Low product yield per fermentation or culture is often a particular problem noted at the primary extraction stage; expression of antibody is high within the cells but a high percentage recovery at the primary extraction stage is remarkably difficult to achieve.

A method that partially addresses this latter problem and that permits the production of antibodies acceptable for therapeutic use is described in US 5,655,866. This method involves the use of heat treatment to facilitate the subsequent isolation of functional Fab' fragments of antibodies from non-functional antibodies, the heat treatment being performed at any time during the fermentation or culture, or at any stage during extraction and purification of the antibodies. At elevated temperatures, above room temperature, functional antibodies are remarkably stable, whilst many other proteins including host cell proteins and free light and heavy chain species and non-functional fragments of antibodies, form precipitates and/or aggregates which are easily separated from functional antibody during primary purification procedures such as filtration or centrifugation or fluidised bed chromatography. The cell extracts were prepared in the method described in US 5,655,866 by incubating the intact cells in Tris HCl buffer 100mM pH 7.4 containing EDTA 10mM.

WO2006/054063 describes an increase in the yield of functional antibody at the primary extraction stage by the inclusion of a non-lysing treatment in combination with heat treatment. This method teaches that after centrifugation the cell pellets were resuspended in a sample comprising 1M Tris, pH 7.4 containing 100mM EDTA followed by non-lysing treatment and then heat treatment.

WO2005/019466 describes an increase in yield of recombinant proteins by the inclusion of an interruption step under defined conditions of temperature and pH after fermentation but prior to downstream processing including extraction.

Summary ~~of the Invention~~

This ~~invention~~ disclosure ~~described~~provided herein is based on the surprising and unexpected observation that after a host cell sample transformed with an expression vector encoding a recombinant antibody molecule has been cultured an increase in the pH of the resulting sample during the primary recovery process has a significant beneficial impact on the yield of antibody.

Whilst the antibody may start at a pH in the range 6 - 9 before processing, such as heating, surprisingly even when buffered the pH drops, probably as a result of cell metabolism. The present inventors now believe that this is detrimental to the

yield/recovery and have proposed to address this by, where appropriate, adjusting the pH of the material before and/or during processing to ensure that the pH stays within the target range.

It has been surprisingly found that the pH of the sample prior to a heat treatment step has a considerable affect on the yield of antibody from the cell sample. It has been found that adjusting the pH of the sample such that the pH of the sample is pH 6 to 9 prior to the heat treatment step provides an increase in the yield of antibody of up to 40%. This enables hugely beneficial savings in time and cost of production of quantities of functional antibodies of therapeutic quality. Indeed other steps often used to increase yield, such as homogenisation and hold steps, may no longer required to achieve high levels of antibody yield.

In methods used previously, for example in US 5,655,866, cell extracts were prepared by incubating the intact cells in a buffer having a pH of 7.4. It has been found that despite the addition of a buffer which would be expected to maintain the pH of the sample at a constant level, the pH of the cell sample in fact drops over time. In certain circumstances, such as over long periods of time following addition of a buffer, the pH of the sample has been found to be as low as pH 5.5 prior to the heat treatment step. It has been found that detecting and optionally adjusting the pH prior to heat treatment to ensure that the pH of the sample is 6 to 9 results in a surprising increase in the yield of antibody.

Whilst not wishing to be bound by theory, it is thought that it is important to maintain pH in the range 6 to 9 during the processing step, such as heat treatment. Adjusting the pH prior to processing (such as a heat treatment step) helps to maintain the pH in the right range. Therefore in one ~~aspect~~embodiment there is provided an antibody extraction step wherein the pH is maintained substantially in the range 6 to 9, for substantially the duration of the process.

Without being bound by theory it is thought that the methods provided by the present invention allow the recovery of recombinant protein from the periplasm during primary isolation which is not released under standard extraction conditions.

According to one aspect, there is provided a method for the manufacture of recombinant antibody molecules comprising (a) culturing a host cell sample of gram-negative bacteria transformed with an expression vector encoding a recombinant antibody molecule; (b) adding an extraction buffer having a pH of 7.5 to 9 to the sample; and (c) subjecting the sample to a heat treatment step within a range of 40°C to 70°C for 1 to 24

hours; wherein the pH of the sample is detected after addition of the extraction buffer and adjusted to ensure that the pH of the sample is 7 to 9 prior to the heat treatment step and wherein the antibody molecule is selected from the group consisting of VH, VL, VHH, Fab, modified Fab, an altered hinge Fab, Fab', $F(ab')_2$ or Fv fragment; a light chain or heavy chain monomer or dimer; a single chain antibody, a single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker, and a Fab-dAb.

~~Accordingly, in~~ In one ~~a first aspect of the present invention~~ embodiment, there is provided a method for the manufacture of recombinant antibody molecules comprising culturing a host cell sample transformed with an expression vector encoding a recombinant antibody molecule; adding an extraction buffer to the sample; and subjecting the sample to a heat treatment step; wherein the pH of the sample is detected after addition of the extraction buffer, and

optionally adjusted, to ensure that the pH of the sample is 6 to 9 prior to the heat treatment step.

Monitoring of the pH at this stage is essential for establishing control over the pH.

In an alternative aspect there is provided a method of for extraction of recombinant antibody molecules from a host cell sample transformed with an expression vector encoding a recombinant antibody molecule; comprising the steps of:

adjusting the pH of a composition of said cells to be in the range 6 to 9, such that the pH is maintained in the range during a subsequent extraction step,

subjecting the cells to an extraction step, such as a heat treatment step,

wherein the pH is monitored at least at one time point immediately before and/or during the extraction step.

It has also been found that an increase in the pH of the extraction buffer provides a surprising increase in the yield of antibody after the sample is subjected to a heat treatment step.

Accordingly, in another ~~a second aspect of the present invention~~ embodiment, there is provided a method for the manufacture of recombinant antibody molecules comprising culturing a host cell sample transformed with an expression vector encoding a recombinant antibody molecule; adding an extraction buffer to the sample having a pH of 7.5 to 9.0; and subjecting the sample to a heat treatment step.

Detailed Description of the Invention

Antibody molecule as employed herein is intended to refer to a whole antibody or a binding fragment thereof, in particular a whole antibody or a Fab fragment.

In the first aspect of the present invention, the sample has or is adjusted to have a pH of 6 to 9 prior to the heat treatment step.

In a preferred embodiment the sample has a pH of 6.5 to 8.5, pH 6.5 to 8.0, pH 7.0 to 9.0, pH 7.0 to 8.5, pH 7.0 to 8.0, pH 7.1 to 8.0, pH 7.5 to 8.0, pH 7.0 to 7.8, pH 7.1 to 7.8, pH 7.1 to 7.7, pH 7.2 to 7.6, pH 7.3 to 7.5, pH 7.1, pH 7.2, pH 7.3, pH 7.4, pH 7.5, pH 7.6, pH 7.7, pH 7.8 or pH 7.9, such as pH 7.4, in particular pH 6.8 prior to the heat treatment step.

The pH measurement referred to herein are generally normalised to 20 degees C.