analytical Development team within Vaccine and Product Development (VPD), performed Haemagglutinin (HA) Potency analysis utilising the compendial Single Radial Immunodiffusion (SRID) assay and internally developed alternative assay; Enzyme Immunoassay (EIA), on iX Synrix Sublingual H1N1 Vaccine Wafers, formulated at 15µg HA/mL.

I.2 RESULTS

[00474] Please refer to Tables 25 and also Figures 35 for the results.

[00475] **TABLE 25: HA Potency testing on MPH used in formulation and the formulated wafers (Composition M = BN221114; Composition N = BN221115; Composition O = BN221116).**

Lot	Expected HA Potency (µg HA/mL)	Mean HA Potency by <u>SRID</u> (n=8) (µg HA/mL)	Recovery by <u>SRID</u> (%)	Loss by <u>SRID</u> (%)	Mean HA Potency by <u>EIA</u> (n=6) (µg HA/mL)	Recovery by <u>EIA</u> (%)	Loss by <u>EIA</u> (%)
MPH 287971 {Seqirus}	1403 ¹	890.0	63	37	825.0	59	42
MPH 287971 {iX Synrix}	1403 ¹	738.0	53	47 ³	NT	NT	NT
Wafer BN221114	30 ²	16.3	54	46	12.7	42	58
Wafer BN221115	30 ²	16.0	53	47	11.4	38	62
Wafer BN221116	30 ²	16.1	54	46	12.1	40	60
Mean Wafer	N/A	16.1 (SD -0.152) (%CV - 1%)	54	46	12.1 (SD -0.851) (%CV - 5%)	40	60

¹ Previously reported SRID potency.
² reconstitution of 500 µL with distilled water, therefore each wafer has an expected potency 30 µg HA/mL. Target formulation of each wafer is 15mg HA/ml.
³ loss of 47% HA from initial SRID testing performed at batch release
NT – Not Tested, SD – Standard Deviation, CV – Coefficient Variation

[00476] **TABLE 26: HA recovery post formulation by SRID and EIA using adjusted values to account for target formulation discrepancies due to loss of potency of MPH drug substance.**

Lot	Expected HA Potency ($\mu\text{g HA/mL}$)	Mean adjusted HA Potency by <u>SRID</u> (n=8) ($\mu\text{g HA/mL}$)	Recovery by <u>SRID</u> (%)	Mean HA Potency by <u>EIA</u> (n=6) ($\mu\text{g HA/mL}$)	Recovery by <u>EIA</u> (%)
Wafer BN221114	30	30.8 ¹	103	24.0	80
Wafer BN221115	30	30.2	101	21.5	72
Wafer BN221116	30	30.4	101	22.8	76
Mean Wafer	N/A	30.5 (SD = 0.152) (%CV = 1%)	102	22.8 (SD = 0.651) (%CV = 5%)	76

¹Example calculation used to derive adjusted value: 16.3mcg HA/mL divided by $0.53 = 30.8\text{ mcg HA/mL}$

- [00477] In view of some discrepancies, we re-calculated the EIA plates.
- [00478] (1) EIA potency ~25% lower than SRID, EIA had much better CVs.
- [00479] (2) There was no matrix effect regardless of increased mucoadhesive, in either assay i.e., no statistical difference in potencies.
- 5 [00480] (3) Both SRID and EIA are reading lower than the expected dose 15mcg/wafer. We dissolved each wafer in 0.5mL WFI so expected 30mcg HA/mL, BUT I believed they formulated on the expected MPH conc of 1403mcg HA/mL, our SRID of the MPH they sent back was 738 mcg HA/mL so they would have under-formulated by 47% (if
- 10 1403 is the number they used). So if they had used the re-calibrated MPH value to formulate the potencies would be approx. 17.6mcg HA/mL EIA and 23.6mcg HA/mL SRID, approx. 79% recovery.
- [00481] **TABLE 27: SRID of MPH 287971 (Seqirus reserve and material returned by iX Syrinx) and iX Syrinx Wafer Vaccines against NIBSC standard 19/306 (calibrated potency 59 $\mu\text{g HA/mL}$), with recovery compared to a predicted concentration of 30 $\mu\text{g HA/mL}$ (Composition M = BN221114; Composition N = BN221115; Composition O = BN221116).**
- 15

	R1	R2	R3	R4	R5	R6	R7	R8		Average (µg HA/mL)	ST Dev	CV	Recovery (from 1403mcgHA/mL expected)
MPH 287971 (Seqirus)	916	908	900	830	938	860	851	924		890	39	4%	64%
MPH 287971 (iX Syrinx)	666	855	674	608	815	N/A	729	818		738	93	13%	53%
	Wafer 1		Wafer 2		Wafer 3		Wafer 3						
	R1	R2	R1	R2	R1	R2	R1	R2					Recovery (15mcg per wafer)
Wafer BN221114*	15.4	12.5	15	15	17.6	16.4	20.4	18.1		16.3	2.4	15%	54%
Wafer BN221115*	13.6	13.2	14.8	16.5	17.9	18.1	16.7	17.5		16.0	1.9	12%	53%
Wafer BN221116*	13	13.9	16.5	14	18.5	15.5	18.5	17.5		16.1	2.3	15%	54%
*reconstituted in 500 µL dH2O, expected potency 30 µg HA/mL													

[00482] **TABLE 28: EIA of MPH 287971 (Seqirus reserve) and iX Syrinx Wafer Vaccines against MPH 287971 material returned by iX Syrinx (calibrated potency 59 µg HA/mL), with recovery compared to a predicted concentration of 30 µg HA/m**
5 (Composition M = BN221114; Composition N = BN221115; Composition O = BN221116).

	R1	R2	R3	R4	R5	R6				Average (µg HA/mL)	ST Dev	CV	Recovery (from 1403mcgHA/mL expected)
MPH 287971 (Seqirus)	792	771	846	816	889	841				825	42	5%	59%
	Wafer 1		Wafer 2		Wafer 3		Wafer 3						
	R1	R2	R1	R2	R1	R2	R1	R2					Recovery (15mcg per wafer)
Wafer BN221114*	13.2	12.1	12.8	12.5	12.7	12.2	13.7	12.8		12.7	0.5	4%	42%
Wafer BN221115*	11.2	11.2	11.4	11.1	11.4	12.1	11.4	11.6		11.4	0.3	3%	38%
Wafer BN221116*	13.4	12.2	11.7	11.5	12.7	11.1	12.7	11.9		12.1	0.8	6%	40%
*reconstituted in 500 µL WFI, expected potency 30 µg tHA/mL													

1.3 CONCLUSION

[00483] Testing of MPH lot 287971, used to formulate wafers (Composition M = BN221114; Composition N = BN221115; Composition O = BN221116), determined a drop in HA potency by SRID of 47% since the initial testing at batch release. This drop in potency would have impacted the target potency used to formulate wafers as the original SRID value for MPH drug substance was used. It is therefore reasonable to apply a correction to the mass balance of HA through wafer formulation which once applied suggests little or minimal loss of antigen when tested by SRID. Potency testing by EIA however suggests an average 24% loss of HA after wafer formulation. The reason for this discrepancy between EIA and

SRID potency methods is not known for certain, however it may be due to the EIA's sensitivity to antigen presentation. Variables such as aggregation and formulation buffer matrix can impact accessibility and affinity of mAb binding thus affecting accuracy of measurement.

5

J EXAMPLE 9 – DISCOVERY OF FORMULATION PARAMETERS FOR DISPENSING AND VIABLE WAFER FORMATION

J.1 PURPOSE

[00484] To determine whether a viable wafer could be prepared that could be dispensed
10 under commercially viable settings, formed a suitable wafer matrix after freeze drying, and
disintegrated in a time of under 60s, whilst retaining moderate viscosity from the added
polymers after disintegration.

J.2 MATERIALS AND METHODS

[00485] A range of placebos were made according to the ingredients listed in Table 30
15 below.

J.3 RESULTS

[00486] Please refer to Table 29 and 30 for the results.

[00487] Table 29 details the different ratios of CMC and sodium alginate used to discover
this desirable feature. Total polymer concentrations ranged from 13.8% to 4.4% of the wafer
20 solids. Note that amylopectin was not a viscosity-altering agent, and functionally served as a
matrix scaffold to strengthen the low MW water soluble ingredients. Different total solids in
the initial bulk solution, as well as different wafer volumes, were prepared

[00488] **TABLE 29:** The different ratios of CMC and sodium alginate used to discover this desirable feature.

Formulation	Able to be Dispensed?	Forms Suitable wafers?	Disintegrates <60s?	Overall Viability
Placebo 1	No	NA	NA	Unviable
Placebo 2	Yes (manually)	Yes	No	Unviable
Placebo 3	Yes (manually)	Yes	No	Unviable
Placebo 4	Yes (manually)	Yes	Yes	Unviable
Placebo 5	Yes (automated) ¹	Yes	Yes	Semi-Viable
Placebo 6	Yes (automated)	Yes	Yes	Viable
Placebo 7	Yes (automated) ²	Yes	Yes	Semi-Viable
Placebo 8	Yes (manually)	Yes	No	Unviable

1. Wafer had poor mechanical properties due to the low solids content.
2. Slowest dispensing setting possible, not commercially viable.

[00489] Table 30 summarises the results of the trials.

