SSPC, the SFI Research Centre for Pharmaceuticals

The monitoring and optimization of cell culture medium preparation to support bioprocess intensification.

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Background:

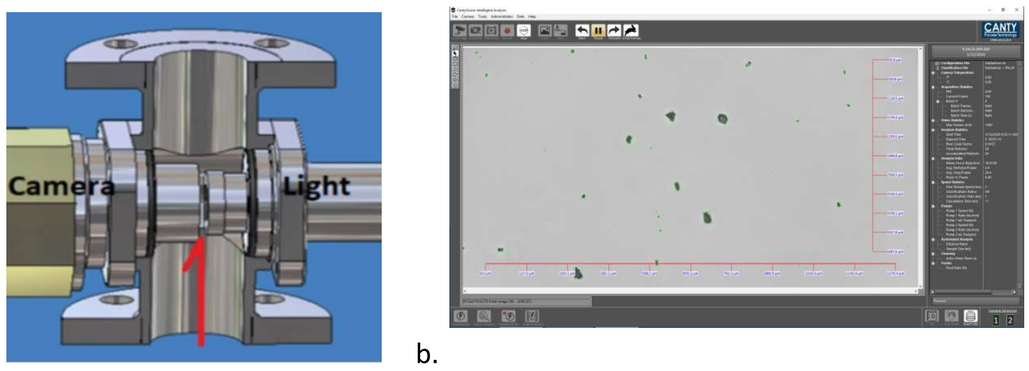
The demands on cell culture medium are ever increasing due to the drive for process intensification. Cell culture medium must supply all nutrients to support cellular growth and protein production. The greater the cell density and product titre in a process, the greater the nutrient demand and the more challenging it is to achieve and maintain dissolution of the required concentrations of nutrients. Cell culture media can be broadly defined as liquid or gel formulations that support cell growth by providing essential components such as growth factors, vitamins, minerals, glucose, and amino acids (O'Flaherty, Bergin et al. 2020). The current market trends in media supply for large-scale biopharmaceutical manufacturing indicate a predominant preference for powdered formats that are then formulated in-house, which account for over 90% of media supplied (Langer and Rader 2014). These multicomponent powdered formats can be often be milled and sometimes granulated. Media preparation is affected by parameters such as mixing time, temperature, pH and addition order of the constituent components. Currently process development relies on trial and error due to the challenges associated with monitoring dissolution endpoint. There is currently no platform of analysis/monitoring for media dissolution.

Objective:

To develop a PAT method using the Canty image analysis system to monitor and determine the endpoint of dissolution in media prep.

Technology:

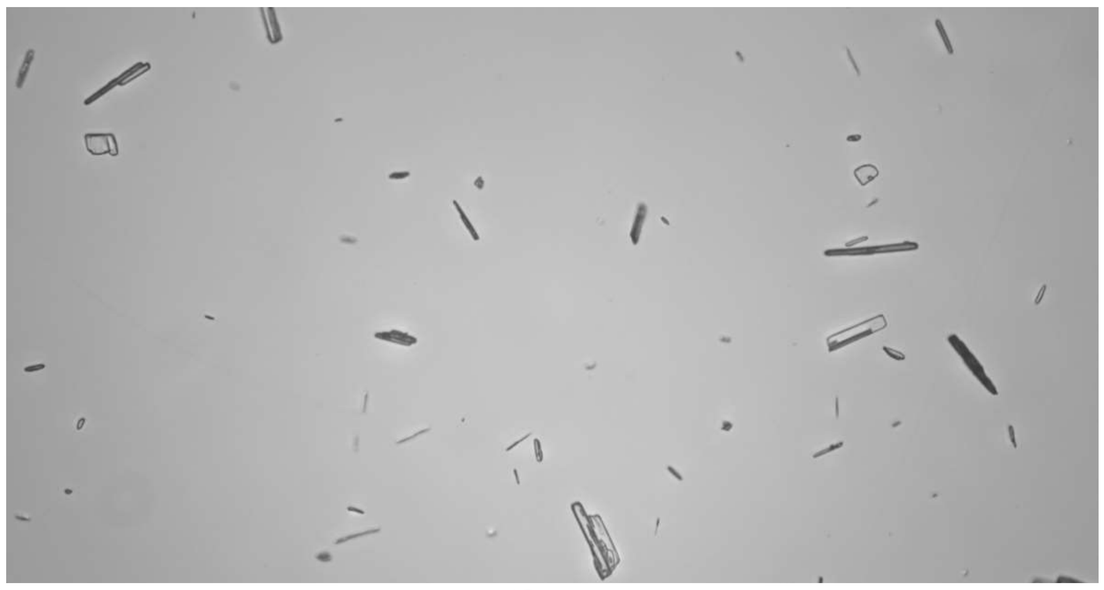
CantyVision Software is able to process images at high-speed real time. The vision system with integral lighting features precision optics designed to enhance the image prior to display or analysis. The analyser utilises a high-resolution image sensor couples with microscopic lens system (J.M.CANTY (2022).



a. b.

