BRIEF SUMMARY

[0005] In some aspects, provided is a method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising: a capture step; one or more ion exchange (IEX) chromatography steps; and a depth filtration step, thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the depth filtration step.

[0006] In some examples, each of the one or more IEX chromatography steps is selected from the group consisting of: an anion exchange (AEX) chromatography step, a cation exchange (CEX) chromatography step, and a multimodal ion exchange (MMIEX) chromatography step. In some examples, the MMIEX chromatography step comprises a multimodal cation exchange/ anion exchange (MM-AEX/CEX) chromatography step.

[0007] In some examples, the method further comprises a virus filtration step.

[0008] In some examples, the method further comprises an ultrafiltration/diafiltration (UF/DF) step.

[0009] In some examples, the purification platform comprises, in order: the capture step; the CEX chromatography step; the AEX chromatography step; the depth filtration step; the virus filtration step; and the UF/DF step.

[0010] In some examples, the depth filtration step comprises processing via a depth filter, and wherein the depth filter is a X0SP depth filter, a C0SP depth filter, a D0SP depth filter, or an EMPHAZE™ depth filter.

[0011] In some examples, the method further comprises a HIC step comprising processing via Sartobind® phenyl.

[0012] In other examples, provided is a method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising: a capture step; a multimodal hydrophobic interaction/ ion exchange (MM-HIC/IEX) chromatography step; and a hydrophobic interaction chromatography

(HIC) step, thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the HIC step and/or the MM-HIC/IEX chromatography step.

[0013] In some examples, the MM-HIC/IEX chromatography step comprises processing via a MM-HIC/IEX chromatography medium and is performed at a pH of about 5.5 to about 8. In some examples, the MM-HIC/IEX chromatography step is a multimodal hydrophobic interaction/ anion exchange (MM-HIC/AEX) chromatography step. In some examples, the MM-HIC/ AEX chromatography step comprises processing via Capto™ Adhere or Capto™ Adhere ImpRes. In some examples, the MM-HIC/IEX chromatography step is a multimodal hydrophobic interaction/ cation exchange (MM-HIC/CEX) chromatography step. In some examples, the MM-HIC/CEX chromatography step comprises Capto™ MMC or Capto™ MMC ImpRes.

[0014] In some examples, the purification platform comprises, in order: the capture step; the MM-HIC/IEX chromatography step; and the HIC step.

[0015] In some examples, the method further comprises a virus filtration step.

[0016] In some examples, the method further comprises an ultrafiltration/diafiltration (UF/DF) step.

[0017] In some examples, the purification platform comprises, in order: the capture step; the MM-HIC/AEX chromatography step; the HIC step; the virus filtration step; and the UF/DF step.

[0018] In some examples, the method further comprises a depth filtration step.

[0019] In some examples, the purification platform comprises, in order: the capture step; the depth filtration step; the MM-HIC/AEX chromatography step; and the HIC step. In some examples, the depth filtration step comprises processing via a depth filter, and wherein the depth filter is a X0SP depth filter. In some examples, the depth filtration step comprises processing via a depth filter, and the depth filter is an EMPHAZE™ depth filter.

[0020] In some examples, the depth filter is used as a load filter in conjunction with the MM-HIC/AEX chromatography step.

[0021] In some examples, the MM-HIC/ AEX chromatography step comprises processing via Capto™ Adhere or Capto™ Adhere ImpRes.

[0022] In some examples, the purification platform comprises, in order: the capture step; the MM-HIC/AEX chromatography step; the depth filtration step; and the HIC step. In some examples, the depth filtration step comprises processing via a depth filter, and wherein the depth filter is a X0SP depth filter, a C0SP depth filter, or a D0SP depth filter. In some examples, the depth filtration step comprises processing via a depth filter, and the depth filter is an EMPHAZE™ depth filter. In some examples, the MM-HIC/ AEX chromatography step comprises processing via Capto™ Adhere or Capto™ Adhere ImpRes.

[0023] In other examples, provided is a method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising: a capture step; one or more ion exchange (IEX) chromatography steps; and a hydrophobic interaction chromatography (HIC) step, thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the HIC step.

[0024] In some examples, the one or more IEX chromatography steps is a cation exchange (CEX) chromatography step.

