

PREPARATION SYSTEM

CROSS-REFERENCE TO RELATED APPLICATION

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This application claims priority from and the benefit of Singapore patent application No. 10202403509Y, which was filed on 11 November 2024. The entire content of this application is incorporated herein by reference.

10 FIELD OF THE INVENTION

The present invention relates generally to an automated sample preparation system or method for preparing a sample containing polypeptides, such as one or more proteins, for an analysis such as by mass spectrometry.

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BACKGROUND

Various techniques for preparing samples containing proteins for analysis are known. Such techniques typically involve steps such as diluting the sample and mixing the sample with various different agents. These sample preparation steps are relatively laborious and time consuming for laboratory workers.

SUMMARY

25 From a first aspect the present invention provides an automated sample processing system for preparing a sample comprising polypeptides for analysis, comprising: a first valve (e.g. Valve 5) having a plurality of ports connected to a plurality of respective fluid lines, and configured such that a fluid path through the first valve is switchable between different pairs of its ports; a second valve (e.g. Valve 3) having a plurality of ports
30 connected to a plurality of respective fluid lines, and configured such that a fluid path through the second valve is switchable between different pairs of its ports, where a port of the first valve is in fluid communication with a port of the second valve; a third valve (e.g. Valve 1) having a plurality of ports connected to a plurality of respective fluid lines, and configured such that a fluid path through the third valve is switchable between different
35 pairs of its ports, where a port of the third valve is in fluid communication with a port of the first valve or a port of the second valve; a first fluid holding coil (e.g. holding coil 32) connected to a port of the second valve; a second fluid holding coil (e.g. holding coil 5) connected to a port of the third valve; and at least one pump for pumping fluid through the system.

40 The references to Valve 0, Valve 1, Valve 2, Valve 3, Valve 4, Valve 5, Valve 6, Valve 7, holding coil 32, and holding coil 5 in this Summary section refer to the components having these names in the Detailed Description section.

As the system comprises two fluid holding coils, it is able to prepare a first sample for analysis by adding one or more agents to it, when the first sample is in the first holding coil, whilst simultaneously holding a second sample in the second holding coil. After the first sample has been processed for analysis, the system may pump the second sample from the second holding coil to the first holding coil so as to be prepared for analysis in the same manner, or in a different manner, to the way in which the first sample was prepared for analysis. Alternatively, the system is able to prepare a first portion of a first sample for analysis by adding one or more agents to it, when the first portion is in the first holding coil, whilst simultaneously holding a second portion of the first sample in the second holding coil. After the first portion of the first sample has been processed for analysis, the system may pump the second portion of the first sample from the second holding coil to the first holding coil so as to be prepared for analysis in the same manner, or in a different manner, to the way in which the first portion of the first sample was prepared for analysis.

The system may be configured to perform one or more processing step on the sample, such as pumping it through a separation column such as a purification column and/or adding one or more of the agents such as those described herein, and to then separate the sample into different portions. The system may be configured to pump one portion of the sample to a fluid holding coil while another of the portions of the sample is processed further by the system, e.g. by adding one or more agents such as those described herein. The one or more agents may be supplied to said another portion of the sample whilst it is in a different fluid holding coil. The system may be configured to then further process said one portion of the sample, in the same way or in a different way to said another portion of the sample. For example, the system may add one or more agent to said another portion of the sample that is different to the agent(s) added to said one portion.

The system may comprise a liquid chromatography separator for separating a sample processed by the system and/or a mass spectrometer for mass analysing a sample processed by the system.

For example, the system may comprise a liquid chromatography separator arranged to separate a sample before it is provided to the mass spectrometer and mass analysed.

As described above, a port of the first valve is in fluid communication with a port of the second valve. These ports may be directly connected to each other by a fluid line.

The system may comprise control circuitry configured to automatically control the first, second and third valves and the at least one pump so as to: pump a first portion of a first sample comprising polypeptides into the system and into the first fluid holding coil; introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil; and pump a second portion of the first sample into the second fluid holding coil, whilst the first portion of the sample is in the system.

Alternatively, the system may comprise control circuitry configured to automatically control the first, second and third valves and the at least one pump so as to: pump a first sample comprising polypeptides into the system and into the first fluid holding coil; introduce at least a first agent for preparing the first sample for analysis into the system via

a port of the first or second valve, and transmit it to the first fluid holding coil; and pump a second sample comprising polypeptides into the system and into the second fluid holding coil, whilst the first sample is in the system.

Alternatively, the system may comprise control circuitry configured to automatically control the first, second and third valves and the at least one pump so as to: pump a first sample, or a first portion of a first sample, comprising polypeptides into the system and into the first fluid holding coil; and introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil.

The control circuitry of the system described herein may be configured to automatically control the valves and the at least one pump so as to introduce a second, different agent for preparing the first sample for analysis, or first portion of the first sample, into the system, and transmit it to the first fluid holding coil.

The control circuitry may be configured to automatically control the valves and the at least one pump so as to either: (i) pump the first portion of the first sample out of the first fluid holding coil; then to pump the second portion of the first sample from the second fluid holding coil to the first fluid holding coil; and then to introduce at least one agent for preparing the second portion of the sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil to meet the second portion of the sample; or (ii) pump the first sample out of the first fluid holding coil; then to pump the second sample from the second fluid holding coil to the first fluid holding coil; and then to introduce at least one agent for preparing the second sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil to meet the second sample.

In the embodiments in which first and second portions of the first sample are pumped to the first fluid holding coil, the system may automatically prepare the second portion of the sample for analysis in the same manner as the first portion of the sample, i.e. the at least one agent that is introduced to the second portion of the sample may be the same agent or agents that was or were introduced to the first portion of the sample.