Fig.1. (a) Shows the flow cell of the Canty particle sizing system that features a FUSEVIEW glass window that's acts as a product contact barrier. Liquid is pumped through the glass window and the analyser processes the images taken in a certain number of frames. (b) High resolution Canty images of Glutamine with the software bounding box highlighted in green.

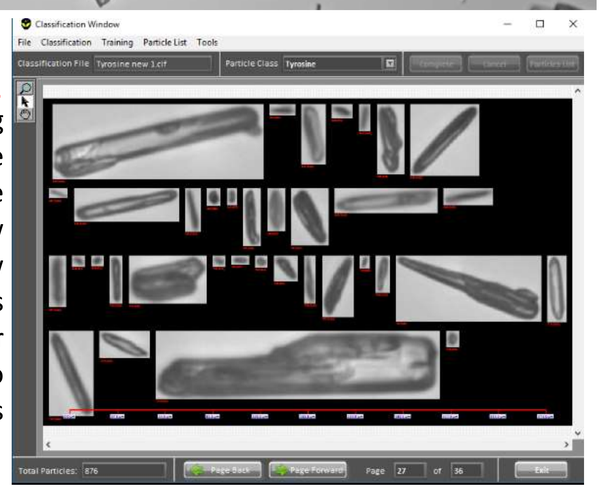
A bounding box is used to measure and calculate multiple dimensions of a particle. Data for particles can be reported using either a batch or incremental update system. Reports on the distribution of particles can be reported using various parameters such as minor or major particle size. The raw data measurements from the analysis system can be reported for all particles measured, including the Dv10 to Dv90 values and moving average.



a.

Particle size, shape & concentration:

Fig.3. (a) Tyrosine and water solution flowing through the Canty flow cell (b) Tyrosine particles selected and classified into one particle class in the classification window system of the Canty. The classification window can be used to select and group particles together with the same characteristics or chosen parameters. It can also be used to exclude certain particles from the results generated.



b.

Tyrosine Dissolution:

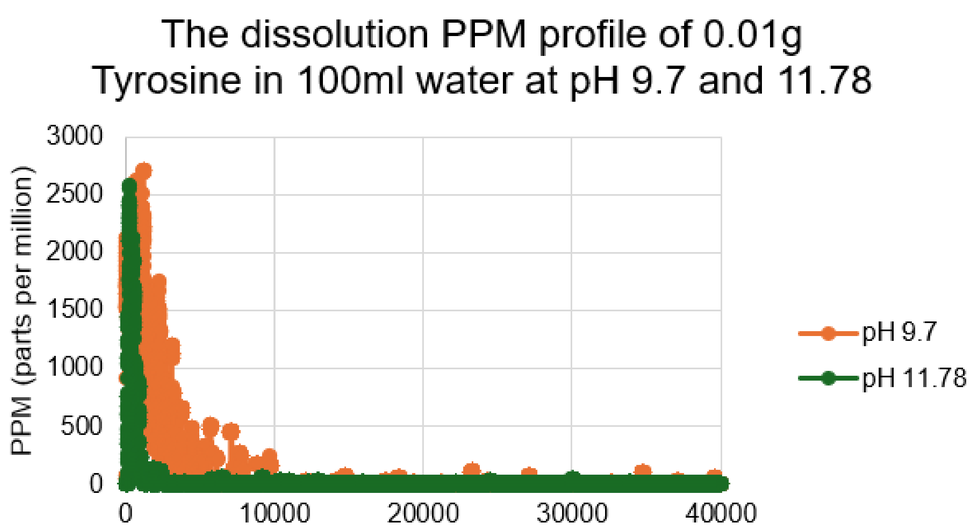


Fig.2. The PPM dissolution profile of 0.01g of Tyrosine in 100ml of water. L-Tyrosine's solubility increases when the pH deviates from its isoelectric point at neutral pH (Hitchcock, D.I. (1924)).