[0025] In some examples, the method further comprises a virus filtration step.

[0026] In some examples, the method further comprises an ultrafiltration/diafiltration (UF/DF) step.

[0027] In some examples, the method further comprises a depth filtration step performed at any stage prior to the UF/DF step.

[0028] In some examples, the purification platform comprises, in order: the capture step; the CEX chromatography step; the HIC step; the virus filtration step; and the UF/DF step.

[0029] In other aspects, provided is a method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising: one or more ion exchange (IEX) chromatography steps; a hydrophobic interaction chromatography (HIC) step; and a depth filtration step, thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample

without the HIC step or the depth filtration step. In some examples, the reduction is as compared to purification of the sample without the HIC and the depth filtration step.

[0030] In some examples, each of the one or more IEX chromatography steps is selected from the group consisting of: an anion exchange (AEX) chromatography step, a cation exchange (CEX) chromatography step, and a multimodal ion exchange (MMIEX) chromatography step. In some examples, the MMIEX chromatography step comprises a multimodal cation exchange/ anion exchange (MM-AEX/CEX) chromatography step.

[0031] In some examples, the method further comprises an ultrafiltration/diafiltration (UF/DF) step.

[0032] In some examples, the purification platform comprises, in order: the CEX chromatography step; the HIC step; the MMIEX chromatography step; the AEX chromatography step; the depth filter step; and the UF/DF step.

[0033] In other aspects, provided is a method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising: a capture step; one or more ion exchange (IEX) chromatography steps; a multimodal hydrophobic interaction/ ion exchange (MM-HIC/IEX) chromatography steps; and one or both of: a hydrophobic interaction chromatography (HIC) step; and a depth filtration step, thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the HIC step or the depth filtration step.

[0034] In some examples, the reduction is as compared to purification of the sample without the HIC and the depth filtration step.

[0035] In some examples, the method further comprises a virus filtration step.

[0036] In some examples, the method further comprises an ultrafiltration/diafiltration (UF/DF) step.

[0037] In some examples, the depth filtration step is performed as a load filter for the MM-HIC/IEX chromatography step, as a load filter for the HIC step, or following the HIC step.

[0038] In some examples, each of the one or more IEX chromatography steps is selected from the group consisting of: a cation exchange (CEX) chromatography step, an anion exchange (AEX) chromatography step, and a multimodal ion exchange (MMIEX) chromatography step.

[0039] In some examples, the MM-HIC/IEX chromatography step is a multimodal hydrophobic interaction/ anion exchange (MM-HIC/AEX) chromatography step. In some examples, the MM-HIC/AEX chromatography step comprises processing via Capto™ Adhere or Capto™ Adhere ImpRes.

[0040] In some examples, the capture step comprises processing via affinity chromatography. In some examples, the capture step is performed in a bind-and-elute mode. In some examples, the affinity chromatography is selected from the group consisting of a protein A chromatography, a protein G chromatography, a protein A/G chromatography, a FcXL chromatography, a protein XL chromatography, a kappa chromatography, and a kappaXL chromatography.

[0041] In some examples, the depth filtration step comprises processing via a depth filter. In some examples, the depth filter is used as a load filter. In some examples, the depth filter comprises a substrate comprising one or more of a diatomaceous earth composition, a silica composition, a cellulose fiber, a polymeric fiber, a cohesive resin, and an ash composition. In some examples, at least a portion of the substrate of the depth filter comprises a surface modification. In some examples, the surface modification is one or more of a quaternary amine surface modification (such as a quaternary ammonium, Q, functionality), a guanidinium surface modification, a cationic surface modification, and an anionic surface modification. In some examples, the depth filter is selected from the group consisting of: a X0SP depth filter, a D0SP depth filter, a C0SP depth filter, an EMPHAZE™ depth filter, a PDD1 depth filter, a PDE1 depth filter, a PDH5 depth filter, a ZETA PLUS™ 120ZA depth filter, a ZETA PLUS™ 120ZB depth filter, a ZETA PLUS™ DELI depth filter, a ZETA PLUS™ DELP depth filter, and a Polisher ST (salt tolerant) depth filter. In some examples, the depth filter is the X0SP depth filter, the D0SP depth filter, or the C0SP depth filter, and wherein the processing via the depth filter is performed at a pH of about 4.5 to about 8. In some examples, the depth filter is the EMPHAZE™ depth filter,

and wherein processing via the depth filter is performed at a pH of about 7 to about 9.5. In some examples, the depth filter is the Polisher ST depth filter, and wherein processing via the depth filter is performed at a pH of about 5 to about 9.