Alternatively, the system may automatically prepare the second portion of the sample for analysis in a different manner to the first portion of the sample, i.e. one or more different agent may be introduced to the second portion of the sample that was not introduced to the first portion of the sample.

Alternatively, in the embodiments in which first and second samples are pumped to the first fluid holding coil, the system may automatically prepare the second sample for analysis in the same manner as the first sample, i.e. the at least one agent that is introduced to the second sample may be the same agent or agents that was or were introduced to the first sample. Alternatively, the system may automatically prepare the second sample for analysis in a different manner to the first sample, i.e. one or more different agent may be introduced to the second sample that was not introduced to the first sample.

It is contemplated that the system may automatically add one or more agent to the second portion of the sample, or to the second sample, whilst it is in the second fluid holding coil.

5 The system may be configured to transmit the first portion of the sample (or first sample) and first agent to the first fluid holding coil such that they meet there, and/or such that the first portion of the sample (or first sample) and second agent are transmitted to the first fluid holding coil such that they meet there.

10 The first fluid holding coil may be connected to a port of the second valve. In such embodiments the control circuitry is configured to automatically introduce the first agent into the system and to the first fluid holding coil by controlling the first valve such that: said fluid path through the first valve is between one of its port that is connected to a source of the first agent and the port that is connected to the second valve; and such that said fluid path through the second valve is between its port that is connected to the first valve and its port that is connected to the first fluid holding coil. Alternatively, the control circuitry may be configured to automatically introduce the first agent into the system and to the first fluid holding coil by controlling the second valve such that said fluid path through the second valve is between one of its port that is connected to a source of the first agent and its port that is connected to the first fluid holding coil. Similarly, the control circuitry may be configured to automatically introduce the second agent into the system and to the first fluid holding coil by controlling the first valve such that: said fluid path through the first valve is between one of its port that is connected to a source of the second agent and the port that is connected to the second valve; and such that said fluid path through the second valve is between its port that is connected to the first valve and its port that is connected to the first fluid holding coil. Alternatively, the control circuitry may be configured to automatically introduce the second agent into the system and to the first fluid holding coil by controlling the second valve such that said fluid path through the second valve is between one of its port that is connected to a source of the second agent and its port that is connected to the first fluid holding coil.

30 The control circuitry may be configured to automatically control the first and second valves and the at least one pump so as to transmit the first agent to the first fluid holding coil such that it meets the first portion of the sample (or the first sample) before the second agent is transmitted to the first holding coil such that it meets the first portion of the sample (or the first sample), or vice versa.

35 The control circuitry may be configured to automatically control the first and/or second valves and the at least one pump so as to: i) load the first agent and first portion of the sample, or first sample, into the first fluid holding coil such that the first agent is sandwiched between volumes of sample in the first fluid holding coil, or such that the sample is sandwiched between volumes of the first agent, and then control the at least one pump so as to move the sandwiched first agent and sample along one or more fluid lines within the system so as to cause the first agent and sample to mix together; and/or ii) load the second agent and first portion of the sample, or first sample, into the first fluid holding coil such that the second agent is sandwiched between volumes of sample in the first fluid holding coil, or such that the sample is sandwiched between volumes of the second agent,

and then control the at least one pump so as to move the sandwiched second agent and sample along one or more fluid lines within the system so as to cause the second agent and sample to mix together.

For example, the control circuitry may be configured to automatically control the first
5 and/or second valves and the at least one pump so as to load a first volume of sample into the first fluid holding coil, and then to load the first agent into the first fluid holding coil, and then to load a second volume of the sample into the first fluid holding coil such that the first agent is sandwiched between the first and second volumes of the sample; and to control
10 the at least one pump so as to move the first agent sandwiched between the first and second volumes of the sample along one or more fluid lines within the system so as to cause the first agent and volumes of the sample to mix together. Additionally, or alternatively, the control circuitry may be configured to automatically control the first and/or second valves and the at least one pump so as to load a first volume of the sample into the first fluid holding coil, and then to load the second agent into the first fluid holding coil, and
15 then to load a second volume of the sample into the first fluid holding coil such that the second agent is sandwiched between the first and second volumes of the sample; and to control the at least one pump so as to move the second agent sandwiched between the first and second volumes of the sample along one or more fluid lines within the system so cause the second agent and portions of the sample to mix together.

20 The control circuitry may be configured to control the at least one pump so as to move the sandwiched first agent and sample back and forth within the first fluid holding coil so as to cause the first agent and sample to mix together. Additionally, or alternatively, the control circuitry may be configured to control the at least one pump so as to move the sandwiched second agent and sample back and forth within the first fluid holding coil so as
25 to cause the first agent and sample to mix together.

Alternatively, the control circuitry may be configured to automatically control the at least one pump and the first and/or second valve so as to: (i) move the sandwiched first agent and sample from the first fluid holding coil to another fluid holding coil in the system so as to cause the first agent and sample to mix together; and optionally to then return the
30 sample to the first fluid holding coil; and/or (ii) move the sandwiched second agent and sample from the first fluid holding coil to another fluid holding coil in the system so as to cause the second agent and sample to mix together; and optionally to then return the first sample to the first fluid holding coil.

Said another fluid holding coil may be a fluid holding coil connected to a port of the
35 first or second valve.

Said one or more fluid lines may comprise a degasser configured to remove air from the sandwiched first agent and sample, and/or from the sandwiched second agent and sample, as it is transmitted along said one or more fluid lines so as to cause the agent and sample to mix together.