5 [00490] **TABLE 30: Results**

	Placebo 1	Placebo 2	Placebo 3	Placebo 4	Placebo 5	Placebo 6	Placebo 7	Placebo 8
Ingredient Name	% w/w solids	% w/w solids	% w/w solids	% w/w solids	% w/w solids	% w/w solids	% w/w solids	% w/w solids
Active	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CMC	6.9	5.3	4.9	4.3	2.2	2.3	3.7	4.3
Sodium alginate	6.9	5.3	4.9	4.3	2.2	2.3	3.7	4.3
Mannitol	69.0	49.8	50.3	50.9	53.3	53.2	51.7	50.9
Lactose monohydrate	0.0	15.3	15.4	15.6	16.4	16.3	15.8	15.6
Amylogum CLS	2.4	13.7	13.9	14.0	14.7	14.7	14.2	14.0
Glycine	6.9	6.1	6.2	6.3	6.5	6.5	6.3	6.2
PEG 1500	6.9	1.7	1.7	1.7	1.8	1.8	1.7	1.7
MCC	1.0	2.8	2.8	2.8	3.0	3.0	2.9	2.8
% solids in bulk solution	20.9	16.7	16.4	16.3	15.6	24.9	25.7	25.0
wafer volume (uL)	550	550	400	400	400	250	250	250

J.4 CONCLUSION

[00491] It was clear that, in order to produce a suitable wafer with the maximum potential viscosity after disintegration, without compromising manufacturing and mechanical properties, the total polymer concentration was ideally in the range 4-6%, with each viscosity agent in the range 2-3%.

K EXAMPLE 10 – EFFECT OF THE TEST FORMULATION ON THE MUCOSAL IMMUNE RESPONSE IN RAT MODEL

K.1 PURPOSE

- 5 [00492] To evaluate the effect of test formulation on mucosal immune response in rat model.

K.2 BACKGROUND

- [00493] Mucosal immune responses are characterized by the production of secretory immunoglobulin A (S-IgA) and its specific transport across the epithelium to the mucosal surface. Mucosal immune responses include a major B-cell component that is characterized by secretory IgA (S-IgA) Ab, as well as T-helper cell and CTL (Cytotoxic T lymphocyte) responses. These responses can be induced by pathogens triggering the organized mucosal inductive sites.

K.3 MATERIALS AND METHODS

- 15 [00494] *Test System.*

[00495] **TABLE 31.**

Species	: <i>Rattus norvegicus</i>
Strain	: Sprague Dawley
Sex	: Male
Age	: 8-10 weeks old (~250g) (Exact age will be provided in the report)
Source	: Hylasco Biotechnology Pvt. Ltd. 4B, M.N. Park, Turkapilly (Vil), Shameerpet (Md), Medchal Dist, -500078
No of groups	: 3
Total number of animals	: 44
Number of animals per group	: 12-16 (Group-1, n=3 animal/time point; Group- 2 & 3, n=4 animal/time point)
Acclimatization	: After clinical examination for good health and suitability for the study, animals will be acclimatized for at least 4 days before the start of the treatment. During acclimatization, animals will be observed once daily for any abnormal observation.
Animal identification	: Animals will be identified by tail marking.

[00496] *Treatment details*[00497] **TABLE 32.**

Group No.	Treatment	Treatment Dose	Number of animals per group	Number of animals/ time point	Route of Administration & Frequency of Dosing
1	Vehicle control (placebo)	-	12	3	Sublingual route; Single administration
2	Test item dose 1	2,000IU Interferon alpha 2b	16	4	
3	Test item dose 2	10,000 IU Interferon alpha 2b	16	4	

[00498] *Test Items*

5 [00499] Interferon alpha 2-beta 1000 IU

[00500] **TABLE 33. Example formulation for 1000IU/wafer**

TRIAL FORMULATION – 1000IU		BATCH SIZE: 600 wafers (1000 IU per wafer)		
ITEM NO.	RAW MATERIAL	BATCH QTY (grams)	SOLIDS CONT. (% w/w)	WAFER CONT. (mg/wafer)
1	Interferon Alpha 2-beta 1000 IU/wafer	6.0	0.21	0.21
2	Stabilization Buffer pH 7-7.4 (initial charge)	160.00	5.72	5.76
3	CMC	1.0	1.65	1.69
4	Sodium alginate	1.0	1.65	1.69
5	Mannitol	27.69	47.8	46.2
6	Lactose monohydrate	18.99	31.4	31.7
7	Amylogum CLS	6.32	10.4	10.6
8	Glycine	0.29	0.47	0.5
9	PEG 1500	0.83	1.38	1.4
10	MCC	0.56	0.93	0.9
11	Stabilization Buffer pH 7-7.4 qs (rinse).	10.5	0.38	0.38
	TOTAL	233.18	100.00	100.8

	Theoretical number of wafers (at SG 1.11, bulk solids 25.95%, pH 7-7.4)	600.2 wafers		-
--	---	--------------	--	---

[00501] Time point animals sacrifice details.

[00502] TABLE 34.

5	Group No	Treatment	Time points (Hours)			
			1h	4h	6h	12h
	1	Vehicle control (placebo)	3			
				3		
					3	
						3
	2	Test item dose 1	4			
				4		
					4	
						4
3	Test item dose 2	4				
			4			
				4		
					4	
Total no. of animals per time point			11	11	11	11

10							
----	--	--	--	--	--	--	--

[00503] Route of administration. Test item will be administered by sublingual route at the doses listed in the **Error! Reference source not found..**

[00504] Environmental conditions. Animals will be housed in an environment-controlled room at 22 ± 3°C and a relative humidity of 30 to 70 percent. The photoperiod will be 12 hours of light and 12 hours of darkness. The experimental room will maintain an adequate fresh air supply of 12 - 15 air changes/hour.

[00505] Housing. Two rats per cage will be housed in sterilized suspended standard, polysulfone cages (Size: approximately L 425 x B 266 x H 185 mm), with stainless steel top grills having the facility for holding pelleted food and drinking water in polycarbonate bottles with stainless steel sipper tubes and enrichment object. During the study period, animals will be housed in a single room.

[00506] Bedding. Steam-sterilized corn cob will be used as bedding material and changed along with the cage at least once a week. Bedding material analysis reports will be included in the study report.

[00507] Diet. A Standard Rodent Maintenance Diet will be provided ad libitum to all animals. Diet analysis reports will be included in the study report.

- [00508] Water. Bore-well water passed through an activated charcoal filter. It is exposed to UV rays in an Aquaguard water filter-cum-purifier manufactured by Eureka Forbes Ltd., Mumbai—400 001, India, and will be provided ad libitum to the animals. Water analysis reports will be included in the study report.
- 5 [00509] Randomization. Animals will be assigned to different groups according to body weight, maintaining an inter-group variation of no more than $\pm 15\%$ of the mean body weight.
- [00510] Protocol.
- [00511] Experimental procedure. Male SD rats, age ~8 weeks, will be procured from Hylasco, India, and quarantined at the test facility for one week. After quarantine, animals
- 10 will be acclimatized for at least 4 days in the experimental room before starting the in-life experimental phase. During acclimatization and throughout the study period, animals will be observed at least once daily for clinical signs & mortality. Animals will be randomized into three treatment groups based on body weight. All the treatment group animals will receive their respective treatment.
- 15 [00512] All the animals will be observed for clinical signs & mortality throughout the study period. All the treatment groups animals will be dosed at 0 h by sublingual route. From the group 1, n=3 animals and group 2 & 3 n=4 animals will be sacrificed at 1h, 4h, 6h & 12h time points. Sublingual mucosal layer and intestinal mucosal layer will be collected for cytokines analysis.
- 20 [00513] Necropsy. At the end of each time point (1,4, 6 & 12h), animals will be sacrificed by using gaseous isoflurane anesthesia. Sublingual mucosa, and intestinal mucosal layer will be collected, weighed and stored at -80°C for further cytokines analysis.
- [00514] Endpoints. Animals will be sacrificed by time points as mentioned above. ELISA will be carried out as per manufacturer's instructions. Detailed procedure will be incorporated
- 25 in report.
- [00515] Biomarker Analysis. A. Sublingual mucosal layer: IL-6, IL-12, CRP & TNF α ; B. Intestinal mucosal layer : IL-6, IL-12, CRP & TNF α .
- [00516] Current scope of study below parameters will be optional and will be carried out based on results of A and B. SIgA level : Sublingual mucosal layer (Optional). SIgA level :
- 30 Intestinal mucosal layer (Optional).
- [00517] Data Compilation. All individual animal data will be presented in Appendices, summarized, and presented in tables. The report will present all findings according to the standard reporting format.

[00518] Statistical Analysis. All analyses and comparisons will be evaluated at the ($p < 0.05$) level. Statistically significant differences ($p \leq 0.05$), indicated by the tests, will be designated throughout the report as stated below: statistical analysis will be carried out with GraphPad Prism software version 9.1. Parameters will be analyzed using One-way ANOVA
5 followed by Dunnett's or Tukey's multiple comparison tests.

[00519] Data, if required, may also be subjected to other statistical tests and will be mentioned in the report.

K.4 RESULTS

[00520] The results assessed will include:

- 10 • Individual animal data for body weights;
- Sublingual mucosal layer: IL-6, IL-12, CRP & TNF α levels;
- Intestinal mucosal layer: IL-6, IL-12, CRP & TNF α levels;
- SIgA levels: Sublingual mucosal layer; and
- SIgA levels: Intestinal mucosal layer.

15 K.5 CONCLUSION

[00521] The inventors expect to detect an increase in one or more specific cytokines and inflammatory markers from baseline in the oral mucosa, and to a lesser extent in the intestinal mucosa:

- 20 a. baseline IL-6, ~20-50 pg/mL. We expect to see a 2-5 fold increase; i.e. result of ~50-100pg/ml post wafer administration. In the alternative, we expect to see any increase over baseline. In another alternative, we expect to see any increase over baseline in at least one animal.
- 25 b. baseline IL-12, ~30-50 pg/mL. We expect to see a 2+ fold increase, i.e. result of 60-100pg/ml post wafer administration. In the alternative, we expect to see any increase over baseline. In another alternative, we expect to see any increase over baseline in at least one animal.
- 30 c. baseline TNF α , ~30-50 pg/mL. We expect to see a 2+ fold increase, i.e. result of 60-100pg/ml post wafer administration. In the alternative, we expect to see any increase over baseline. In another alternative, we expect to see any increase over baseline in at least one animal.