[0042] In some examples, the HIC step comprises processing via a HIC membrane or a HIC column. In some examples, processing via the HIC membrane or the HIC column is performed using low salt concentrations. In some examples, processing via the HIC membrane or the HIC column is performed in flow-through mode. In some examples, the HIC membrane or HIC column comprises a substrate comprising one or more of an ether group, an ethyl group, a propyl group, an isopropyl group, a butyl group, a hexyl group, an octyl group, and a phenyl group. In some examples, the HIC membrane or the HIC column is selected from the group consisting of Bakerbond WP HI-Propyl™, Phenyl Sepharose® Fast Flow (Phenyl-SFF), Phenyl Sepharose® Fast Flow Hi-sub (Phenyl-SFF HS), Toyopearl® Hexyl-650C, Toyopearl® Hexyl-650M, Toyopearl® Hexyl-650S, Poros™ Benzyl Ultra, and Sartobind® phenyl. In some examples, processing via the HIC membrane or the HIC column is performed at a pH of about 4.5 to about 7.

[0043] In some examples, each of the one or more IEX chromatography steps comprises processing via an IEX chromatography membrane or an IEX chromatography column. In some examples, the IEX chromatography membrane or the IEX chromatography column is selected from the group consisting of: SPSFF, QSFF, SPXL, Streamline™ SPXL, ABx™, Poros™ XS, Poros™ 50HS, DEAE, DMAE, TMAE, QAE, and MEP-Hypercel™.

[0044] In some examples, the purification platform is for purification of a target from the sample, and wherein the sample comprises the target and one or more host cell impurities. In some examples, the target comprises a polypeptide. In some examples, the target is an antibody moiety. In some examples, the antibody moiety is a monoclonal antibody. In some examples, the antibody moiety is a human, humanized, or chimeric antibody. In some examples, the antibody moiety is selected from the group consisting of: an anti-TAU antibody, an anti-TGFβ3 antibody, an anti-VEGF-A antibody, an anti-CD20 antibody, an anti-CD40 antibody, an anti-HER2 antibody, an anti-IL6 antibody, an anti-IgE antibody, an anti-IL13 antibody, an anti-TIGIT antibody, an anti-PD-L1 antibody, an anti-VEGF-A/ANG2 antibody, an anti-CD79b

antibody, an anti-ST2 antibody, an anti-factor D antibody, an anti-factor IX antibody, an anti-factor X antibody, an anti-abeta antibody, an anti-CEA antibody, an anti-CEA/CD3 antibody, an anti-CD20/CD3 antibody, an anti-FcRH5/CD3 antibody, an anti-Her2/CD3 antibody, an anti-FGFR1/KLB antibody, a FAP-4-1 BBL fusion protein, a FAP-IL2v fusion protein, and a TYRP1 TCB antibody. In some examples, the antibody moiety is selected from the group consisting of: ocrelizumab, pertuzumab, ranibizumab, trastuzumab, tocilizumab, faricimab, polatuzumab, gantenerumab, cibisatamab, crenezumab, mosunetuzumab, tiragolumab, bevacizumab, rituximab, atezolizumab, obinutuzumab, lampalizumab, omalizumab, ranibizumab, emicizumab, selicrelumab, prasinezumab, RO6874281, and RO7122290.

[0045] In some examples, the one or more host cell impurities comprises a host cell protein. In some examples, the host cell protein is a hydrolytic enzyme. In some examples, the hydrolytic enzyme is a lipase, an esterase, a thioesterase, a phospholipase, carboxylesterase, hydrolase, cutinase, or a ceramidase.

[0046] In some examples, the sample comprises a host cell or components originating therefrom. In some examples, the sample is, or is derived from, a cell culture sample. In some examples, the cell culture sample comprises a host cell, and wherein the host cell is a Chinese hamster ovary (CHO) cell or an *E. coli* cell.

[0047] In some examples, the method further comprises a sample processing step.

[0048] In some examples, the reduction in the hydrolytic enzyme activity rate is at least about 20%.