40 Any air bubbles that are present in the one or more fluid lines may prevent the agent and sample mixing together. The degasser removes such air bubbles so as to enhance the mixing. The degasser may be a portion of a fluid line that is gas permeable

but liquid impermeable, e.g. a portion having apertures such as pores or slits, so as to let air out of the fluid line but not liquid.

5 The first valve may comprise one or more further ports connected to one or more respective further fluid lines for transmitting fluid therethrough so that the fluid enters or leaves the sample processing system at or from the first valve; and/or the second valve may comprise one or more additional ports connected to one or more respective additional fluid lines for transmitting fluid therethrough so that the fluid enters or leaves the sample processing system at or from the second valve.

10 A source of a, or the, first agent may be connected to a port of the first and/or second valve by a fluid line, wherein the source of the first agent is a source of denaturing agent for denaturing the polypeptides.

A source of a, or the, second agent may be connected to a port of the first and/or second valve by a fluid line, wherein the source of the second agent is a digestion agent, such as an enzyme, for digesting the polypeptides.

15 A source of a quenching agent for inhibiting a digestion agent from digesting the polypeptides may be connected to a port of the first and/or second valve by a fluid line; and the control circuitry may be configured to automatically control the first and/or second valves and the at least one pump so as to pump the quenching agent from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of a sample or first sample is located in the first fluid holding coil.

20 A source of a neutralising agent for neutralising the pH of a sample may be connected to a port of the first and/or second valve by a fluid line; and the control circuitry may be configured to automatically control the first and/or second valve and the at least one pump so as to pump the neutralising agent from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of a sample or first sample is located in the first fluid holding coil.

25 A source of a reducing agent for breaking disulphide bonds of the polypeptides may be connected to a port of the first and/or second valve by a fluid line; and the control circuitry may be configured to automatically control the first and/or second valves and the at least one pump so as to pump the reducing agent from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of a sample or the first sample is located in the first fluid holding coil.

30 A source of an alkylating agent for preventing disulphide bonds of the polypeptides reforming may be connected to a port of the first and/or second valve by a fluid line; and the control circuitry may be configured to automatically control the first and/or second valves and the at least one pump so as to pump the alkylating agent from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of a sample or first sample is located in the first fluid holding coil.

35 The system may be configured to pump each of the various agents to the first portion of the sample, or to the first sample, individually, e.g. so as to meet the sample in the first holding coil. Alternatively, the system may be configured to pump multiple ones of the various agents into the system and to mix them prior to supplying the resulting mixture to the sample.

It is contemplated that rather than having a separate source for each of the agents mentioned herein, multiple ones of the agents may be provided as a mixture from a single source. This increases the speed at which the sample can be processed and therefore increases the sample throughput through the system. For example, a source containing a mixture of a neutralising agent for neutralising the pH of a sample and a reducing agent for breaking disulphide bonds of the polypeptides and/or a denaturing agent for denaturing the polypeptides may be connected to a port of the first and/or second valve by a fluid line; and the control circuitry may be configured to automatically control the first and/or second valves and the at least one pump so as to pump the mixture from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of the sample or first sample is located in the first fluid holding coil.

The control circuitry may be configured to control the valves and the at least one pump so as to cause any one of the agents, or the mixture of agents, to be mixed with the first portion of the sample or first sample in a manner corresponding to the mixing procedure described above, e.g. by moving the sandwiched agent and sample from the first fluid holding coil to another fluid holding coil so as to cause the agent and sample to mix together.

As described above, after the various agent(s) has been mixed with the first portion of the sample or first sample, the system may move the first portion of the sample or first sample out of the first fluid holding coil and move the second portion of the sample or second sample from the second fluid holding coil to the first fluid holding coil. The second portion of the sample or second sample may then be prepared in the same way as is described herein in relation the first portion of the sample or first sample. Alternatively, the second portion of the sample or second sample may be prepared in a different way, e.g. by adding one or more agent that is different to the agent(s) added to the first portion of the sample or first sample.

The system may comprise a reaction coil having a heater coupled thereto, connected via a fluid line to a port of one of the first or second valves; and the control circuitry may be configured to automatically control the heater to heat the reaction coil and control the valves and the at least one pump so as to pump the sample to the heated reaction coil.

In the embodiments that process multiple portions of a sample or multiple different samples in overlapping timescales, one or more additional reaction coil may be provided so that the different portions or different samples can be sent to different reaction coils. Additionally, or alternatively, one or more additional pump may be provided so that the different portions or different samples can be pumped, e.g. to their respective reaction coils.

The first valve may comprise between 4 and 15 ports that are connected to respective fluid lines; and/or the second valve may comprise between 4 and 15 ports that are connected to respective fluid lines.

Additionally, or alternatively, the third valve may comprise between 4 and 15 ports that are connected to respective fluid lines.

For example, the first valve and/or the second valve and/or third valve may each comprise x ports that are connected to a respective x fluid lines, where $x \leq 15, \leq 14, \leq 13, \leq 12, \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 . Additionally, or alternatively, each of the first and/or the second and/or the third valves may have at least 5 ports, at least 6 ports, at least 7 ports or
5 at least 8 ports that are connected to respective fluid lines.

Each of the first and/or second and/or third valves may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

Providing the valve with such relatively few ports connected to fluid lines helps
10 ensure that fluid does not leak from the ports under pressure. This is particularly beneficial when a separation column is connected to one of the ports, since fluid is then pumped through the valve to the separation column at relatively high pressure. The relatively low number of ports connected to fluid lines is also beneficial if the valve is located in a refrigerated compartment with one or more sources of fluid to be introduced into one or
15 more ports of the valve, as it enables the valve to be smaller and have fewer connections to the ports.

