Feed batch cultivation of mammalian cells is widely utilised for the production of mammalian cells. In this work, feed batch processes are used for cGMP production at scales up to 20,000L. In order to meet ever increasing productivity and economic targets, these processes inevitably have to operate at very high cell concentrations, in some cases in excess of 20x10^6 cells/mL. Improving control of process parameters such as pH, osmolality, dissolved oxygen, feeding strategy, all of which are tightly inter-linked, together with feed composition, become vital for improving bioreactor performance and process robustness. Factors considered here are feed composition, feed strategy and optimising pH.

Where highly concentrated complex nutrient feeds are used, problems can arise with the solubility and the chemical compatibility of the feed components. Also the rate of feeding is highly important. In addition to the obvious nutrient depletion resulting from insufficient feeding, excessive feeding of amino acids may result in an increase of osmolality sufficient to inhibit cell growth. These problems have been solved by reformulating the nutrient feeds and adjusting feeding rates prescribed by cell concentration and a pre-determined knowledge of metabolic rates.

Carbon metabolism (but not, to a significant degree, amino acid metabolism) was found to be highly dependent on culture pH. Operating at high pH resulted in rapid cell growth but inefficient cell concentration and a pre-determined knowledge of metabolic rates. Reformulating the nutrient feeds and adjusting feeding rates prescribed by cell concentration and a pre-determined knowledge of metabolic rates. Operating at high pH resulted in rapid cell growth but inefficient cell concentration and a pre-determined knowledge of metabolic rates.

One strategy to inhibit lactate metabolism is to operate at low culture pH, however lowering pH too much below physiological levels will inhibit cell growth. To find a suitable pH level, batch cultures in 10 litre airlift bioreactors were set up with pH control set points ranging from 6.7 to 7.5. The results indicate that lowering pH to 6.8-6.8 did not have any detrimental effect on specific rate of product accumulation or amino acid concentrations (Figures 2.5). Running fed batch cultures at a constant pH of 6.8 compared to 7.0 with a drift to 6.8 resulted in a significant reduction in lactate levels (Figure 5).

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INTRODUCTION
Fed batch mammalian cell culture is a widely successful technique for recombinant protein production and monoclonal antibody production in particular. These cultures can reach cell concentrations in excess of 20x10^6 cells/mL in a 25-35 day culture. Increasing product concentrations (without re-engineering the cell line) could therefore be a matter of increasing cell densities and/or extending culture duration. What sets the limit to this, however, is the build up of carbon metabolism with consequent high lactate accumulation. Lower pH inhibited cell growth but resulted in a lower specific lactate production rate. The best operating pH with low lactate concentration and acceptable cell growth was found to be around 6.80.

Methods
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Implementing these combined improvements led to an extension of culture duration of a GS-CHO model cell line from 15 to 23 days and an increase of harvest titre from 3.1 g/L to 6.5 g/L.

pH CONTROL AND OPTIMISATION
Standard practice in the biopharmaceutical industry is to control pH using a sodium bicarbonate - carbon dioxide system. In aqueous solutions bicarbonate is a weak base, while carbonic acid is a weak acid. Where highly concentrated complex nutrient feeds are used, problems can arise with the solubility and the chemical compatibility of the feed components. Also the rate of feeding is highly important. In addition to the obvious nutrient depletion resulting from insufficient feeding, excessive feeding of amino acids may result in an increase of osmolality sufficient to inhibit cell growth. These problems have been solved by reformulating the nutrient feeds and adjusting feeding rates prescribed by cell concentration and a pre-determined knowledge of metabolic rates.

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Feeding strategies are linked to amino acid consumption rates to try to match the availability of amino acids in solution with amino acid concentrations in the cells. Where amino acid concentrations are high, gluconeogenesis becomes vital for good pH control and the carbon dioxide necessary for this can arise with the solubility and the chemical compatibility of the feed components. Also the rate of feeding is highly important. In addition to the obvious nutrient depletion resulting from insufficient feeding, excessive feeding of amino acids may result in an increase of osmolality sufficient to inhibit cell growth. These problems have been solved by reformulating the nutrient feeds and adjusting feeding rates prescribed by cell concentration and a pre-determined knowledge of metabolic rates.

Another important factor that affects pH control is the rate of amino acid consumption. This can be assessed by measuring the rate of increase in osmolality and the rate of product accumulation. A high rate of product accumulation is indicative of a low rate of amino acid consumption. This is because amino acids are involved in protein synthesis and therefore a low rate of amino acid consumption indicates a low rate of protein synthesis.

Figure 1: Increases in osmolality through increased amino acid concentrations inhibit growth of the investigated GS-CHO cell line.

FEED RATES AND FORMULATION
In mammalian fed batch cell cultures, the feed(s) will have a highly complex formulation due to cellular nutrient requirements, and must be in a very concentrated form due to volume limitation in the bioreactor. The feed components have varying chemical properties and aqueous solubilities. They may also be chemically reactive, forming unwanted chemical compounds with other feed components. Solubilising some feed components requires adding acid or alkali, which makes an undesirable contribution to culture osmolality. Acidic and alkaline feeds also interact with control of culture pH, alkaline feeds in particular elicit addition of carbon dioxide to counteract increases in culture pH, and this carbon dioxide can react with feed bicarbonate to form carbonic acid and create a carbon dioxide pressure that is toxic to cells. Thus, the rate of amino acid consumption must be matched to the rate of carbon dioxide production to maintain a constant culture pH.

A combination of feed reformulation and feed rate adjustment, together with a lower culture pH of 6.80 (previously operated with a pH 7.0 to 6.8 dead band) resulted in an improved process compared to Lonza's original generic GS-CHO process. Lactate levels were significantly reduced, osmolality was maintained at a low level for longer (Figure 5) and high culture viability was maintained for longer (Figure 4). Growth was slightly reduced, but due to a longer culture duration, from 15 to 23 days, and higher specific production rates, the product concentration at harvest was increased more than twofold from 3.1 g/L to 6.8 g/L (Figure 6).

RESULTS
An initial slower growth-rate was seen when operating at a constant pH of 6.8, compared to the previous process operating with a pH 7.0 to 6.8 dead band, but combined with reformulation of feeds and feed strategy, this led to a much extended culture duration and higher viability.

Figure 4: An initial slower growth-rate was seen when operating at a constant pH of 6.8, compared to the previous process operating with a pH 7.0 to 6.8 dead band, but combined with reformulation of feeds and feed strategy, this led to a much extended culture duration and higher viability.

Figure 5: Lactate accumulation is substantially reduced at the constant pH of 6.8. Due to the reduced lactate accumulation, feed reformulation and adjusted feed rates, osmolality was maintained lower for longer. Both cultures exhibited high lactate accumulation in the decline phase.

Figure 6: Harvest product concentration of the modified process, with reformulation of feeds and feed strategy, was increased more than twofold to the original process of 6.8 g/L.

CONCLUSIONS
Culture performance and robustness was shown to be improved by:

- Reformulating nutrient feeding to avoid chemical instability and reducing the need of potentially harmful components.
- Adjusting feed rates by pre-determined knowledge of metabolic rates thus avoiding detrimental osmolality increases caused by excess amino acid concentrations.
- Adjusting pH down to achieve a more effective carbon metabolism and lower lactate accumulation reducing the need for alkali additions for pH control and subsequent later stage high CO2.

These combined modifications increased culture duration from 15 to 23 days and increased harvest product concentration from 3.1 g/L to 6.8 g/L.

ACKNOWLEDGMENTS
CellCulture and ProcessDevelopment and Purification and AssayDevelopment departments at Lonza.

ABSTRACT
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