enzymes from yeast to produce epoxidized oils and describes detecting lipase activity of these concentrated lipase-nanoparticle conjugates using fluorescent substrate and without disclosing the exact conditions. Likewise, Yoo et al., (Cell Chemical Biology, 2020, 27: 143-157) discloses a fluorogenic substrate assay for detecting lipase activity and uses Triton X-100 for solubilizing the highly concentrated lipase rPfMAGLLP prior to analysis, but not as part of the reaction solution. WO 2010/024924 discloses an assay for screening for lipases expressed in *E.coli* using a fluorogenic substrate and hence again the assay is not used for detecting contaminating lipase activity in recombinant protein samples purified from eukaryotic cells. Yet none of these prior art assays determine contaminating lipase activity in a recombinant protein sample produced in eukaryotic cells.

[007] Menden et al., 2019 (Journal of Enzyme Inhibition of Medicinal Chemistry, 34(1): 1474-1480) reports a lipase activity assay in which lipase activity of a defined enzyme extract of Candida rugosa lipase (CRL) isoforms is detected to verify the mode of action of the inhibitor tropolone using 4-methylumbelliferryl butyrate (4-MUB) and palmitate (4-MUP) as substrate. Limitations to the assay are reported, which include the intrinsic decrease in solubility of the hydrophobic fatty acid tail with length and the autocatalysis of the substrate in the basic pH range. Moreover, no surfactant is used in the assay. More recently, Jahn et al., 2020 (Pharm. Res. 37(118): 2-13) reports a chromophore-based lipase activity assay for use in determining polysorbate degradation in samples of harvested cell culture fluids using 4-methylumbelliferyl oleate (4-MuO) as substrate. However, the moderate sensitivity still requires incubation times of 24 hour or more.

[008] Accordingly, there is still a need for an improved method with high sensitivity that can determine lipase activity in a relevant sample in a short period of time.

SUMMARY

[009] The present disclosure relates to a method for detecting (contaminating) lipase activity in a sample comprising a recombinant protein comprising (a) providing at least one sample comprising a recombinant protein produced in a eukaryotic cell; (b) contacting the at least one sample with a reaction solution to form a reaction mixture, wherein the reaction solution comprises: (i) a buffer having a pH of about pH 4 to about pH 9, (ii) a non-denaturing surfactant not having an ester-bond, wherein the surfactant is a non-ionic or zwitter-ionic surfactant, (iii) a substrate comprising the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the 4-MU ester is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester, and (iv) optionally a non-buffering salt; (c) incubating the sample and the substrate in the reaction mixture; and (d) detecting (contaminating) lipase activity by measuring hydrolysis of the 4-MU ester and detecting the fluorescence intensity of the released chromophore 4-MU (which is a 4-MU ester hydrolysis product); wherein optionally hydrolysis is measured by detecting the fluorescence intensity of the released chromophore 4-MU over time, while incubating the sample and the substrate in the reaction mixture according to step (c).

In one aspect, the present disclosure relates to a method for detecting lipase activity in a sample comprising a recombinant protein comprising (a) providing at least one sample comprising a recombinant protein produced in a eukaryotic cell; (b) contacting the at least one sample with a reaction solution to form a reaction mixture, wherein the reaction solution comprises: (i) a buffer having a pH of about pH 4 to about pH 9, (ii) a non-denaturing surfactant not having an ester-bond, wherein the surfactant is a non-ionic or zwitter-ionic surfactant, (iii) a substrate comprising the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the 4-MU ester is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester, and (iv) optionally a non-buffering salt; (c) incubating the sample and the substrate in the reaction mixture; (d) detecting lipase activity by measuring hydrolysis of the 4-MU ester and detecting the fluorescence intensity of the released chromophore 4-MU; optionally measuring hydrolysis by detecting the fluorescence intensity of the released chromophore 4-MU over time, while incubating the sample and the substrate in the reaction mixture according to step (c). The method is to be understood to refer to an *in vitro* method. In certain examples the sample and the substrate in the reaction mixture are incubated for any time period between 2 min and less than 5 hours, less than 3 hours, less than 2 hours, or less than 0.5 hours. The at least one sample may be a

harvested cell culture fluid (HCCF), an in-process control (IPC) sample, a drug substance sample or a drug product sample. The recombinant protein in the sample for detecting lipase activity is preferably a therapeutic protein, such as an antibody, an antibody fragment, an antibody derived molecule or an fusion protein (e.g., an Fc fusion protein). According to the disclosure, the recombinant protein in the sample for detecting lipase activity is not a lipase and/or does not comprise lipase activity. Thus, any lipase activity detected in the at least one sample is contaminating lipase activity and/or derived from at least one contaminating protein having lipase activity, such as host cell proteins (HCPs) derived from the eukaryotic cell.