The system may comprise a desalting column connected, via a fluid line, to a port of one of the first or second valves; and the control circuitry may be configured to perform a first routine in which it automatically controls the valves and the at least one pump so as to
20 pump the sample through the desalting column so as to desalt the sample. Alternatively, or additionally, the system may comprise a source of diluent for diluting the sample; wherein the control circuitry is configured to perform a second routine in which it automatically controls the valves and the at least one pump so as to pump the source of diluent to the sample so as to dilute salts in the sample.

25 In the first routine the control circuitry may be configured to pump the sample from the first holding coil through the desalting column.

The system may comprise a user interface configured to enable a user to select which of the first or second routines the system is to operate; and the system may be configured to perform the selected first or second routine in response thereto.

30 The system may comprise a refrigeration compartment, and also a liquid chromatography separator for separating a sample processed by the system and/or a mass spectrometer for mass analysing a sample processed by the system; wherein the system is configured to pump a portion of the sample processed by the system to the liquid chromatography separator and/or a mass spectrometer, and to pump at least part of the
35 remainder of the sample processed by the system to the refrigeration compartment.

The system may include a storage vessel in the refrigeration compartment and the system may be configured to pump said at least part of the remainder of the sample to the storage vessel.

40 In the system disclosed herein, one of the first and second valves may be located in a refrigeration compartment; and optionally the other of the first or second valves may be located outside of the refrigeration compartment. This refrigeration compartment may be the same one as that mentioned above, i.e. to which said remainder of the sample is

pumped, or it may be a different refrigeration compartment. The apparatus may be configured such that said other of the first or second valves is at ambient temperature.

5 The valve located in the refrigeration compartment may comprise at least one port connected to at least one respective fluid line for transmitting fluid from at least one source of fluid in the refrigerated compartment into said at least one port; wherein each of said at least one fluid lines has a length of ≤ 20 cm. For example, each of said at least one fluid lines may have a length of ≤ 15 cm or ≤ 10 cm.

The at least one fluid lines may have a relatively small internal diameter, such as ≤ 0.3 mm or ≤ 0.4 mm.

10 Providing the at least one fluid line with such relatively small lengths and diameters is advantageous as the volume of the fluid in the fluid line is relatively low. This is beneficial, for example, because the at least one source of fluid may be an expensive agent and providing a fluid line with such a relatively low volume enables only a relatively small volume of the agent to be used to prime the fluid line. As the agent(s) may be
15 required to be replaced every few days it is important to reduce wastage where possible.

The apparatus may comprise said at least one source of fluid located in the refrigerated compartment.

20 The at least one source of fluid in the refrigerated compartment may be at least one of: a reducing agent for breaking disulphide bonds of the polypeptides; an alkylating agent for preventing disulphide bonds of the polypeptides combining; and a digestion agent, such as an enzyme, for digesting the polypeptides. Any two of the above sources, or all three of the above sources may be located in the refrigerated compartment.

25 One or more sources of the following agents may be connected to a port of said other of the first and second valves: a source of denaturing agent for denaturing the polypeptides; a neutralising agent for neutralising the pH of the sample; and a quenching agent for inhibiting a digestion agent from digesting the polypeptides. This one or more sources may be located outside of the refrigerated compartment.

30 Although one of the first and second valves and said at least one source of fluid have been described as being located in a refrigeration compartment, it is contemplated that the valve may be located outside of the refrigeration compartment. In such embodiments the portion of the fluid line from each source of fluid (i.e. source of agent) to the valve that extends outside of the refrigeration compartment preferably has a low volume. This minimises the heating of the agent in the fluid line, which is beneficial where the agents are temperature dependent. For example, the portion of the fluid line that
35 extends outside of the refrigeration compartment may have a length of ≤ 20 cm, and preferably ≤ 15 cm or ≤ 10 cm. The internal diameter is preferably ≤ 0.3 mm or ≤ 0.4 mm.

40 The third valve (e.g. Valve 1) may be in fluid communication with a port of the first valve, and the system may comprises a fourth valve (e.g. Valve 6) in fluid communication with a different port of the first valve, wherein the third and fourth valves are in fluid communication in a manner such that fluid can pass from the third valve to the fourth valve whilst bypassing the first and second valves. The system may comprise a mass spectrometer in fluid communication with the fourth valve. The control system may be configured to operate in a first mode in which it automatically controls the third valve, the

first valve, optionally the second valve, and the at least one pump so as to pump a first portion of the sample, or a first sample, into the first fluid holding coil for a first agent to be added thereto; and at a subsequent time to control these valves and the fourth valve so that this first portion of the sample or the first sample is pumped to the mass spectrometer.

5 Alternatively, or additionally, the control system may also be configured to operate in a second, different mode in which it automatically controls the third and fourth valves and the at least one pump so as to pump a different portion of the sample or a different sample to the fourth valve and then to the mass spectrometer whilst bypassing the first and second valves and the first holding coil.

10 In the second mode sample therefore does not pass to the first and second valves or into the first holding coil and does not have the first and second agents added to it.

The fourth valve may comprise at least 4 ports that are connected to respective fluid lines. For example, the fourth valve may comprise x ports that are connected to a respective x fluid lines, where $x \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 .

15 The fourth valve may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

The system may comprise a sensor for determining the concentration of a protein or polypeptide in the first portion of the sample, or the first sample, passing through a fluid line between the third valve and the first valve, in the first mode, and producing a signal
20 representative of the determined concentration. The control system may be configured to automatically perform a third routine that controls the amount of a first agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil for mixing with the sample based on said signal representative of the determined concentration, and/or control the amount of a second agent introduced into the
25 system via a port of the first or second valve and transmitted to the first fluid holding coil for mixing with the sample based on said signal. Alternatively, or additionally, the control system may be configured to perform a fourth routine that controls the amount of a first agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil so as to be a first pre-determined amount, and controls the
30 volume of the sample transmitted to the first fluid holding coil for mixing with the first agent based on said signal representative of the determined concentration and based on said first pre-determined amount.