L-Tyrosine, an amino acid crucial for protein synthesis and cellular metabolism, has a solubility of less than 0.453 g/L in water at neutral pH. This limited solubility often becomes a constraint in the preparation of concentrated media essential for bioprocesses. To address the solubility issue, a common strategy has been to utilize separate feeds of L-Tyrosine with extreme pH values. The introduction of feeds with extreme pH can lead to higher salt concentrations in the culture medium, adversely affecting cell health and metabolic activities, potentially leading to suboptimal performance. Overdosing with L-Tyrosine solutions at extreme pH can cause precipitation. This precipitation not only wastes the L-Tyrosine but also can clog feeding lines and disrupt the overall process.

Media preparation:

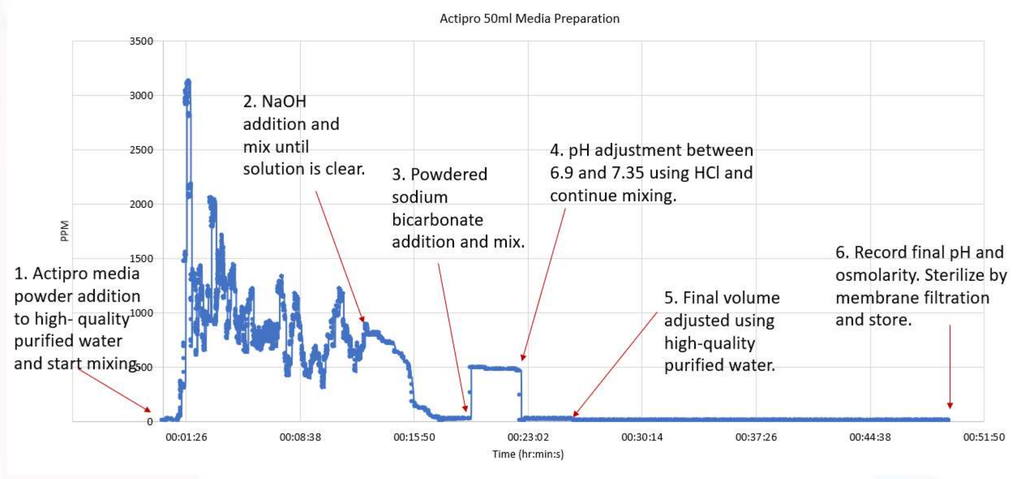
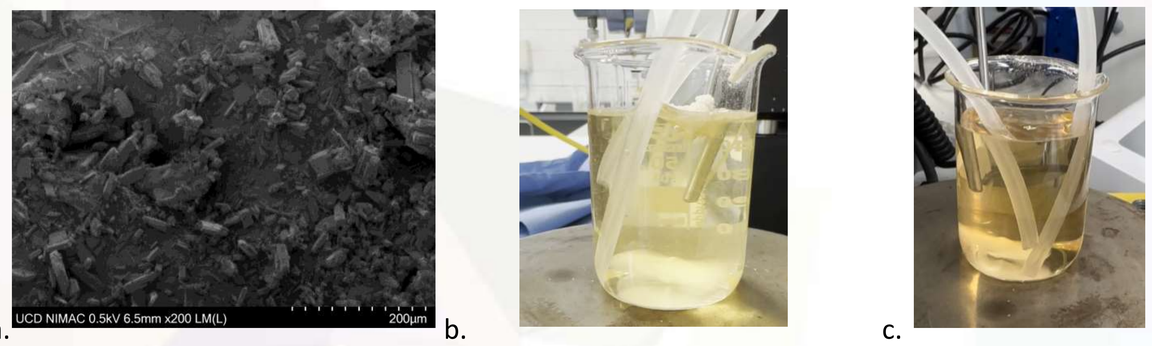


Fig.4. Shows a Canty PPM profile of the basal media preparation stages for one batch of 50ml Actipro media prepared. The stages of addition are labelled on the graph as Media addition, NaOH, Sodium Bicarbonate and HCl.



a. b. c.

Fig.5. Shows images of Actipro media powder. (a) In Scanning Electron Microscope (SEM) image (b) Actipro powder media addition preparation step (c) Media at the end of preparation before membrane filtration and storage.

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References:
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O'Flaherty, R., et al. (2020). "Mammalian cell culture for production of recombinant proteins: A review of the critical steps in their biomanufacturing." Biotechnol Adv **43**: 107552
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