- d. baseline IL-6, ~20-50 pg/mL. We expect to see a result of 50-100pg/ml post wafer administration. In the alternative, we expect to see any increase over baseline. In another alternative, we expect to see any increase over baseline in at least one animal.
- 5 e. baseline CRP, ~5-10 ug/ml. We expect to see a 2-10 fold increase, i.e. a result of 10-50 ug/ml. In the alternative, we expect to see any increase over baseline. In another alternative, we expect to see any increase over baseline in at least one animal.
- f. baseline sIgA levels of ~5–10 µg/mL in saliva or mucosal secretions. Expected Concentration with IFN-α Stimulation: May rise to 15–25 µg/mL (representing a 50–150% increase). In the alternative, we expect to see any increase over baseline. In another alternative, we expect to see any increase over baseline in at least one animal.
- 10
- [00522] The inventors expect to see a significant elevation in one or more cytokines above in mucosal tissue and will determine sIgA levels in both oral and intestinal mucosa. The inventors expect to detect a significant increase in sIgA levels in the oral mucosa, and to
- 15 a lesser extent in the intestinal mucosa.

CLAIMS

1. A composition wherein said composition comprises a biologically active material wherein the composition is adapted to release the biologically active material to a mucosal surface of a patient in need thereof.
5
2. The composition of claim 1, wherein the composition is adapted to induce a biological or immunological response at the epithelial layer of the mucosal system.
3. The composition of claims 1 of 2, wherein the composition is a solid dosage form.
4. The composition of any one of the above claims, wherein the composition comprises one or more agents selected from the group consisting of: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a low MW ionic matrix agent; a second non-ionic matrix agent; and a non-ionic crystalline matrix forming agent.
10
5. The composition of any one of the above claims, wherein the composition comprises the following agents: an ionic water soluble post-disintegration viscosity agent; a low MW ionic matrix agent; a second non-ionic matrix agent; and a non-ionic crystalline matrix forming agent.
15
6. The composition of any one of the above claims, wherein the composition comprises one or more agents selected from the group consisting of: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a second water soluble post-disintegration viscosity agent; a low MW ionic matrix agent; a second non-ionic matrix agent; and a non-ionic crystalline matrix forming agent.
20
7. The composition of any one of the above claims, wherein the composition comprises the following agents: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a second water soluble post-disintegration viscosity agent; a low MW ionic matrix agent; a second non-ionic matrix agent; and a non-ionic crystalline matrix forming agent.
25
8. The composition of any one of the above claims, wherein the composition comprises one or more agents selected from the group consisting of: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a second water soluble post-disintegration viscosity agent; an adjuvant; a low MW ionic matrix agent; a second non-ionic matrix agent; a non-ionic crystalline matrix forming agent; a second non-ionic crystalline matrix forming agent; and a further non-ionic matrix agent.
30

9. The composition of any one of the above claims, wherein the composition comprises the following agents: an amorphous non-ionic matrix forming agent; an ionic water soluble post-disintegration viscosity agent; a second water soluble post-disintegration viscosity agent; an adjuvant; a low MW ionic matrix agent; a second non-ionic matrix agent; a non-ionic crystalline matrix forming agent; a second non-ionic crystalline matrix forming agent; and a further non-ionic matrix agent.
10. The composition of any one of the above claims, wherein the composition is a rapidly disintegrating solid dosage form.
11. The composition of any one of the above claims, wherein the biologically active material is present in the composition at a concentration selected from the group consisting of: between 1 nanogram and 100 milligrams; and between 0.01 nanogram and 1000 milligrams.
12. The composition of any one of the above claims, wherein the biologically active material is present in the composition at a concentration selected from the group consisting of: 1% (w/w); 2% (w/w); 3% (w/w); 4% (w/w); 5% (w/w); 6% (w/w); 7% (w/w); 8% (w/w); 9% (w/w); 10% (w/w); 11% (w/w); 12% (w/w); 13% (w/w); 14% (w/w); 15% (w/w); 16% (w/w); 17% (w/w); 18% (w/w); 19% (w/w); 20% (w/w); 21% (w/w); 22% (w/w); 23% (w/w); 24% (w/w); 25% (w/w); 26% (w/w); 27% (w/w); 28% (w/w); 29% (w/w); 30% (w/w); 31% (w/w); 32% (w/w); 33% (w/w); 34% (w/w); 35% (w/w); 36% (w/w); 37% (w/w); 38% (w/w); 39% (w/w); 40% (w/w); 41% (w/w); 42% (w/w); 43% (w/w); 44% (w/w); 45% (w/w); 46% (w/w); 47% (w/w); 48% (w/w); 49% (w/w); 50% (w/w); and 2-95% (w/w); 3-65% (w/w); 4-55% (w/w); 5-45% (w/w); 6-35% (w/w); 7-25% (w/w); 8-15% (w/w); and within 2 decimal points of each figure.
13. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: haemagglutinins; vaccines; immunoglobulins; allergens; interferons; monoclonal immunoglobulins; and monoclonal antibodies.
14. The composition of any one of the above claims, wherein the biologically active molecules are components in vaccines for respiratory viruses which initially contact a mucosal surface prior to replication and spread of infection, including those selected from the group consisting of: influenza; COVID-19 (coronavirus disease 2019); and RSV (respiratory syncytial virus).
15. The composition of any one of the above claims, wherein the biologically active material is one of the family of biological molecules designed to elicit an immunogenic response at a mucosal surface, selected from the group consisting of: hemagglutinins; monoclonal antibodies; and viral fragments.

16. The composition of any one of the above claims, wherein the biologically active material is preferably selected from the group consisting of: hemagglutinins (HA).
17. The composition of any one of the above claims, wherein the HA is present at a level selected from the group consisting of: 0.1 to 1000µg; 1-100µg; 1-50µg; and 5-20µg.
- 5 18. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: from vitamins; pharmaceutical actives; small chemical entities; biologics; amino acids; proteins; peptides; polypeptides; nucleotides; nucleic acids; oligonucleotides; vaccines; monoclonal antibodies; nucleic acids; and a pharmaceutically acceptable salt; analog; homolog; derivative; solvate; hydrate; and
- 10 polymorph thereof.
19. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: anti-obesity drugs; central nervous system stimulants; carotenoids; corticosteroids; elastase inhibitors; anti-fungals; oncology therapies; anti-emetics; analgesics; cardiovascular agents; anti-inflammatory agents; such as NSAIDs
- 15 and COX-2 inhibitors; anthelmintics; anti-arrhythmic agents; antibiotics (including penicillins); anticoagulants; antidepressants; antidiabetic agents; antiepileptics; antihistamines; antihypertensive agents; antimuscarinic agents; antimycobacterial agents; antineoplastic agents; immunosuppressants; antithyroid agents; antiviral agents; anxiolytics; sedatives (hypnotics and neuroleptics); astringents; alpha-adrenergic receptor blocking agents; beta-
- 20 adrenoceptor blocking agents; blood products and substitutes; cardiac inotropic agents; contrast media; cough suppressants (expectorants and mucolytics); cytokines; diagnostic agents; diagnostic imaging agents; diuretics; dopaminergics (anti-parkinsonian agents); haemostatics; immunological agents; lipid regulating agents; muscle relaxants; parasympathomimetics; parathyroid calcitonin and biphosphonates; prostaglandins; radio-
- 25 pharmaceuticals; sex hormones (including steroids); anti-allergic agents; stimulants and anoretics; sympathomimetics; thyroid agents; vaccines; vasodilators; xanthines; and a pharmaceutically acceptable salt, analog, homolog, derivative, solvate, hydrate, and polymorph thereof.
20. The composition of any one of the above claims, wherein the biologically active material
- 30 is selected from the group consisting of: an anti-viral vaccine; anti-bacterial vaccine; and a cytokine.
21. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: vaccines that generate mucosal immunity against an antigen.

22. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: Vaccines that aim to generate mucosal immunity include: Oral Polio Vaccine (OPV): The oral polio vaccine helps induce a protective mucosal immune response against the poliovirus in the digestive tract; Rotavirus Vaccines aim to protect against severe diarrhea and gastroenteritis caused by rotaviruses, which primarily affect the gastrointestinal mucosa; Cholera Vaccine: Cholera vaccines target the mucosal immune response in the gut to protect against cholera, a bacterial infection that affects the intestinal mucosa; Influenza Vaccine: These vaccines aim to generate mucosal immunity against influenza viruses in the respiratory tract; Intranasal COVID-19 Vaccines; Mucosal HSV-2 Vaccine; and Norovirus Vaccine.
23. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: FDA-approved influenza vaccines in the United States include Afluria, Fluarix, Flublok, Flucelvax, Flulaval, Fluzone (available in various formulations including standard-dose, high-dose, and intradermal), FluMist Quadrivalent, Fluzone High-Dose (for individuals aged 65 and older), Fluad (for individuals aged 65 and older), Fluad Quadrivalent, and Flucelvax Quadrivalent.
24. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: Approved vaccines, by brand name, that are associated with the mucosal immune response include: Vaccines: FluMist Quadrivalent: A live attenuated influenza vaccine (LAIV) administered intranasally to stimulate mucosal immunity against influenza; Rotarix: An oral vaccine used to protect against rotavirus, which primarily infects the gastrointestinal mucosa; RotaTaq: Another oral vaccine designed to prevent rotavirus infection; Oral Polio Vaccine (OPV): Used to induce a mucosal immune response against poliovirus in the digestive tract; Cholera Vaccine (Vaxchora): Administered orally to generate mucosal immunity against *Vibrio cholerae*, the bacterium causing cholera; Shigella Vaccine: Several experimental Shigella vaccines are in development to combat Shigella infections, which affect the gastrointestinal mucosa; Adenovirus Type 4 and Type 7 Vaccine (Barr Labs): Used to protect military personnel from acute respiratory disease caused by adenovirus. The adenovirus primarily affects respiratory mucosa; Adenovirus Vaccine (Ad26.COV2.S): One of the COVID-19 vaccines, Janssen's (Johnson & Johnson) Ad26.COV2.S, primarily targets the respiratory mucosa to induce immunity against the SARS-CoV-2 virus.
25. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: biologics.

26. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: Biologics that can influence the mucosal immune response include: Anti-TNF Biologics: Tumor necrosis factor (TNF) is a proinflammatory cytokine that plays a role in various autoimmune diseases. Biologics like Adalimumab (Humira), Infliximab (Remicade), and Etanercept (Enbrel) target TNF and are used to treat conditions such as inflammatory bowel disease (IBD) and rheumatoid arthritis; Anti-IL Biologics: Interleukins (IL) are signalling molecules that regulate immune responses. Biologics like Ustekinumab (Stelara) and Vedolizumab (Entyvio) target specific interleukins to treat conditions like Crohn's disease and psoriasis; Anti-IL-17 Biologics: Some biologics, like Secukinumab (Cosentyx), target interleukin-17 (IL-17) to treat conditions like psoriasis and ankylosing spondylitis, which can involve mucosal inflammation; Anti-IL-23 Biologics: Biologics like Guselkumab (Tremfya) target interleukin-23 (IL-23) and are used to treat conditions like plaque psoriasis and psoriatic arthritis; Anti-CD Biologics: Monoclonal antibodies targeting cluster of differentiation (CD) markers on immune cells, such as Vedolizumab (Entyvio), can modulate the mucosal immune response in the gastrointestinal tract and treat inflammatory bowel diseases like Crohn's disease and ulcerative colitis; Biologics Targeting B Cells: Rituximab (Rituxan) is a biologic that depletes B cells, which are involved in autoimmune responses. It is used to treat conditions like rheumatoid arthritis and certain types of vasculitis; Anti-Integrin Biologics: Natalizumab (Tysabri) is an integrin receptor antagonist that modulates immune cell migration and is used in the treatment of multiple sclerosis; Biologics for Allergic Asthma: Omalizumab (Xolair) is a biologic that targets IgE antibodies and is used in the treatment of allergic asthma.

27. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: Approved antibodies, by brand name, that are associated with the mucosal immune response include: Antibodies and Immunotherapies: Palivizumab (Synagis): An antibody used to provide passive immunity against respiratory syncytial virus (RSV) in high-risk infants. RSV primarily affects the respiratory mucosa; Casirivimab and Imdevimab (REGN-COV2): A combination of monoclonal antibodies authorized for the treatment of COVID-19. They target the spike protein of the SARS-CoV-2 virus, which infects the respiratory mucosa; Tocilizumab (Actemra): Tocilizumab is an immunosuppressive drug used to treat conditions like cytokine release syndrome (CRS), which can be associated with severe respiratory symptoms in some cases.

28. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: H1N1 influenza vaccines include: Flublok Quadrivalent: Developed by Sanofi Pasteur, this vaccine was designed to protect against multiple influenza strains, including H1N1; Flud Quadrivalent: An adjuvanted influenza

- vaccine from Seqirus that includes protection against H1N1; Vaxigrip Tetra: This is a quadrivalent inactivated influenza vaccine developed by Sanofi Pasteur. It targets several influenza strains, including H1N1; QIVc: An H1N1-specific quadrivalent inactivated vaccine candidate; Influvac Tetra: This is a quadrivalent inactivated influenza vaccine from Mylan
- 5 that includes protection against H1N1; NanoFlu: Developed by Novavax, NanoFlu is designed to protect against several influenza strains, including H1N1. The composition of any one of the above claims, wherein the biologically active material is selected from the group consisting of: H1N1 influenza vaccine; interferon alpha-2a; interferon alpha-2b; infliximab and rituximab.
- 10 29. The composition of any one of the above claims, wherein the biologically active material is one of the family of interferons or a pharmaceutically acceptable derivative, such as a pegylated derivative or a non-pegylated.
30. The composition of any one of the above claims, wherein the biologically active material is preferably selected from the group consisting of: interferon.
- 15 31. The composition of any one of the above claims, wherein the biologically active material is preferably selected from the group consisting of: interferon type I; interferon type II; interferon type III.
32. The composition of any one of the above claims, wherein the biologically active material is preferably selected from the group consisting of: interferon alpha; and interferon beta.
- 20 33. The composition of any one of the above claims, wherein the biologically active material is preferably selected from the group consisting of: interferon alpha-2a; interferon alpha-2b or a pharmaceutically acceptable derivative, such as a pegylated derivative or a non-pegylated.
34. The composition of any one of the above claims, wherein the biologically active material
- 25 is preferably selected from the group consisting of: interferon alpha (α), beta (β), and gamma (γ) and the two types: type I includes the alpha and beta forms and type II consists of gamma form.
35. The composition of any one of the above claims, wherein the interferon may be present in each dosage preferably selected from the group consisting of: 0.01 ng to 1000 μ g; 0.1ng to
- 30 100 μ g; 1ng to 10 μ g; 1 ng to 1 μ g; 1 ng to 100ng; 1 nanogram to 20ng.
36. The composition of any one of the above claims, wherein the concentration of the interferon may be stated in International Units (IU), where 1ng interferon comprises 100IU.

37. The composition of any one of the above claims, wherein the interferons may be present from 1IU to 10,000,000IU, preferably 100IU to 1,000,000IU, more preferably 500IU to 10,000IU.
38. The composition of any one of the above claims, wherein the interferons may be present
5 from 1IU to 100,000,000IU, preferably 10IU to 1,000,000IU, more preferably 100IU to 10,000IU, most preferably 100IU to 2000IU.
39. The composition of any one of the above claims, wherein the biologically active molecules are components in vaccines for respiratory viruses which initially contact a mucosal surface prior to replication and spread of infection, including those selected from
10 the group consisting of: influenza; COVID-19 (coronavirus disease 2019); and RSV (respiratory syncytial virus).
40. The composition of any one of the above claims, wherein the biologically active material is one of the family of biological molecules designed to elicit an immunogenic response at a mucosal surface, selected from the group consisting of: hemagglutinins; monoclonal
15 antibodies; and viral fragments.
41. The composition of any one of the above claims, wherein the biologically active material is preferably selected from the group consisting of: hemagglutinins (HA).
42. The composition of any one of the above claims, wherein the HA is present at a level selected from the group consisting of: 0.1 to 1000µg; 1-100µg; 1-50µg; and 5-20µg.
- 20 43. The composition of any one of the above claims, wherein the biologically active material has a molecular weight selected from the group of ranges consisting of: greater than 1000Da; from 1MDa to 100000MDa; from 1kDa to 100000kDa; from 1kDa to 10000kDa; from 1kDa to 1000kDa; from 1kDa to 100kDa; from 1kDa to 50kDa; from 1kDa to 40kDa; from 1kDa to 30kDa; from 1kDa to 20kDa; from 1kDa to 10kDa; from 1kDa to 500kDa; from
25 2kDa to 400kDa; from 3kDa to 300kDa; from 4kDa to 200kDa; from 5kDa to 100kDa;
44. The composition of any one of the above claims, wherein the monoclonal antibodies (MAB) are biologically active against cancer cells.
45. The composition of any one of the above claims, wherein the MAB initiates action against cancer cells through contact with the mucosal surface.
- 30 46. The composition of any one of the above claims, wherein the composition comprises an amorphous non-ionic matrix forming agent.

47. The composition of any one of the above claims, wherein the amorphous non-ionic matrix forming agent is selected from the group consisting of: amylopectin; an agent selected from the amylopectin family; and amylogum CLS.
- 5 48. The composition of any one of the above claims, wherein the amorphous non-ionic matrix forming agent is selected from the group consisting of: amylopectin.
49. The composition of any one of the above claims, wherein the amorphous non-ionic matrix forming agent is present at a concentration selected from the group consisting of: 5-20%; 8-10%; or 13-14%; 5%; 6%; 7%; 8%; 9%; 10%; 11%; 12%; 13%; 14%; 15%; 16%; 17%; 18%; 19%; 20% and within 2 decimal points of each figure (w/w%).
- 10 50. The composition of any one of the above claims, wherein the amylopectin is not in the form of starch or modified starch.
51. The composition of any one of the above claims, wherein the amylopectin, which has a low viscosity even at elevated concentrations, essential for providing a scaffold for the monosaccharide particles and allowing non-friability and handleability, was found to be most
15 useful at 10-20% w/w.
52. The composition of any one of the above claims, wherein the composition comprises an ionic water soluble post-disintegration viscosity agent.
53. The composition of any one of the above claims, wherein the ionic water soluble post-disintegration viscosity agent is selected from the group consisting of: anionic substituted
20 cellulose or starch derivatives, such as salts of carboxymethyl celluloses (CMC) or starch glycolates.
54. The composition of any one of the above claims, wherein the ionic water soluble post-disintegration viscosity agent is present at a concentration selected from the group consisting of: 0.5%-10%; 0.5%; 1%-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and
25 within 2 decimal points of each figure (w/w).
55. The composition of any one of the above claims, wherein the ionic water soluble post-disintegration viscosity agent is present at a concentration 1.5-2.5% (w/w).
56. The composition of any one of the above claims, wherein the ionic water soluble post-disintegration viscosity agent is present at a concentration 1.0-5.0% (w/w).
- 30 57. The composition of any one of the above claims, wherein the optimum levels of alginic acid and CMC were discovered by experiment to be about 2.0% w/w solids content in the final wafer, in the ratio 1:1, with a useful range of 1.5-2.5% w/w.

