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Compaction of chemically defined cell culture media increases its dissolution rate through an increase of solvent accessible surface area

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ABSTRACT

Cultivation of mammalian cells for the generation of therapeutics requires the use of cell culture media which is a defined mixture of nutrients including carbohydrates, amino acids, lipids, vitamins, and inorganic salts. Its composition influences the cell density, yield, and quality of the molecule expressed. These chemically defined cell culture media are manufactured as powders since they offer advantages of storage, handling and stability. To prevent de-mixing of the powders and maintain homogeneity, these powders are milled down to small particle size ranges. However, this small particle size allows for inter-particle forces to impact powder flow properties, and decreases the surface area available to dissolution. Hence a method is required that improves these powder parameters, without altering their composition. A deeper understanding of the dissolution mechanism of multi-component mixtures like cell culture media is also needed. Here, cell culture media granules, generated from homogeneous powders by roller compaction are evaluated. The granulation process did not alter the composition of the media and properties of solutions generated thereof. The analysis and comparison of the dissolution profile of powder and compacted media enabled the calculation of the actual surface area available to dissolution. The improvement in granule dissolution can be attributed to dispersion and disintegration of the granules on wetting increasing available surface area. Decrease in inter-particle forces in granules also improves flow-ability properties. Surface character and pores on the granules further augments the improvement in dissolution.

1. Introduction

The cultivation of mammalian cells in the production of peptides and protein therapeutics for the treatment of various diseases including cancers, diabetes, and auto immune diseases requires the use of cell culture media (CCM) [1,2]. These CCM include carbohydrates, amino acids, vitamins, fatty acids, and inorganic salts. Whereas earlier mammalian cell cultivation was supported by serum, chemically defined media (CDM) with known chemical identity of all ingredients are currently used. The achievable cell density, titer, metabolism and culture duration is dependent on the compositions of cell culture media [3]. For the predominantly used Chinese hamster ovary (CHO) cells, research shows that individual components of mammalian cell culture media (CCM), even in trace amounts, influence the post translational modifications crucial to the function of the recombinant proteins produced [4–6]. The formulation of these media is done in small scale in liquid using stock solution of ingredients. However, for its use in large scale, media are generated by dissolving and filter sterilizing a mixture of all the ingredients in the form of, ideally, a single powder. Powdered CCM offer increased stability during storage and therefore preferred. Dry powder cell culture media (DPM) used in large scale processes needs to be easy to handle, and should dissolve quickly. In these large scale setups, CCM solutions are often heated or subject to drastic pH changes to increase the powder dissolution rate which can detrimental to, heat labile and pH sensitive components such as riboflavin [7–9]. To maintain homogeneity of CCM and to maximize solvent accessible surface area during dissolution, CCM is milled to a small particle size range. This can be counterproductive since, fine CCM powders are more prone to influence inter-particle forces [10,11]. These forces cause formation of agglomerates in aqueous solvents, limiting the available surface area for dissolution. Therefore, the available surface area differs from the total particle surface area in powdered CCM.

In this study, compacted CCM generated by roller compaction of homogeneous CCM powders are evaluated. Additives are not used in the compaction of CCM. The compaction process was found to have no

Abbreviations: CCM, Cell culture media; CDM, Chemically defined media; CHO, Chinese hamster ovary; DPM, Dry Powder Media; FBRM, Focused Beam Reflectance Measurement; FTIR, Fourier transformed Infrared spectroscopy; mAb, Monoclonal Antibody; ppm, parts per million; UPLC, Ultra high performance liquid chromatography; ρ_b, Bulk density; ρ_t, Tapped density.

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significant influence on physicochemical properties of CCM and did not alter the cellular performance. The composition of the media remains unaltered allowing for the comparison of CCM powder and granule dissolution. Compacted CCM were capable of overcoming inter-particle adhesion forces resulting in improvement of solid flow properties and increase in the solvent accessible surface area. The dissolution of granules was further increased by their rough surface character and worm holing effects caused by the presence of pores. Analysis of the dissolution profile of granules and comparison to their corresponding powder, supplemented with surface area measurements, allows the evaluation of the available surface area of a powder during dissolution.

2. Material & methods

2.1. Cell culture media

Merck Life science proprietary Cellvento™CHO 210 media and Cellvento™Feed 210 was used in this study, hence forth referred to as CHO210 and Feed210 respectively. CHO210 is a cell culture media and Feed210 is a cell culture feed designed for fed batch cultivation of CHO cells. The compaction was performed using a RC-100 (Powtec, Germany) roller compactor.

2.2. Fine residual analysis

An online Aerosol spectrometer (GRIMM, Germany) was used to measure the dust generated in the transfer of cell culture media to a DasGip DS0700TPSS bioreactor (DasGip, Eppendorf, Germany) in a controlled environment. A mass equal of 23.13 g for CHO210 media and 81.60 g for Feed210 was used. This corresponds to the mass required to generate working concentrations of these media. Measurements were made every 6 s for a total of 300 s. The cell culture media (powder or compacted media) was transferred manually after 40 s. The baseline was subtracted from the reading for each run. Measurements were performed in six replicates. The fine dust particles measured in this system was correlated with the free particles not incorporated into CCM granules.