The sensor may be a spectroscopic sensor such an ultra-violet spectroscope.

35 The system may comprise a user interface configured to enable a user to select which of the third or fourth routines the system is to operate; wherein the system is configured to perform the selected third or fourth routine in response thereto.

The system may comprise a separation column, such as a purification column, between the third valve and the sensor for separating the protein or polypeptide from other components in the sample such that the protein or polypeptide arrives at the sensor
40 separated from the other components.

A source of binding buffer may be connected to a port of the third valve, and the system may be configured to automatically prepare the separation column, prior to

pumping sample to the separation column, by controlling the third valve and the one or more pump so as to pump the binding buffer from its source to the separation column.

A source of elution buffer may be connected to a port of the third valve, and the control circuitry may be configured to cause the sample to elute from the separation column to the sensor, after the sample has been supplied to the separation column, by controlling the third valve and the one or more pump so as to pump the elution buffer to the separation column.

The system may comprise a fifth valve (e.g. Valve 2) between the sensor and the first valve, the fifth valve having a port connected to the sensor by a fluid line, a port connected to a fluid line for supplying sample to the first valve, a port connected to the third valve by a fluid line that bypasses the sensor, ports connected to a first sample loop, and ports connected to a second sample loop. The control circuitry may be configured, in the first mode, to control the fifth valve and the at least one pump such that sample is pumped from the sensor into the first sample loop and such that, in a further mode, sample is pumped from the third valve into the second sample loop through the fluid line that bypasses the sensor.

The system may comprise a user interface configured to enable a user to select which of the first or further modes the system is to operate, wherein the system is configured to perform the selected first or further mode in response thereto.

When the system operates in the first mode then it may subsequently control the fifth valve and pump so as to pump sample from the first sample loop to the first valve. In contrast, when the system operates in the further mode then it may subsequently control the fifth valve and pump so as to pump sample from the second sample loop to the first valve.

The control circuitry may be configured to control the valves and at least one pump such that the sample in the first sample loop is processed as described herein, i.e. by mixing with the first and second agents etc., and then the sample in the second sample loop is processed in the same manner or in a different manner.

The fifth valve may comprises between 4 and 15 ports that are connected to respective fluid lines. For example, the fifth valve may comprise x ports that are connected to a respective x fluid lines, where $x \leq 15, \leq 14, \leq 13, \leq 12, \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 . Additionally, or alternatively, the fifth valve may have at least 5 ports, at least 6 ports, at least 7 ports or at least 8 ports that are connected to respective fluid lines.

The fifth valve may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

The system may comprise a source of diluent connected to the fluid line between the third and fourth valves and a pump for pumping the diluent; and the control circuitry may be configured, in the second mode, to control the pump so as to pump diluent into the fluid line between the third and fourth valves at the time that the sample is passing therethrough.

The fluid line into which the diluent is pumped whilst the sample is passing therethrough may have a relatively large internal diameter, such as ≥ 0.4 mm or ≥ 0.5 mm, such that the back pressure in the fluid line is relatively low.

The system may comprise a sixth valve (e.g. Valve 7) having a port connected to the fourth valve for receiving sample from the fourth valve, ports connected to a first sample loop, ports connected to a second, different sample loop, and a port in fluid communication with a mass spectrometer by a fluid line. The control circuitry may be configured to control the sixth valve and the at least one pump such that sample is pumped from the fourth valve into one of the first or second sample loop that is connected to the sixth valve.

The sixth valve may comprise between 4 and 15 ports that are connected to respective fluid lines. For example, the sixth valve may comprise x ports that are connected to a respective x fluid lines, where $x \leq 15$, $x \leq 14$, $x \leq 13$, $x \leq 12$, $x \leq 10$, $x \leq 9$, $x \leq 8$, $x \leq 7$, $x \leq 6$, or $x \leq 5$. Additionally, or alternatively, the sixth valve may have at least 5 ports, at least 6 ports, at least 7 ports or at least 8 ports that are connected to respective fluid lines.

The sixth valve may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

The first and second sample loops may have different sample receiving volumes, and the system may comprise a user interface configured to enable a user to select a method of processing or analysing the sample from a plurality of different methods, wherein the control system is configured to selectively supply the sample to either the first or the second sample loop in response to the selected method.

Alternatively, the system may be configured to control the fourth and sixth valves such that sample is supplied from the fourth valve into the first sample loop connected to the sixth valve whilst sample is pumped from the second sample loop connected to the sixth valve through the port in fluid communication with the mass spectrometer. Optionally, the system is configured to control the fourth and sixth valves such that sample is supplied from the fourth valve into the second sample loop connected to the sixth valve whilst sample is pumped from the first sample loop connected to the sixth valve through the port in fluid communication with the mass spectrometer.

The system described herein may comprise a mass spectrometer for mass analysing the sample, and the control circuitry may be configured to control the valves and at least one pump so as to pump the sample to the mass spectrometer. The system may comprise a liquid chromatography separator upstream of the mass spectrometer.

Accordingly, a liquid chromatography separator may be provided in the fluid line between the mass spectrometer and the port of the sixth valve that is in fluid communication with the mass spectrometer, for separating the sample being provided to the mass spectrometer.

The system may comprise a seventh valve (e.g. Valve 0) having a plurality of ports, wherein a plurality of sources of sample are connected to the plurality of ports by a plurality of respective fluid lines; and the control circuitry may be configured to control the seventh valve and the one or more pumps so as to select which source of sample to pump through the system.