58. The composition of any one of the above claims, wherein the absolute level of the two polymers used for this invention, alginic acid salt and carboxymethyl cellulose salt (CMC), is important to the performance of the wafer.
59. The composition of any one of the above claims, wherein levels too low do not provide
5 enough viscosity for the disintegrated film to survive too long.
60. The composition of any one of the above claims, wherein levels too high make the wafer too viscous to manufacture end dispense, and form a poorly-disintegrating wafer.
61. The composition of any one of the above claims, wherein the composition comprises a second ionic water soluble post-disintegration viscosity agent.
- 10 62. The composition of any one of the above claims, wherein the second ionic water soluble post-disintegration viscosity agent is selected from the group consisting of: anionic substituted natural polysaccharides, such as salts of alginates or pectinate, including sodium alginate.
63. The composition of any one of the above claims, wherein the second ionic water soluble
15 post-disintegration viscosity agent is present at a concentration selected from the group consisting of: 0.5%; 1%-10%; 1%-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w%).
64. The composition of any one of the above claims, wherein the second ionic water soluble post-disintegration viscosity agent is present at a concentration 1.5-2.5% (w/w).
- 20 65. The composition of any one of the above claims, wherein the second ionic water soluble post-disintegration water soluble viscosity agent is present at a concentration 1.0-5.0% (w/w).
66. The composition of any one of the above claims, wherein the ionic water soluble post-disintegration viscosity agent and the second ionic water soluble post-disintegration viscosity
25 agent are preferably present in the composition in the ratio 0.5-1.5, with a useful range of 1.5-2.5% w/w for each agent.
67. The composition of any one of the above claims, wherein the total polymer concentration is at or within the range of 4-6% (w/w), with each viscosity agent is at or within the range of 2-3%.
- 30 68. The composition of any one of the above claims, wherein the composition comprises an adjuvant.

69. The composition of any one of the above claims, wherein the composition comprises a plurality of adjuvants.
70. The composition of any one of the above claims, wherein the adjuvant is selected from the group consisting of: squalene, aluminium salts, such as aluminium hydroxide, aluminium phosphate, and aluminium potassium sulfate and alpha-tocopherol.
71. The composition of any one of the above claims, wherein the adjuvant is selected from the group consisting of squalene.
72. The composition of any one of the above claims, wherein the adjuvant is present at a concentration selected from the group consisting of: 0.1%-10%; 0.5%, 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w). The composition of any one of the above claims, wherein the adjuvant is present at a concentration 0.5-5% (w/w).
73. The composition of any one of the above claims, wherein the adjuvant selected from the group consisting of: aluminium salts, such as aluminium hydroxide, aluminium phosphate, and aluminium potassium sulfate.
74. The composition of any one of the above claims, wherein the aluminium salt is present at a concentration selected from the group consisting of: 0.1%-10%; 0.5%, 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w).
75. The composition of any one of the above claims, wherein the aluminium salt is present at a concentration 0.1-5% (w/w).
76. The composition of any one of the above claims, wherein the adjuvant selected from the group consisting of alpha-tocopherol.
77. The composition of any one of the above claims, wherein the alpha-tocopherol is present at a concentration selected from the group consisting of: 0.01%-5%; and within 2 decimal points of each figure (w/w).
78. The composition of any one of the above claims, wherein the alpha tocopherol is present at a concentration 0.01-0.5% (w/w).
79. The composition of any one of the above claims, wherein the composition comprises a low MW ionic matrix agent.
80. The composition of any one of the above claims, wherein the low MW ionic matrix agent is selected from the group consisting of: amino acids.

81. The composition of any one of the above claims, wherein the low MW ionic matrix agent is glycine.
82. The composition of any one of the above claims, wherein the low MW ionic matrix agent is present at a concentration selected from the group consisting of: 1%-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w).
83. The composition of any one of the above claims, wherein the composition comprises a second non-ionic matrix agent.
84. The composition of any one of the above claims, wherein the second non-ionic matrix agent is selected from the group consisting of: microcrystalline cellulose (MCC).
85. The composition of any one of the above claims, wherein the second non-ionic matrix agent is present at a concentration selected from the group consisting of: 1-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w%).
86. The composition of any one of the above claims, wherein the composition comprises a non-ionic crystalline matrix forming agent.
87. The composition of any one of the above claims, wherein the non-ionic crystalline matrix forming agent is selected from the group consisting of: mannitol
88. The composition of any one of the above claims, wherein the non-ionic crystalline matrix forming agent is present at a concentration selected from the group consisting of: 20-50%; 5-50%; 5%; 6%; 7%; 8%; 9%; 10%; 11%; 12%; 13%; 14%; 15%; 16%; 17%; 18%; 19%; 20%; 21%; 22%; 23%; 24%; 25%; 26%; 27%; 28%; 29%; 30%; 31%; 32%; 33%; 34%; 35%; 36%; 37%; 38%; 39%; 40%; 41%; 42%; 43%; 44%; 45%; 46%; 47%; 48%; 49%; 50%; and within 2 decimal points of each figure (w/w).
89. The composition of any one of the above claims, wherein monosaccharides, preferably lactose and mannitol, were useful for the preparation of an ionically neutral, highly water-soluble matrix interpenetrated with a network of pores for rapid disintegration and dissolution.
90. The composition of any one of the above claims, wherein, the combined concentration was found to be preferably most useful at 65-80% w/w.
91. The composition of any one of the above claims, wherein the composition comprises a second non-ionic crystalline matrix forming agent.

92. The composition of any one of the above claims, wherein the second non-ionic crystalline matrix forming agent is selected from the group consisting of: lactose; and lactose monohydrate.
93. The composition of any one of the above claims, wherein the second non-ionic matrix agent is present at a concentration selected from the group consisting of: 5-50%; 5%; 6%; 7%; 8%; 9%; 10%; 11%; 12%; 13%; 14%; 15%; 16%; 17%; 18%; 19%; 20%; 21%; 22%; 23%; 24%; 25%; 26%; 27%; 28%; 29%; 30%; 31%; 32%; 33%; 34%; 35%; 36%; 37%; 38%; 39%; 40%; 41%; 42%; 43%; 44%; 45%; 46%; 47%; 48%; 49%; 50%; and within 2 decimal points of each figure (w/w%).
94. The composition of any one of the above claims, wherein the composition comprises a further non-ionic matrix agent.
95. The composition of any one of the above claims, wherein the further non-ionic matrix agent is selected from the group consisting of: PEG1000-20,000, preferably PEG 1500.
96. The composition of any one of the above claims, wherein the further non-ionic matrix agent is present at a concentration selected from the group consisting of: 1%-10%; 1%; 2%; 3%; 4%; 5%; 6%; 7%; 8%; 9%; 10%; and within 2 decimal points of each figure (w/w%).
97. The composition of any one of the above claims, wherein the composition comprises has a pH selected from the group consisting of between 5 to 6; between 4 to 7.5; and between 7.0 – 7.5.
98. The composition of any one of the above claims, wherein the composition comprises a pharmaceutically acceptable buffering reagent.
99. The composition of any one of the above claims, wherein the buffering reagent is selected from the group consisting of: salts of phosphates, carbonates, Tris; preferably combinations of phosphate salts; including sodium carbonate.
100. The composition of any one of the above claims, wherein the buffering reagent is present at a concentration selected from the group consisting of: 0.1-0.5%; 0.1%; 0.2%; 0.3%; 0.4%; 0.5%; and within 2 decimal points of each figure (w/w%).
101. The composition of any one of the above claims, wherein the wafers are buffered with a suitable buffer, most preferably a blend of mono- and di-alkaline salt phosphates, adjusted to afford a pH optimal for the stability of the chosen biological active, generally between 4 and 7.5.

102. The composition of any one of the above claims, wherein the composition comprises a colouring agent.
103. The composition of any one of the above claims, wherein the composition comprises a flavouring agent.
- 5 104. The composition of any one of the above claims, wherein the composition comprises a stability agent.
105. The composition of any one of the above claims, wherein the stability agent is selected from the group consisting of: metal chelators such as EDTA, pharmaceutically acceptable surfactant including polysorbate 80.
- 10 106. The composition of any one of the above claims, wherein the composition comprises an anti-protease agent, such as such as antipeptidases or antitrypsin.
107. The composition of any one of the above claims, wherein the composition disintegrates once placed in the oral cavity in a time period selected from the group consisting of: <10s; <20s; <30s; <40s; <50s; <60s; <70s; <80s; <90s; <100s; <110s; <120s
15 (seconds).
108. The composition of any one of the above claims, wherein the composition disintegrates once placed in the oral cavity in a time period selected from the group consisting of: <10s; <20s; <30s; <40s; <50s; <60s; <70s; <80s; <90s; <100s; <110s; <120s;
20 <130s; <140s; <150s; <160s; <170s; <180s; <190s; <200s; <210s; <220s; <230s; <240s; <250s; <260s; <270s; <280s; <290s; <300s; <310s; <320s; <330s; <340s; <350s; <360s; <370s; <380s; <390s; <400s; <410s; <420s; <430s; <440s; <450s; <460s; <470s; <480s; <490s; <500s; <510s; <520s; <530s; <540s; <550s; <560s; <570s; <580s; <590s; <600s; <610s; <620s; <630s; <640s; <650s; <660s; <670s; <680s; <690s; <700s; <710s; <720s; <730s; <740s; <750s; <760s; <770s; <780s; <790s; <800s; <810s; <820s; <830s; <840s;
25 <850s; <860s; <870s; <880s; <890s; <900s; <900s; <930s; <960s; <990s; <1020s; <1050s; <1080s; <1110s; <1140s; <1170s; <1200s; <1230s; <1260s; <1290s; <1320s; <1350s; <1380s; <1410s; <1440s; <1470s; <1500s; <1530s; <1560s; <1590s; <1620s; <1650s; <1680s; <1710s; <1740s; <1770s; <1800s; <1830s; <1860s; <1890s; <1920s; <1950s; <1980s; <2010s; <2040s; <2070s; <2100s; <2130s; <2160s; <2190s; <2220s; <2250s;
30 <2280s; <2310s; <2340s; <2370s; <2400s; <2430s; <2460s; <2490s; <2520s; <2550s; <2580s; <2610s; <2640s; <2670s; <2700s; <2730s; <2760s; <2790s; <2820s; <2850s; <2880s; <2910s; <2940s; <2970s; <3000s; <3030s; <3060s; <3090s; <3120s; <3150s; <3180s; <3210s; <3240s; <3270s; <3300s; <3330s; <3360s; <3390s; <3420s; <3450s; <3480s; <3510s; <3540s; <3570s; <3600s; <3630s; <3660s; <3690s; <3720s; <3750s;

<3780s; <3810s; <3840s; <3870s; <3900s; <3930s; <3960s; <3990s; <4020s; <4050s; <4080s; <4110s; <4140s; <4170s; <4200s; and <4230s (seconds).