[010] In a preferred example the substrate is selected from the group consisting of 4-methylumbelliferyl octanoate, 4-methylumbelliferyl nonanoate, 4-methylumbelliferyl decanoate (4-MUD), 4-methylumbelliferyl undecanoate and 4-methylumbelliferyl dodecanoate, more preferably the substrate is selected from the group consisting of 4-methylumbelliferyl octanoate, 4-methylumbelliferyl decanoate (4-MUD) and 4-methylumbelliferyl dodecanoate. According to preferred examples of the disclosure, the surfactant has a final concentration in the reaction mixture above its critical micelle concentration in the reaction mixture. The non-denaturing non-ionic or zwitter-ionic surfactant may be CHAPS, CHAPSO, Zwittergent (such as Zwittergent 3-12) or a saponin. Preferably, the non-denaturing non-ionic or zwitter-ionic surfactant is CHAPS. CHAPS may be provided at a final concentration in the reaction mixture of about 8 mM to about 20 mM, preferably at about 8 mM to about 15 mM, more preferably at about 10 mM. A suitable buffer comprises one or more buffer substances selected from the group consisting of formic acid, acetic acid, lactic acid, citric acid, malic acid, maleic acid, glycine, glycylglycine, succinic acid, TES (2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)), MES (2-(N-morpholino)ethanesulfonic acid), Tris base, Bis-Tris, Bis-Tris-Propane, Bicine (N,N-bis(2-hydroxyethyl)glycine), HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid), TAPS (3-([tris(hydroxymethyl)methyl]amino}pro-panesulfonic acid), Tricine (N-tris(hydroxymethyl)methylglycine), Na_2HPO_4 and NaH_2PO_4. Preferably, the buffer has a pH of about 5 to about 7.5, preferably the buffer has a pH of about 5.5 to about 7.5. In certain examples the buffer is a multi-component buffer having a buffering range from at least about pH 5 to at least about pH 7.5, preferably from at least about pH 4 to at least about pH 8.

[011] The optional non-buffering salt may be, e.g., NaCl, KCl and $CaCl_2$ and is preferably NaCl or KCl. It may be provided at a concentration of about 100 mM to about 200 mM, preferably about 130 mM to about 170 mM, more preferably about 140 mM to about 150 mM in the reaction mixture. The ionic strength of non-buffering salt in the reaction mixture is preferably about 200 mM or less, more preferably about 170 mM or less and more preferably about 150 mM or less, such as from about 100 mM to about 200 mM, preferably about 130 mM to about 170 mM, more preferably about 140 mM to about 150 mM in the reaction mixture. Alternatively or in addition the cumulative ionic strength of the buffer and the non-buffering salt in the reaction mixture may be about 450, preferably about 400 mM or less, more preferably about 350 mM or less.

[012] The disclosure relates to a method of manufacturing a recombinant protein of interest comprising the steps of (i) cultivating a eukaryotic cell expressing a recombinant protein of