The sources of sample may be bioreactors.

The seventh valve may comprise between 3 and 15 ports that are connected to respective fluid lines. For example, the seventh valve may comprise x ports that are

connected to a respective x fluid lines, where $x \leq 15, \leq 14, \leq 13, \leq 12, \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 . Additionally, or alternatively, the third valve may have at least 3 ports, at least 4 ports, at least 5 ports or at least 6 ports that are connected to respective fluid lines.

5 The seventh valve may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

The term fluid used herein may refer to liquid.

Where components are described herein as being connected, this may refer to the components being directly connected. In contrast, where components are described herein as being in fluid communication they may either be directly connected to each other
10 by a fluid line, or may be connected to each other via further components.

Although the valves have been referred to as first, second, third, fourth, sixth or seventh valves, this nomenclature is used to distinguish between the different valves and does not necessarily mean that that number of valves are present in the system.

15 The present invention also provides a method of preparing a sample comprising polypeptides for analysis by using the sample preparation system described herein.

Accordingly, the present invention provides a method of preparing a sample comprising polypeptides for analysis, comprising: providing a sample preparation system comprising: a first valve having a plurality of ports connected to a plurality of respective fluid lines; a second valve having a plurality of ports connected to a plurality of respective
20 fluid lines, where a port of the first valve is in fluid communication with a port of the second valve; a third valve having a plurality of ports connected to a plurality of respective fluid lines, where a port of the third valve is in fluid communication with a port of the first valve or a port of the second valve; a first fluid holding coil connected to a port of the second valve; a second fluid holding coil (e.g. holding coil 5) connected to a port of the third valve; and at
25 least one pump for pumping fluid through the system; and controlling the ports in each of the first, second and third valves that are connected to each other and the at least one pump so as to pump the sample through the valves to at least one of the fluid holding coils.

The method may comprise pumping the sample to a liquid chromatography separator and/or mass spectrometer.

30 The method may comprise pumping the sample through a chromatography separator so as to separate it prior to mass analysing the sample in the mass spectrometer.

The method may comprise controlling the first, second and third valves and the at least one pump so as to: pump a first portion of a first sample comprising polypeptides into
35 the first fluid holding coil; introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil; and pump a second portion of the first sample into the second fluid holding coil, whilst the first portion of the sample is in the system.

Alternatively, the method may comprise controlling the first, second and third valves
40 and the at least one pump so as to: pump a first sample comprising polypeptides into the system and into the first fluid holding coil; introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil; and pump a second different sample comprising polypeptides

into the system and into the second fluid holding coil, whilst the first sample is in the system. The second sample may be from a different source of sample to the first sample.

Alternatively, the method may comprise controlling the first, second and third valves and the at least one pump so as to: pump a first sample, or a first portion of a first sample, comprising polypeptides into the system and into the first fluid holding coil; and introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil.

The methods described herein may comprise controlling the valves and the at least one pump so as to introduce into the system a second, different agent for preparing the first sample, or first portion of the first sample, for analysis, and transmit the second agent to the first fluid holding coil.

The method may comprise controlling the valves and the at least one pump so as to either: (i) pump the first portion of the first sample out of the first fluid holding coil; then to pump the second portion of the first sample from the second fluid holding coil to the first fluid holding coil; and then to introduce at least one agent for preparing the second portion of the sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil to meet the second portion of the sample; or (ii) pump the first sample out of the first fluid holding coil; then to pump the second sample from the second fluid holding coil to the first fluid holding coil; and then to introduce at least one agent for preparing the second sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil to meet the second sample.

In the embodiments in which first and second portions of the first sample are pumped to the first fluid holding coil, the method may prepare the second portion of the sample for analysis in the same manner as the first portion of the sample, i.e. the at least one agent that is introduced to the second portion of the sample may be the same agent or agents that was or were introduced to the first portion of the sample. Alternatively, the method may prepare the second portion of the sample for analysis in a different manner to the first portion of the sample, i.e. one or more different agent may be introduced to the second portion of the sample that was not introduced to the first portion of the sample.

Alternatively, in the embodiments in which first and second different samples are pumped to the first fluid holding coil, the method may prepare the second sample for analysis in the same manner as the first sample, i.e. the at least one agent that is introduced to the second sample may be the same agent or agents that was or were introduced to the first sample. Alternatively, the method may prepare the second sample for analysis in a different manner to the first sample, i.e. one or more different agent may be introduced to the second sample that was not introduced to the first sample.

It is contemplated that the method may automatically add one or more agent to the second portion of the sample, or to the second sample, whilst it is in the second fluid holding coil.

The method may comprise transmitting the first portion of the sample (or first sample) and first agent to the first fluid holding coil such that they meet there, and/or such that the first portion of the sample (or first sample) and second agent are transmitted to the first fluid holding coil such that they meet there.

The first fluid holding coil may be connected to a port of the second valve. In such embodiments the method introduces the first agent into the system and to the first fluid holding coil by controlling the first valve such that: said fluid path through the first valve is between one of its port that is connected to a source of the first agent and the port that is connected to the second valve; and such that said fluid path through the second valve is between its port that is connected to the first valve and its port that is connected to the first fluid holding coil. Alternatively, the method may introduce the first agent into the system and to the first fluid holding coil by controlling the second valve such that said fluid path through the second valve is between one of its port that is connected to a source of the first agent and its port that is connected to the first fluid holding coil. Similarly, the method may introduce the second agent into the system and to the first fluid holding coil by controlling the first valve such that: said fluid path through the first valve is between one of its port that is connected to a source of the second agent and the port that is connected to the second valve; and such that said fluid path through the second valve is between its port that is connected to the first valve and its port that is connected to the first fluid holding coil. Alternatively, the method may introduce the second agent into the system and to the first fluid holding coil by controlling the second valve such that said fluid path through the second valve is between one of its port that is connected to a source of the second agent and its port that is connected to the first fluid holding coil.