109. The composition of any one of the above claims, wherein the composition disintegrates in the oral cavity without leaving a residue of said dosage form in the oral cavity
5 that is detectable by a subject, thereby avoiding the urge for the subject to swallow the composition.

110. The composition of any one of the above claims, wherein the composition dissolves once placed in the oral cavity in a time period selected from the group consisting of: <10s; <20s; <30s; <40s; <50s; <60s; <70s; <80s; <90s; <100s; <110s; <120s; 180s, preferably 15-
10 60s (seconds).

111. The composition of any one of the above claims, wherein the composition dissolves once placed in the oral cavity in a time period selected from the group consisting of: <10s; <20s; <30s; <40s; <50s; <60s; <70s; <80s; <90s; <100s; <110s; <120s; <130s; <140s; <150s; <160s; <170s; <180s; <190s; <200s; <210s; <220s; <230s; <240s; <250s; <260s;
15 <270s; <280s; <290s; <300s; <310s; <320s; <330s; <340s; <350s; <360s; <370s; <380s; <390s; <400s; <410s; <420s; <430s; <440s; <450s; <460s; <470s; <480s; <490s; <500s; <510s; <520s; <530s; <540s; <550s; <560s; <570s; <580s; <590s; <600s; <610s; <620s; <630s; <640s; <650s; <660s; <670s; <680s; <690s; <700s; <710s; <720s; <730s; <740s; <750s; <760s; <770s; <780s; <790s; <800s; <810s; <820s; <830s; <840s; <850s; <860s;
20 <870s; <880s; <890s; <900s; <900s; <930s; <960s; <990s; <1020s; <1050s; <1080s; <1110s; <1140s; <1170s; <1200s; <1230s; <1260s; <1290s; <1320s; <1350s; <1380s; <1410s; <1440s; <1470s; <1500s; <1530s; <1560s; <1590s; <1620s; <1650s; <1680s; <1710s; <1740s; <1770s; <1800s; <1830s; <1860s; <1890s; <1920s; <1950s; <1980s; <2010s; <2040s; <2070s; <2100s; <2130s; <2160s; <2190s; <2220s; <2250s; <2280s;
25 <2310s; <2340s; <2370s; <2400s; <2430s; <2460s; <2490s; <2520s; <2550s; <2580s; <2610s; <2640s; <2670s; <2700s; <2730s; <2760s; <2790s; <2820s; <2850s; <2880s; <2910s; <2940s; <2970s; <3000s; <3030s; <3060s; <3090s; <3120s; <3150s; <3180s; <3210s; <3240s; <3270s; <3300s; <3330s; <3360s; <3390s; <3420s; <3450s; <3480s; <3510s; <3540s; <3570s; <3600s; <3630s; <3660s; <3690s; <3720s; <3750s; <3780s;
30 <3810s; <3840s; <3870s; <3900s; <3930s; <3960s; <3990s; <4020s; <4050s; <4080s; <4110s; <4140s; <4170s; <4200s; and <4230s (seconds).

112. The composition of any one of the above claims, wherein the composition dissolves in the oral cavity without leaving a residue of said dosage form in the oral cavity that is detectable by a subject, thereby avoiding the urge for the subject to swallow the
35 composition.

113. The composition of any one of the above claims, wherein the composition has a viscosity profile of: CMC and sodium alginate.
114. The composition of any one of the above claims, wherein the disintegrated bolus forms a "dome" with structure given by a judicious level of two main water-soluble polymeric chains.
115. The composition of any one of the above claims, wherein the polymers give the "dome" viscosity, keeping it in position and delaying drainage and swallowing, and also delay the ingress of saliva, the long chain mucopolysaccharides of which have difficulty in penetrating the "dome".
116. The composition of any one of the above claims, wherein the viscosity is not so great that bioactive are prevented from diffusing to the epithelial layer continuously (unlike solid film wafers which "melt" and trap the active in a highly viscous matrix with very little water present).
117. The composition of any one of the above claims, wherein the difference is engineered by the highly porous nature of the wafer, which rapidly absorbs extant rinse-water by capillary action to cause rapid disintegration and dissolution (wafer films have no pores, and so trap the water on the outside of the matrix).
118. The composition of any one of the above claims, wherein by these features, the bioactive molecules are given a useful time of contact before being overwhelmed with excess saliva and inadvertent mouth movement (2-10 minutes).
119. The composition of any one of the above claims, wherein the composition has a solubility profile selected from the group consisting of: wherein the composition is greater than 96% water soluble.
120. The composition of any one of the above claims, wherein the composition has a solubility profile generated by an agent selected from the group consisting of: amylopectin; mannitol; lactose; viscosity agents; and wherein the composition is greater than 96% water soluble.
121. The composition of any one of the above claims, wherein the composition has a non-ionisable profile selected from the group consisting of: >96% non-ionisable; >90% non-ionisable; and 65-75% non-ionisable.
122. The composition of any one of the above claims, wherein the composition has a non-ionisable profile generated by an agent selected from the group consisting of: amylopectin,

mannitol, lactose; and wherein the composition is >96% non-ionisable; >90% non-ionisable; 65-75% non-ionisable.

123. The composition of any one of the above claims, wherein water is removed from the combined agents by a freeze-drying process to a residual level of 0-10%, preferably 0-5%,
5 most preferably 3-5%.

124. The composition of any one of the above claims, wherein the composition has a porosity profile selected from the group consisting of: the proportion of water removed by lyophilization is 65-75%; and the proportion of water removed by lyophilization is >60%.

125. The composition of any one of the above claims, wherein the composition has a
10 porosity profile generated by an agent selected from the group consisting of: amylopectin; mannitol; lactose; and wherein the proportion of water removed by lyophilization is 65-75%; and the proportion of water removed by lyophilization is >60%.

126. The composition of any one of the above claims, wherein the composition comprises components as listed in any one of Compositions A to O.

15 127. The composition of any one of the above claims, wherein the composition comprises components as listed in any one of Compositions A to H.

128. The composition of any one of the above claims, wherein the composition is selected from the group consisting of: Composition A; Composition B; Composition C; Composition D; Composition E; Composition F; Composition G; Composition H – Option A; Composition H –
20 Option B; and Composition H – Option C; Composition I; Composition J; Composition K; Composition L; Composition M; Composition N; Composition O.

129. The composition of any one of the above claims, wherein the composition is selected from the group consisting of: Composition H – Option A; Composition H – Option B; and Composition H – Option C.

25 130. The composition of any one of the above claims, wherein the composition is selected from the group consisting of: Composition I; Composition J; and Composition L.

131. The composition of any one of the above claims, wherein the composition is selected from the group consisting of: Composition I; Composition J; Composition K; Composition L.

132. The composition of any one of the above claims, wherein the mucosal surface is
30 selected from the group consisting of: oral; buccal; rectal; vaginal; oculi; otic; nasal.

133. The composition of any one of the above claims, wherein the mucosal surface is selected from the group consisting of: oral; buccal; nasal.

134. The composition of any one of the above claims, wherein the composition is stable for a time period selected from the group consisting of: 1 week; 2 weeks; 3 weeks; 4 weeks; 5 weeks; 6 weeks; 7 weeks; 8 weeks; 9 weeks; 10 weeks; 11 weeks; 12 weeks; 13 weeks; 14 weeks; 15 weeks; 16 weeks; 17 weeks; 18 weeks; 19 weeks; 20 weeks; 21 weeks; 22 weeks; 23 weeks; 24 weeks; 25 weeks; 26 weeks; 27 weeks; 28 weeks; 29 weeks; 30 weeks; 31 weeks; 32 weeks; 33 weeks; 34 weeks; 35 weeks; 36 weeks; 37 weeks; 38 weeks; 39 weeks; 40 weeks; 41 weeks; 42 weeks; 43 weeks; 44 weeks; 45 weeks; 46 weeks; 47 weeks; 48 weeks; 49 weeks; 50 weeks; 51 weeks; 52 weeks; 1 month; 2 months; 3 months; 4 months; 5 months; 6 months; 7 months; 8 months; 9 months; 10 months; 11 months; 12 months; 13 months; 14 months; 15 months; 16 months; 17 months; 18 months; 19 months; 20 months; 21 months; 22 months; 23 months; 24 months; 25 months; 26 months; 27 months; 28 months; 29 months; 30 months; 31 months; 32 months; 33 months; 34 months; 35 months; 36 months; 37 months; 38 months; 39 months; 40 months; 41 months; 42 months; 43 months; 44 months; 45 months; 46 months; 47 months; 48 months; 49 months; 50 months; 51 months; 52 months; 53 months; 54 months; 55 months; 56 months; 57 months; 58 months; 59 months; 60 months; 1 month to 12 months; 12 months to 24 months; 12 months to 36 months; 12 months to 48 months; 1 year to 2 years; 1 year to 3 years; and 1 year to 5 years. The composition of any one of the above claims, wherein the composition is stable as demonstrated by a potency assay run against a reference biological active material.

135. A pharmaceutical composition comprising the composition of any one of claims 1 to 134 together with a pharmaceutical excipient or carrier.

136. A dosage form comprising the composition of any one of claims 1 to 134.

137. The dosage form of claim 136, wherein the dosage form comprises the biologically active material at a dose selected from the group consisting of: between 0.001 to 100mg; between 2 and 50mg; between 5 and 40mg; between 10 and 30mg; and between 20 and 25mg.

138. The dosage form of any one of claims 136 to 137, wherein the dosage form comprises the biologically active material at a dose selected from the group consisting of: between 1 nanogram to 100mg; 1 nanogram to 10mg; 1 nanogram to 1mg; 1 nanogram to 100µg; 1 nanogram to 1µg.

139. The dosage form of any one of claims 136 to 138, wherein the dosage form comprises the biologically active material at a dose selected from the group consisting of:

between 0.1 ng to 1000 µg; 1 nanogram to 10µg; 1 nanogram to 1µg; 1 nanogram to 100ng; 1 nanogram to 10ng.