interest in cell culture; (ii) harvesting the recombinant protein; (iii) purifying the recombinant protein; and (iv) optionally formulating the recombinant protein into a pharmaceutically acceptable formulation suitable for administration; and (v) obtaining at least one sample comprising the recombinant protein in steps (ii), (iii) and/or (iv); wherein the method further comprises detecting (contaminating) lipase activity in a sample comprising the recombinant protein comprising: (a) providing the at least one sample comprising the recombinant protein produced in a eukaryotic cell of step (v); (b) contacting the at least one sample with a reaction solution to form a reaction mixture, wherein the reaction solution comprises: (i) a buffer having a pH of about pH 4 to about pH 9, (ii) a non-denaturing surfactant not having an ester-bond, wherein the surfactant is a non-ionic or zwitter-ionic surfactant, (iii) a substrate comprising the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the 4-MU ester is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester, and (iv) optionally a non-buffering salt; (c) incubating the sample and the substrate in the reaction mixture; (d) detecting (contaminating) lipase activity by measuring hydrolysis of the 4-MU ester and detecting the fluorescence intensity of the released chromophore 4-MU; optionally measuring hydrolysis by detecting the fluorescence intensity of the released chromophore 4-MU over time, while incubating the sample and the substrate in the reaction mixture according to step (c). In another aspect, the present disclosure relates to a method of manufacturing a recombinant protein of interest comprising the steps of (i) cultivating a eukaryotic cell expressing a recombinant protein of interest in cell culture; (ii) harvesting the recombinant protein; (iii) purifying the recombinant protein; and (iv) optionally formulating the recombinant protein into a pharmaceutically acceptable formulation suitable for administration; and (v) obtaining at least one sample comprising the recombinant protein in steps (ii), (iii) and/or (iv); wherein the method further comprises detecting lipase activity in a sample comprising the recombinant protein comprising: (a) providing the at least one sample comprising the recombinant protein produced in a eukaryotic cell of step (v); (b) contacting the at least one sample with a reaction solution to form a reaction mixture, wherein the reaction solution comprises: (i) a buffer having a pH of about pH 4 to about pH 9, (ii) a non-denaturing surfactant not having an ester-bond, wherein the surfactant is a non-ionic or zwitter-ionic surfactant, preferably wherein the surfactant is not polyethylene glycol *tert*-octylphenyl ether (Triton X-100) and not polyethylene glycol nonylphenyl ether (NP-40), (iii) a substrate comprising the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the 4-MU ester is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester, and (iv) optionally a non-buffering salt; (c) incubating the sample and the substrate in the reaction mixture; and (d) detecting lipase activity by measuring hydrolysis of the 4-MU ester and detecting the fluorescence intensity of the released chromophore 4-MU; optionally measuring hydrolysis by detecting the fluorescence intensity of the released chromophore 4-MU over time, while incubating the sample and the substrate in the reaction mixture according to step (c). In certain examples, the method comprises obtaining at least one sample comprising the recombinant protein in step (ii), wherein the sample is a harvested cell culture fluid (HCCF) or a cell

lysate; step (iii), wherein the sample is an in-process control (IPC) sample; and/or step (iv), wherein the sample is a drug substance sample or a drug product sample; preferably comprising obtaining at least one sample comprising the recombinant protein in step (iii), comprising obtaining at least one sample before and after affinity chromatography, and/or before and after acid treatment, before and after depth filtration following acid treatment, and/or before and after ion exchange chromatography, preferably anion exchange chromatography or cation exchange chromatography.

[013] The disclosure relates to a kit for determining contaminating lipase activity in a sample comprising a recombinant protein, such as an IPC sample, comprising: (i) a buffer having a pH of about pH 4 to about pH 9, (ii) a non-denaturing surfactant not having an ester-bond, wherein the surfactant is a non-ionic or zwitter-ionic surfactant, (iii) a substrate comprising the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the substrate is a saturated unbranched-chain fatty acid (C6 to C16) 4-MU ester, and (iv) optionally a non-buffering salt, and/or (iiv) optionally water for dilution. In another aspect, the present disclosure relates to a kit for determining contaminating lipase activity in a sample comprising a recombinant protein comprising: (i) a buffer having a pH of about pH 4 to about pH 9; (ii) a non-denaturing surfactant not having an ester-bond, wherein the surfactant is a non-ionic or zwitter-ionic surfactant; (iii) a substrate comprising the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the substrate is a saturated unbranched-chain fatty acid (C6 to C16) 4-MU ester; and (iv) optionally a non-buffering salt; and/or (v) optionally water for dilution. In certain examples, the buffer, the surfactant and the optional non-buffering salt are premixed as an assay buffer. Preferably, said assay buffer is at least about 3-fold concentrated or about 3-fold to about 5-fold concentrated relative to a final reaction mixture. Alternatively, the assay buffer is provided as a dry mixture. Such dry mixture may be reconstituted with water to provide said at least about 3-fold concentrated or 5-fold concentrated assay buffer relative to a final reaction mixture. Alternatively or in addition the buffer, the surfactant, the substrate and the optional non-buffering salt are premixed and added as a master mix to the sample, wherein the master mix is provided at about 80 % (v/v) to about 70 % (v/v) of the reaction mixture, preferably at about 75 % of the reaction mix.