[0049] In some examples, the method further comprises determining the hydrolytic enzyme activity rate of the composition.

[0050] In some examples, the method further comprises determining the level of one or more hydrolytic enzymes in the composition.

[0051] In some examples, the composition comprises a polysorbate. In some examples, the polysorbate is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80.

[0052] In other examples, provided is a pharmaceutical composition obtained from a method described herein. In one aspect, provided is a pharmaceutical composition obtained from the method as disclosed herein.

[0053] In other examples, provided is a formulated antibody moiety composition comprising an antibody moiety and a polysorbate, wherein the composition has a reduced rate of polysorbate hydrolysis, wherein the shelf-life of the composition is more than 12 months.

[0054] In other examples, provided is a formulated antibody moiety composition comprising an antibody moiety and a polysorbate, wherein the composition has a reduced rate of polysorbate hydrolysis activity, wherein the shelf-life of the composition is extended compared to the shelf-life indicated in documents filed with a health authority related to the formulated antibody moiety composition, wherein the shelf-life is extended by at least 3 months compared to the shelf-life indicated in said documents.

[0055] In some examples, the rate of polysorbate hydrolysis is reduced by at least about 20%.

[0056] In other examples, provided is a formulated antibody moiety composition comprising an antibody moiety, wherein the formulated antibody moiety composition has a reduced degradation of polysorbate, wherein the degradation is reduced by at least about 20% compared to the degradation indicated in documents filed with a health authority related to the formulated antibody moiety composition.

[0057] In other examples, provided is a formulated antibody moiety composition comprising an antibody moiety and a polysorbate, wherein the polysorbate is degraded during storage of the liquid composition by 50% or less per year.

[0058] In some examples, the antibody moiety is a monoclonal antibody. In some examples, the antibody moiety is a human, humanized, or chimeric antibody. In some examples, the antibody is selected from the group consisting of an anti-TAU antibody, an anti-TGFβ3 antibody, an anti-VEGF-A antibody, an anti-CD20 antibody, an anti-CD40 antibody, an anti-HER2 antibody, an anti-IL6 antibody, an anti-IgE antibody, an anti-IL13 antibody, an anti-TIGIT antibody, an anti-PD-L1 antibody, an anti-VEGF-A/ANG2 antibody, an anti-CD79b antibody, an anti-ST2 antibody, an anti-factor D antibody, an anti-factor IX antibody, an anti-factor

X antibody, an anti-abeta antibody, an anti-CEA antibody, an anti-CEA/CD3 antibody, an anti-CD20/CD3 antibody, an anti-FcRH5/CD3 antibody, an anti-Her2/CD3 antibody, an anti-FGFR1/KLB antibody, a FAP-4-1 BBL fusion protein, a FAP-IL2v fusion protein, and a TYRP1 TCB antibody. In some examples, the antibody moiety is selected from the group consisting of ocrelizumab, pertuzumab, ranibizumab, trastuzumab, tocilizumab, faricimab, polatuzumab, gantenerumab, cibisatamab, crenezumab, mosunetuzumab, tiragolumab, bevacizumab, rituximab, atezolizumab, obinutuzumab, lampalizumab, omalizumab ranibizumab, emicizumab, selicrelumab, prasinezumab, RO6874281, and RO7122290.

[0059] In some examples, the polysorbate is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80.

[0060] Those skilled in the art will recognize that several examples are possible within the scope and spirit of the disclosure of this application. The disclosure is illustrated further by the examples below, which are not to be construed as limiting the disclosure in scope or spirit to the specific procedures described therein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0061] **FIGS. 1A-1E** show schematics of exemplary purification platforms described herein.

[0062] **FIG. 2** shows a bar graph of the hydrolytic activity (as normalized to the control) of compositions obtained from exemplary purification platforms. The hydrolytic activity rates were measured using a FAMS assay.

[0063] **FIG. 3** shows a bar graph of the hydrolytic activity (as normalized to the control) of compositions obtained from exemplary purification platforms. The hydrolytic activity rates were measured using a FAMS assay.

[0064] **FIG. 4** shows a bar graph of the hydrolytic activity (as normalized to the control) of compositions obtained from exemplary purification platforms. The hydrolytic activity rates were measured using a FAMS assay.