140. The dosage form of any one of claims 136 to 139, wherein the dosage form comprises the composition of HA.

5 141. The dosage form of any one of claims 136 to 140, wherein the dosage form for influenza vaccines comprises the biologically active material at a dose selected from the group consisting of: from 0.1 to 1000µg; 1-100µg; 1-50µg, preferably 5-20µg.

142. The dosage form of any one of claims 136 to 140, wherein the dosage form for COVID-19 vaccines comprises the biologically active material at a dose selected from the
10 group consisting of: from 0.1µg to 100mg; 10µg-10mg; 100µg to 5mg, preferably 200µg to 2mg.

143. The dosage form of any one of claims 136 to 142, wherein the dosage form comprises the biologically active material at a dose selected from the group consisting of: from 0.1 to 1000µg; 1-100µg; 1-50µg; and 5-20µg.

15 144. The dosage form of any one of claims 136 to 143, wherein the biologically active material is selected from the group consisting of: interferon alpha-1a (or alpha 1 beta); interferon alpha-2b (or alpha 2 beta); interferon beta-1a; or any other interferon or combination, including pharmaceutically acceptable pegylates and non-pegylates.

145. A vaccine comprising the composition of any one of claims 1 to 134.

20 146. The vaccine of claim 145, wherein the vaccine develops a biological response to treat or prevent disease or disorder selected from the group consisting of: H1N1; RSV; COVID-19; and any mucosal viral or bacterial infection targeting the respiratory tract.

147. A method of treating or preventing or delaying a disease or disorder, said method comprising administering to a patient in need thereof a therapeutically effective amount of
25 the dosage form of claims 136 to 144.

148. The method of claim 147, wherein the disease or disorder is selected from the group consisting of: influenza, RSV, whooping cough (pertussis) or COVID-19.

149. The method of any one of claims 147 to 148, wherein the disease or disorder is selected from the group consisting of: Infectious Diseases: (such as Bacterial infections, viral
30 infections, fungal infections, parasitic infections, sexually transmitted infections (STIs), tropical diseases, zoonotic diseases); Cardiovascular Diseases: (such as Coronary artery disease, hypertension (high blood pressure), heart failure, arrhythmias, stroke, vascular

diseases); Respiratory Diseases: (such as Asthma, chronic obstructive pulmonary disease (COPD), pneumonia, tuberculosis, lung cancer); Neurological Diseases: (such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy, stroke, autoimmune diseases); Gastrointestinal Diseases: (such as Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophageal reflux disease (GERD), cirrhosis, colorectal cancer); Endocrine and Metabolic Diseases: (such as Diabetes mellitus, thyroid disorders, obesity, Cushing's syndrome, Addison's disease, hormonal disorders); Autoimmune Diseases: (such as Rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type 1 diabetes, psoriasis, autoimmune skin diseases, autoimmune thyroid diseases, autoimmune gastrointestinal diseases); Cancer: (such as Breast cancer, lung cancer, prostate cancer, leukemia, skin cancer); Genetic Disorders: (such as Down syndrome, cystic fibrosis, Huntington's disease, hemophilia, sickle cell anemia, rare genetic syndromes); Musculoskeletal Diseases: (such as Osteoarthritis, rheumatoid arthritis, osteoporosis, muscular dystrophy, fibromyalgia, rheumatic diseases); Renal (Kidney) Diseases: (such as Chronic kidney disease, acute kidney injury, polycystic kidney disease, nephrotic syndrome, kidney stones); Skin Diseases: (such as Eczema, psoriasis, acne, skin cancer, vitiligo); Hematological Diseases: (such as Anemia, thrombocytopenia, hemophilia, leukemia, lymphoma); Psychiatric Disorders: (such as Depression, anxiety disorders, schizophrenia, bipolar disorder, obsessive-compulsive disorder (OCD)); Immunodeficiency Disorders: (such as HIV/AIDS, primary immunodeficiency diseases, severe combined immunodeficiency (SCID)); Eye and Ear Disorders: (such as Glaucoma, cataracts, macular degeneration, deafness, and hearing disorders); Reproductive and Gynecological Diseases: (such as Polycystic ovary syndrome (PCOS), endometriosis, infertility, ovarian cancer, testicular cancer, reproductive system diseases); Oral and Dental Diseases: (such as Tooth decay (cavities), gum disease, oral cancer, temporomandibular joint disorder (TMJ)); Allergic Diseases: (such as Allergic rhinitis (hay fever), food allergies, eczema, asthma); Rare and Orphan Diseases: (such as Huntington's disease, amyotrophic lateral sclerosis (ALS), Pompe disease, Niemann-Pick disease, Prader-Willi syndrome); Nutritional Diseases: (such as Malnutrition, obesity, anorexia nervosa, bulimia nervosa, vitamin deficiencies); Rheumatic Diseases: (such as Spondyloarthropathies, juvenile idiopathic arthritis, polymyalgia rheumatica, giant cell arteritis); Vascular Diseases: (such as Atherosclerosis, varicose veins, deep vein thrombosis (DVT), peripheral artery disease (PAD)); Liver Diseases: (such as Hepatitis (A, B, C, etc.), non-alcoholic fatty liver disease (NAFLD), primary biliary cholangitis, Wilson's disease); Urinary Tract Diseases: (such as Urinary tract infections (UTIs), bladder disorders, interstitial cystitis, urolithiasis (kidney stones)); Sleep Disorders: (such as Insomnia, sleep apnea, narcolepsy, restless legs syndrome, circadian rhythm disorders).

150. The method of any one of claims 147 to 149, wherein the disease or disorder is selected from the group consisting of: infectious diseases: (bacterial infections, viral infections, fungal infections, parasitic infections); cardiovascular diseases: (coronary artery disease, hypertension (high blood pressure), heart failure, arrhythmias); neurological disorders: (alzheimer's disease, parkinson's disease, epilepsy, multiple sclerosis);
 5 respiratory diseases: (asthma, chronic obstructive pulmonary disease (COPD), pneumonia, tuberculosis); gastrointestinal disorders: (irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophageal reflux disease (GERD), liver cirrhosis); endocrine disorders: (diabetes mellitus, thyroid disorders, cushing's syndrome, addison's disease);
 10 autoimmune diseases: (rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, crohn's disease); musculoskeletal disorders: (osteoarthritis, rheumatoid arthritis, osteoporosis, muscular dystrophy); cancer: (breast cancer, lung cancer, colon cancer, leukemia); mental health disorders: (depression, anxiety disorders, bipolar disorder, schizophrenia); genetic disorders: (down syndrome, cystic fibrosis, huntington's disease,
 15 hemophilia); skin disorders: (eczema, psoriasis, acne, melanoma); Immunodeficiency Disorders: (HIV/AIDS, primary immunodeficiency, severe combined immunodeficiency (SCID); Renal (kidney) diseases: (chronic kidney disease, kidney stones, polycystic kidney disease, glomerulonephritis); ophthalmic (eye) disorders: (glaucoma, cataracts, macular degeneration, retinitis pigmentosa); haematological disorders: (anemia, hemophilia,
 20 thrombocytopenia, sickle cell disease); Allergies: (hay fever (allergic rhinitis), food allergies, allergic asthma, anaphylaxis); reproductive health disorders: (infertility, polycystic ovary syndrome (PCOS), erectile dysfunction, endometriosis); metabolic disorders: (phenylketonuria (PKU), gaucher disease, tay-sachs disease, wilson's disease); rare and orphan diseases: (huntington's disease, amyotrophic lateral sclerosis (ALS), pompe disease,
 25 prader-willi syndrome).

151. The method of any one of claims 147 to 150, wherein the disease or disorder is selected from the group consisting of: infectious diseases: (such as bacterial infections, viral infections, fungal infections, parasitic infections, sexually transmitted infections (STIs), tropical diseases, zoonotic diseases); autoimmune Diseases: (such as rheumatoid arthritis,
 30 systemic lupus erythematosus, multiple sclerosis, type 1 diabetes, psoriasis, autoimmune skin diseases, autoimmune thyroid diseases, autoimmune gastrointestinal diseases); and Cancer: (such as breast cancer, lung cancer, prostate cancer, leukemia, skin cancer).

152. The method of any one of claims 147 to 151, wherein the disease or disorder is a viral infection selected from the group consisting of: Flaviviridae (Dengue fever; Zika virus
 35 infection; Yellow fever); Herpesviridae (Herpes simplex virus (HSV) infection; Varicella-zoster virus (VZV) infection (Chickenpox and Shingles); Epstein-Barr virus (EBV) infection

- (Mononucleosis); Cytomegalovirus (CMV) infection; Human herpesvirus 6 (HHV-6) infection; Retroviridae (Human immunodeficiency virus (HIV) infection (AIDS)); Orthomyxoviridae (Influenza (Flu)); Paramyxoviridae (Measles (Rubeola); Mumps); Poxviridae (Smallpox (Variola)); Coronaviridae (COVID-19 (SARS-CoV-2)); Papillomaviridae (Human papillomavirus (HPV) infection (Causes various types of warts, and some strains are associated with cervical cancer)); Picornaviridae (Common cold (Rhinovirus)); Hepadnaviridae (Hepatitis B); Caliciviridae (Norovirus infection (Viral gastroenteritis)); Togaviridae (Rubella (German Measles)); Adenoviridae (Respiratory adenovirus infections); Bunyaviridae (Hantavirus infection); Rhabdoviridae (Rabies).
- 10 153. The method of any one of claims 147 to 152, wherein the disease or disorder is a bacterial infection selected from the group consisting of: Streptococcus pyogenes (Group A Streptococcus) - Causes strep throat, skin infections, and more; Staphylococcus aureus - Responsible for various skin and soft tissue infections; Escherichia coli (E. coli) - Can lead to urinary tract infections, food poisoning, and other illnesses; Salmonella - Causes foodborne
- 15 infections; Mycobacterium tuberculosis - Responsible for tuberculosis (TB); Neisseria gonorrhoeae - Causes gonorrhea; Chlamydia trachomatis - Responsible for chlamydia infections; Helicobacter pylori - Linked to peptic ulcers and gastritis; Clostridium difficile - Can cause antibiotic-associated diarrhea and colitis; Streptococcus pneumoniae - A common cause of pneumonia and other respiratory infections.
- 20 154. The method of any one of claims 147 to 153, wherein the disease or disorder is treated by initiating a mucosal immune.
155. The method of any one of claims 147 to 154, wherein the disease or disorder is treated by initiating a mucosal immune is selected from the group consisting of: Respiratory
- 25 infections such as the common cold, influenza, and pneumonia, as well as gastrointestinal infections like those caused by rotavirus, norovirus, and foodborne bacteria (e.g., Salmonella, E. coli); Helicobacter pylori infections, which can lead to gastritis and peptic ulcers, involve the stomach's mucosal lining; Sexually transmitted infections like gonorrhea and chlamydia primarily affect the genital and urinary mucosal surfaces; Additionally, conditions like inflammatory bowel diseases, allergic rhinitis, asthma, sinusitis, otitis media,
- 30 and other mucosal inflammations require treatment strategies that target or modulate the mucosal immune response.
156. The method of any one of claims 147 to 144, wherein the dosage form is administered to deliver a therapeutic effective amount of the biological active material.