[014] In a preferred example, the substrate is selected from the group consisting of 4-methylumbelliferyl octanoate, 4-methylumbelliferyl nonanoate, 4-methylumbelliferyl decanoate (4-MUD), methylumbelliferyl undecanoate and methylumbelliferyl dodecanoate. The kit may also further comprise an organic solvent for dissolving the substrate, or the substrate dissolved in an organic solvent, and/or one or more microtiter plate having 96 wells or a multiple of 96 wells. The non-denaturing non-ionic or zwitter-ionic surfactant may be CHAPS, CHAPSO, Zwittergent (such as Zwittergent 3-12) and a saponin. Preferably, the non-denaturing non-ionic or zwitter-ionic surfactant is CHAPS. In certain examples, the buffer comprises one or more buffer substances selected from the group consisting of formic acid, acetic acid, lactic acid, citric acid, malic acid, maleic acid, glycine, glycylglycine, succinic acid, TES (2-{[tris(hydroxyme-thyl)methyl]amino}ethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)), MES (2-(N-morpholino)ethanesulfonic acid), Tris base, Tris, Bis-Tris, Bis-Tris-Propane, Bicine (N,N-bis(2-hydroxyethyl)glycine), HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid), TAPS (3-([tris(hydroxymethyl)methyl]amino-pro-panesulfonic acid), Tricine (N-tris(hydroxymethyl)methylglycine), Na_2HPO_4 and NaH_2PO_4. Preferably the buffer has a pH of about 5 to about 7.5, more preferably the buffer has a pH of about 5.5 to about 7.5. In certain examples the buffer is a multi-component buffer having a buffering range from at least about pH 5 to at least about pH 7.5, preferably from at least about pH 4 to at least about pH 8. The optional non-buffering salt may be, e.g., NaCl, KCl and $CaCl_2$ and is preferably NaCl or KCl.

DESCRIPTION OF THE FIGURES

[015] FIGURE 1: Depicted is a hydrolysis reaction for the substrate 4-MUD.

[016] FIGURE 2: Detection of lipase activity in different bulk drug substances (BDS) at variable pH. Hydrolytic activity of bulk drug substances A-B and D-E (monoclonal antibodies and antibody-like formats) sample was measured at different pH between pH 4 and pH 8 as indicated. All measurements were performed with 30 µM 4-MUD in AMT assay buffer (75 mM acetate, 75 mM MES, 150 mM Tris, 150 mM NaCl, 10 mM CHAPS, pH 5.5) using a microplate reader (λ_{em} = 450 nm, λ_{ex} = 330 nm, top read mode) in a black 96 well plate.

[017] FIGURE 3: Solubility of 4-MUD in the assay buffer. Solubility of 0 to 60 µM 4-MUD was tested with light scattering experiments in AMT assay buffer (75 mM acetate, 75 mM MES, 150 mM Tris, 150 mM NaCl, 10 mM CHAPS, pH 4-8) using a microplate reader (λ_{em} = 450 nm, λ_{ex} = 330 nm, top read mode) in a black 96 well plate.

[018] FIGURE 4: Michaelis-Menten kinetics of the hydrolytic activity in a bulk drug substance (BDS D). Assay was performed in AMT assay buffer (75 mM acetate, 75 mM MES, 150 mM Tris, 150 mM NaCl, 10 mM CHAPS, pH 5.5) with varying concentrations of 4-MUD (1.5625 µM - 150 µM) using a microplate reader (λ_{em} = 450 nm, λ_{ex} = 330 nm, top read mode) in a black 96 well plate.

[019] FIGURE 5: Solubility of 4-MUD was tested with light scattering experiments in AMT assay buffer (75 mM acetate, 75 mM MES, 150 mM Tris, 150 mM NaCl, 20 mM CHAPS, pH 7) using a fluorescent spectrometer (λ_{em} = 400 nm, λ_{ex} = 400 nm) in a 1 cm macro-cuvette.