The method may comprise controlling the first and second valves and the at least one pump so as to transmit the first agent to the first fluid holding coil such that it meets the first portion of the sample (or the first sample) before the second agent is transmitted to the first holding coil such that it meets the first portion of the sample (or the first sample), or vice versa.

The method may comprise controlling the first and/or second valves and the at least one pump so as to: i) load the first agent and first portion of the sample, or first sample, into the first fluid holding coil such that the first agent is sandwiched between volumes of sample in the first fluid holding coil, or such that the sample is sandwiched between volumes of the first agent, and then control the at least one pump so as to move the sandwiched first agent and sample along one or more fluid lines within the system so as to cause the first agent and sample to mix together; and/or ii) load the second agent and first portion of the sample, or first sample, into the first fluid holding coil such that the second agent is sandwiched between volumes of sample in the first fluid holding coil, or such that the sample is sandwiched between volumes of the second agent, and then control the at least one pump so as to move the sandwiched second agent and sample along one or more fluid lines within the system so as to cause the second agent and sample to mix together.

For example, the method may comprise controlling the first and/or second valves and the at least one pump so as to load a first volume of sample into the first fluid holding coil, and then to load the first agent into the first fluid holding coil, and then to load a second volume of the sample into the first fluid holding coil such that the first agent is sandwiched between the first and second volumes of the sample; and to control the at least one pump so as to move the first agent sandwiched between the first and second

volumes of the sample along one or more fluid lines within the system so as to cause the first agent and volumes of the sample to mix together. Additionally, or alternatively, the method may comprise controlling the first and/or second valves and the at least one pump so as to load a first volume of the sample into the first fluid holding coil, and then to load
5 the second agent into the first fluid holding coil, and then to load a second volume of the sample into the first fluid holding coil such that the second agent is sandwiched between the first and second volumes of the sample; and to control the at least one pump so as to move the second agent sandwiched between the first and second volumes of the sample along one or more fluid lines within the system so cause the second agent and portions of
10 the sample to mix together.

The method may comprise moving the sandwiched first agent and sample back and forth within the first fluid holding coil so as to cause the first agent and sample to mix together. Additionally, or alternatively, the method may comprise controlling the at least one pump so as to move the sandwiched second agent and sample back and forth within
15 the first fluid holding coil so as to cause the first agent and sample to mix together.

Alternatively, the method may comprise controlling the at least one pump and the first and/or second valve so as to: (i) move the sandwiched first agent and sample from the first fluid holding coil to another fluid holding coil in the system so as to cause the first agent and sample to mix together; and optionally to then return the sample to the first fluid
20 holding coil; and/or (ii) move the sandwiched second agent and sample from the first fluid holding coil to another fluid holding coil in the system so as to cause the second agent and sample to mix together; and optionally to then return the first sample to the first fluid holding coil.

Said another fluid holding coil may be a fluid holding coil connected to a port of the
25 first or second valve.

Said one or more fluid lines may comprise a degasser that removes air from the sandwiched first agent and sample, and/or from the sandwiched second agent and sample, as it is transmitted along said one or more fluid lines so as to cause the agent and sample to mix together. Any air bubbles that are present in the one or more fluid lines may prevent
30 the agent and sample mixing together. The degasser removes such air bubbles so as to enhance the mixing. The degasser may be a portion of a fluid line having apertures, such as pores or slits, that are configured so as to let air out of the fluid line but not liquid.

The first valve may comprise one or more further ports connected to one or more respective further fluid lines for transmitting fluid therethrough so that the fluid enters or
35 leaves the sample processing system at or from the first valve; and/or the second valve may comprise one or more additional ports connected to one or more respective additional fluid lines for transmitting fluid therethrough so that the fluid enters or leaves the sample processing system at or from the second valve.

A source of a, or the, first agent may be connected to a port of the first and/or
40 second valve by a fluid line, wherein the source of the first agent is a source of denaturing agent for denaturing the polypeptides. Optionally, the method comprises controlling the first and/or second valves and the at least one pump so as to pump the first agent from its

source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

A source of a, or the, second agent may be connected to a port of the first and/or second valve by a fluid line, wherein the source of the second agent is a digestion agent, such as an enzyme, for digesting the polypeptides. Optionally, the method comprises controlling the first and/or second valves and the at least one pump so as to pump the second agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

A source of a quenching agent for inhibiting a digestion agent from digesting the polypeptides may be connected to a port of the first and/or second valve by a fluid line; and the method may comprise controlling the first and/or second valves and the at least one pump so as to pump the quenching agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

A source of a neutralising agent for neutralising the pH of a sample may be connected to a port of the first and/or second valve by a fluid line; and the method may comprise controlling the first and/or second valve and the at least one pump so as to pump the neutralising agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

A source of a reducing agent for breaking disulphide bonds of the polypeptides may be connected to a port of the first and/or second valve by a fluid line; and the method may comprise controlling the first and/or second valves and the at least one pump so as to pump the reducing agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

A source of an alkylating agent for preventing disulphide bonds of the polypeptides reforming may be connected to a port of the first and/or second valve by a fluid line; and the method may comprise controlling the first and/or second valves and the at least one pump so as to pump the alkylating agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

The method may comprise pumping each of the various agents to the first portion of the sample, or to the first sample, individually, e.g. so as to meet the sample in the first holding coil. Alternatively, the method may pump multiple ones of the various agents into the system and to mix them prior to supplying the resulting mixture to the sample.