2.3. Characterization CCM solids

Friability was determined using a TAR friability tester (Erweka, Germany) according to US pharmacopeia guidelines [12]. Water content was determined by Karl-Fischer titration using a Karl Fischer Titrator (Metrohm, Switzerland). Particle size analysis and specific surface was determined by laser diffraction analysis with a Mastersizer3000 (Malvern, UK). Bulk and tab density was determined with a Tapped Density Tester JV1000 (Antech, Ireland) and Carr index ($100(1-\rho_b/\rho_t)$) was calculated from these values. Since Hausner ratio (ρ_t/ρ_b) can be calculated from Carr Index, only Carr index is reported. Based on database data (not shown) the values were categorized. The dynamic avalanche angle and break energy was measured using a Revolution Powder Analyzer (Mercury Scientific Inc., USA). Dynamic avalanche angle was determined as maximum angle achieved before avalanche occurrence and break energy was determined as the difference of the maximum and minimum possible energy of a sample given its mass and volume. All measurements were performed in quadruplicates.

2.4. Detection of amino acids and vitamins

The amino acid analysis was performed using a pre-column derivatization employing the AccQ Tag® Ultrareagent kit (Waters, USA). Derivatization, chromatography and data analysis were performed according to the supplier recommendations (Waters, USA). Vitamins were quantified by using an LC-MS method. All measurements were performed in triplicates.

2.5. Characterization of solutions of CCM

For determination of pH a SevenMulti pH meter (Mettler Toledo, Switzerland) was used, osmolality was measured with an Osmomat 030 (Gonotec, Germany). Color of solutions of CHO210 and Feed210 was evaluated with a Color Flex EZ (Hunterlab, USA) using the lightness value. All measurements were performed in quadruplicates.

2.6. Surface and pore characterization

Scanning electron microscopy (SEM) was performed using a LEO 1530 Gemini instrument (Carl Zeiss, Germany) as per manufacturer instructions. Mercury intrusion porosimetry was performed with a Quantachrome Poremaster (Quantachrome Instruments, USA). The modal volume, surface area, and pore number fraction was determined. The data were analyzed using Quantachrome Poremaster for Windows version 8.0. Mercury volume was normalized by sample weight.

2.7. Fed batch cell culture experiments

Fed batch experiments were conducted in 50 mL shake tubes, with feeding every second or third day. The CHO DG44 clone expressing human monoclonal antibody (mAb) was grown in CHO210 media, and fed Feed210. The starting culture volume was 30 mL and the culture conditions were maintained at 37 °C, 5% CO_2, 80% humidity and agitation at 320 rpm. Experimental conditions were performed as quadruplicates.

2.8. Online dissolution analysis

DasGip DS0700TPSS bioreactor (DasGip, Eppendorf, Germany) with two Rushton impeller rotating at 380 rpm was used in this study. Temperature was maintained at 30 °C. Conductivity, pH and redox potential were measured with a conductivity measuring cell with 5-ring technology with Pt1000 (Metrohm, Switzerland), pH meter (Metrohm, Switzerland), and combined Pt-ring electrode (Metrohm, Switzerland) respectively and controlled with the Tiamo 2.3 software. Changes in particle size distribution were determined using Focused Beam Reflectance Measurement (FBRM) ParticleTrack G400 (Mettler Toledo, USA) and ATR FTIR (Attenuate total reflectance Fourier transformed infrared spectroscopy) was measured with the ReactIR 15 with a DST Fiber Conduit (Mettler Tolledo, USA) controlled with the iC FBRM4.3 (Mettler Tolledo, USA) and iC FTIR4.3 (Mettler Tolledo, USA) software respectively. These software packages were also used for data analysis. Probes were positioned as per manufacturer's instructions. The type of impeller and impeller speed were optimized with a design of experiments study (data not shown). Changes in particle size distribution is measured by FBRM as chord length distributions. A chord length is defined as the length of the path of the laser across a particle. Complete dissolution was defined as standard deviation of FBRM counts <10 μm remaining below twice the standard deviation at 40 min and 55 min for CHO210 and Feed210 respectively. Experiments were performed in triplicates.

2.9. Statistical analysis

All statistical analyses were performed using the Graphpad Prism 6.03 software (GraphPad Software, Inc., USA). The two tailed Mann-Whitney test was used to determine significant differences between physicochemical characters. Amino acids and vitamins were analyzed by a two way ANOVA with a Sidak's multiple comparison test. Cell culture characteristics were compared by determining area under the curve from viable cell density and specific productivity was determined from the slope of titer vs integrated cell viability, corrected for changes in culture volume during the process. Both of these factors were analyzed by two tailed Mann-Whitney tests. The first derivative of the FTIR dissolution profile was also performed with the Graphpad Prism