157. The method of any one of claims 147 to 156, wherein the therapeutically effective amount is administered to the subject utilising a dosing regimen selected from the group consisting of: twice hourly; hourly; once every six hours; once every 8 hours; once every 12 hours; once daily; twice weekly; once weekly; once every 2 weeks; once every 6 weeks; 5 once a month; every 2 months; every 3 months; once every 6 months; once yearly; and once.
158. The method of any one of claims 147 to 157, wherein the dosage form delivers a therapeutic effective amount of the biologically active material.
159. The method of any one of claims 147 to 158, wherein the therapeutically effective 10 amount is an amount of biologically active material selected from the group consisting of: between 0.001 to 100mg/kg; between 2 and 50mg/kg; between 5 and 40mg/kg; between 10 and 30mg/kg; between 20 and 25mg/kg; and 20mg/kg/day.
160. The method of any one of claims 147 to 159, wherein the therapeutically effective amount is an amount of biologically active material selected from the group consisting of: 15 between 0.01 nanogram to 100mg; 1 nanogram to 10mg; 1 nanogram to 1mg; 1 nanogram to 100µg; 1 nanogram to 1µg per dosage event.
161. The method of any one of claims 147 to 160, wherein the therapeutically effective amount is an amount of biologically active material selected from the group consisting of: 20 between 1 nanogram to 100mg; 1 nanogram to 10mg; 1 nanogram to 1mg; 1 nanogram to 100µg; 1 nanogram to 1µg per day.
162. The method of any one of claims 147 to 161, wherein the therapeutically effective amount is an amount of biologically active material selected from the group consisting of: between 0.1 ng to 1000 µg; 1 nanogram to 10µg; 1 nanogram to 1µg; 1 nanogram to 100ng; 1 nanogram to 10ng per day.
- 25 163. The method of any one of claims 147 to 162, wherein the therapeutically effective amount is administered to the subject using a method selected from the group consisting of: orally; sublingually; buccally; rectally; vaginally; mucosally; by the ocular route; by the otic route; nasally; and by inhalation.
164. The method of any one of claims 147 to 163, wherein the therapeutically effective 30 amount is administered to the subject using a method selected from the group consisting of: beneath the tongue or buccally.
165. The method of any one of claims 147 to 164, wherein the biologically active material initiates a mucosal immune response against the antigen.

166. The method of any one of claims 147 to 165, wherein the biologically active material does not enter the circulation induces an immune cascade via surface contact and surface function.
167. The method of any one of claims 147 to 166, wherein the biologically active material induces an immune cascade via surface contact and surface function.
168. The method of any one of claims 147 to 167, wherein the biologically active material does not enter the circulation system.
169. The method of any one of claims 147 to 168, wherein no detectable levels of the biologically active material enters the circulation system.
170. The method of any one of claims 147 to 169, wherein the detectable levels of the biologically active material that enters the circulation system is selected from the group consisting of: less than 0.001%; less than 0.01%; less than 0.1%; less than 2%; less than 3%; less than 4%; less than 5%; less than 6%; less than 7%; less than 8%; less than 9%; less than 10%; less than 11%; less than 12%; less than 13%; less than 14%; less than 15%; less than 16%; less than 17%; less than 18%; less than 19%; less than 20%; less than 21%; less than 22%; less than 23%; less than 24%; less than 25%; less than 26%; less than 27%; less than 28%; less than 29%; less than 30%; less than 31%; less than 32%; less than 33%; less than 34%; less than 35%; less than 36%; less than 37%; less than 38%; less than 39%; less than 40%; less than 41%; less than 42%; less than 43%; less than 44%; less than 45%; less than 46%; less than 47%; less than 48%; less than 49%; less than 50%; less than 51%; less than 52%; less than 53%; less than 54%; less than 55%; less than 56%; less than 57%; less than 58%; less than 59%; less than 60%; less than 61%; less than 62%; less than 63%; less than 64%; less than 65%; less than 66%; less than 67%; less than 68%; less than 69%; less than 70%; less than 71%; less than 72%; less than 73%; less than 74%; and less than 75% (w/w%).
171. The method of any one of claims 147 to 170, wherein said biologically active material is not absorbed by diffusion directly into the systemic circulation.
172. The method of any one of claims 147 to 171, wherein said method comprises one or more phases selected from the group consisting of: PHASE 1: Sublingual Administration: Post administration of sublingual vaccine/biologic onto the sublingual space (0 min); PHASE 2: Adhesion Immunogenic Precursor Phase: Time 1 to 20 mins (from antigens/vaccines/biologics touching the SL mucosa that is after wafer disintegration); adhesion/adsorption/binding of vaccine/biologic to the lamina propria mucosal cells (more specifically to the M cells or Microfold cells, Langerhans cells or LCs or dendritic cells or

DCs) then within 15 to 20 min, reactions with the immune cells (immunogenic precursor). Mucosal M cells and mucosal Langerhans cells are specialized cells found in the mucosal surfaces of the body. They have distinct functions and characteristics. Following the attachment of vaccines/biologics by adhesion/adsorption to the apical membrane of the M cells allow the active transportation of biologics (e.g. vaccine/biologic particles) across the epithelial cell layer where interactions with immune cells can take place. M cells have the unique ability to take up biologics (e.g. vaccines, interferons, biologics) via endocytosis, phagocytosis, or transcytosis. These biologics are delivered to antigen-presenting cells, such as dendritic cells, and B lymphocytes triggering the adaptive immunity (immune potentiators). Langerhans cells are a type of dendritic cell and play a vital role in biologics presentation and initiation of immune responses. They capture vaccines/biologics, process them, and present them to T cells in lymph nodes, which is essential for the initiation of adaptive immune responses. In summary, mucosal M cells primarily function in transporting antigens across the mucosal barrier to initiate immune responses in mucosal-associated lymphoid tissues, while mucosal Langerhans cells function in antigen presentation and immune activation in both the skin and mucosal surfaces. Hence, sublingual vaccines/biologics usually do not reach the bloodstream but are captured by these antigen-presenting M cells, LCs cells and DCs cells, in the epithelia mucosa. This biologic uptake is then process in the cells and present their epitopes to T cells and B cells for the induce effective adaptive immunity (memory immunity cells) which can then be distributed to other parts of the body via lymphatic drainage; PHASE 3: Immune Sentinel Activation Phase: Time 20 mins to 2 hours: antigen/vaccine/biologic attached to the induced immune sentinel activation by LCs and dendritic cells released cytokines and chemokines within 30mins to 2 hours and attracts more immune cells to the site of SL mucosa to fight invasion and infection; and PHASE 4: Fingerprints of Specific Infections Professional Antigen-Presenting T, B and NK cells to Fight Specific Infections: Time by day 3: antigen specific B and T effector cells start to disseminate to other lymph nodes triggering humoral immune responses expected in 2 weeks.

173. The method of any one of claims 147 to 172, wherein the dosage form is administered to deliver a therapeutic effective amount of the interferon or vaccine.

174. The method of any one of claims 147 to 173, wherein the dosage form is administered to deliver a therapeutic effective amount of the interferon.

175. A method of vaccination against a disease or disorder, said method comprising administering to a patient in need thereof a therapeutically effective amount of the dosage form of claims 136 to 144.

176. A method of initiating a mucosal immune response against an antigen, said method comprising administering to a patient in need thereof a therapeutically effective amount of the dosage form of claims 136 to 144 to a mucosal surface, wherein the biologically active material contains an amino acid sequence which is at least partially homologous to a sequence on the antigen.

177. A method of generating IgA immunity against an antigen, said method comprising administering to a patient in need thereof a therapeutically effective amount of the dosage form of claims 136 to 144 to a mucosal surface, wherein the biologically active material contains an amino acid sequence which is at least partially homologous to a sequence on the antigen.

178. Use of the composition of claims 1 to 134 in the manufacture of a medicament for the treatment or prevention of a disease or disorder.

179. A process of preparing the composition of claims 1 to 134, said process comprising the steps of: (1) combining at least one matrix forming agent with a biological active material to form a mixture; and (2) freeze drying the mixture to form the solid dosage form.

180. The process of claim 179, wherein the process further comprises the steps of: (a) Adjusted CMC and alginate to 1.5% (refer to 3x B220506 and 4x B220511); (b) SG taken from lab value for B220506; (c) Phosphate buffer with PS80 and vaccine until homogenous; (d) Measure out initial buffer charge then add to the CMC/alginate mixture and stir until homogenous; and (e) The rest of the excipients can then be added.

181. A kit comprising the dosage form of claims 136 to 144 together with instructions for its use.

182. The kit of claim 181, wherein the kit is directed to treating or preventing a disease or disorder selected from the group consisting of: H1N1; RSV; whooping cough (pertussis) and COVID-19.