It is contemplated that rather than having a separate source for each of the agents mentioned herein, multiple ones of the agents may be provided as a mixture from a single source. This increases the speed at which the sample can be processed and therefore increases the sample throughput through the system. For example, a source containing a mixture of a neutralising agent for neutralising the pH of a sample and a reducing agent for breaking disulphide bonds of the polypeptides and/or a denaturing agent for denaturing the

polypeptides may be connected to a port of the first and/or second valve by a fluid line; and the method may comprise controlling the first and/or second valves and the at least one pump so as to pump the mixture from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

The method may comprise controlling the valves and the at least one pump so as to cause any one of the agents, or the mixture of agents, to be mixed with the first portion of the sample or first sample in a manner corresponding to the mixing procedure described above, e.g. by moving the sandwiched agent and sample from the first fluid holding coil to another fluid holding coil so as to cause the agent and sample to mix together.

As described above, after the various agent(s) has been mixed with the first portion of the sample or first sample, the first portion of the sample or first sample may be moved out of the first fluid holding coil and the second portion of the sample or second sample may be moved from the second fluid holding coil to the first fluid holding coil. The second portion of the sample or second sample may then be prepared in the same way as is described herein in relation the first portion of the sample or first sample. Alternatively, the second portion of the sample or second sample may be prepared in a different way, e.g. by adding one or more agent that is different to the agent(s) added to the first portion of the sample or first sample.

The system may comprise a reaction coil having a heater coupled thereto, connected via a fluid line to a port of one of the first or second valves. The method may comprise heating the reaction coil with the heater, and controlling the valves and the at least one pump so as to pump a or the first portion of sample, or first sample, to the heated reaction coil after an agent for preparing the sample has been added to it.

The first valve may comprise between 4 and 15 ports that are connected to respective fluid lines; and/or the second valve may comprise between 4 and 15 ports that are connected to respective fluid lines.

Additionally, or alternatively, the third valve may comprise between 4 and 15 ports that are connected to respective fluid lines.

For example, the first valve and/or the second valve and/or third valve may each comprise x ports that are connected to a respective x fluid lines, where $x \leq 15, \leq 14, \leq 13, \leq 12, \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 . Additionally, or alternatively, each of the first and/or the second and/or the third valves may have at least 5 ports, at least 6 ports, at least 7 ports or at least 8 ports that are connected to respective fluid lines.

Each of the first and/or second and/or third valves may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

Providing the valve with such relatively few ports connected to fluid lines helps ensure that fluid does not leak from the ports under pressure. This is particularly beneficial when a separation column is connected to one of the ports, since fluid is then pumped through the valve to the separation column at relatively high pressure. The relatively low number of ports connected to fluid lines is also beneficial if the valve is located in a refrigerated compartment with one or more sources of fluid to be introduced into one or

more ports of the valve, as it enables the valve to be smaller and have fewer connections to the ports.

The system may comprise a desalting column connected, via a fluid line, to a port of one of the first or second valves; and the method may comprise performing a first routine in which the valves and the at least one pump are controlled so as to pump the sample through the desalting column so as to desalt the sample. Alternatively, or additionally, the method may comprise performing a second routine in which the valves and the at least one pump are controlled so as to pump diluent to the sample so as to dilute salts in the sample.

In the first routine, the sample may be pumped from the first holding coil through the desalting column.

The system may comprise a user interface configured to enable a user to select which of the first or second routines the system is to operate. The method may comprise the user selecting the first or second routine at the user interface, and the system automatically performing the selected first or second routine in response thereto.

The method may comprise pumping a portion of the sample processed by the system to a liquid chromatography separator and/or mass spectrometer, and pumping at least part of the remainder of the sample processed by the system to a refrigeration compartment.

The method may include pumping said at least part of the remainder of the sample to a storage vessel located in the refrigeration compartment.

One of the first and second valves may be located in a refrigeration compartment; and optionally the other of the first or second valves may be located outside of the refrigeration compartment. Said other of the first or second valves may be maintained at ambient temperature.

The method may comprise transmitting fluid from at least one source of fluid located in the refrigerated compartment into at least one port of the valve located in the refrigeration compartment via at least one respective fluid lines; wherein each of said at least one fluid lines has a length of ≤ 20 cm. For example, each of said at least one fluid lines may have a length of ≤ 15 cm or ≤ 10 cm.

The at least one fluid lines may have a relatively small internal diameter, such as ≤ 0.3 mm or ≤ 0.4 mm.

The method may comprise transmitting fluid from at least one source of fluid located in the refrigerated compartment into at least one port of the valve located in the refrigeration compartment via at least one respective fluid lines; wherein the at least one source of fluid in the refrigerated compartment is at least one of: a reducing agent for breaking disulphide bonds of the polypeptides; an alkylating agent for preventing disulphide bonds of the polypeptides combining; and a digestion agent, such as an enzyme, for digesting the polypeptides. Any two of the above sources, or all three of the above sources may be located in the refrigerated compartment.

One or more sources of the following agents may be connected to a port of said other of the first and second valves: a source of denaturing agent for denaturing the polypeptides; a neutralising agent for neutralising the pH of the sample; and a quenching

agent for inhibiting a digestion agent from digesting the polypeptides. This one or more sources may be located outside of the refrigerated compartment.

5 The third valve (e.g. Valve 1) may be in fluid communication with a port of the first valve, and the system may comprises a fourth valve (e.g. Valve 6) in fluid communication with a different port of the first valve. The third and fourth valves may be in fluid communication in a manner such that fluid can pass from the third valve to the fourth valve whilst bypassing the first and second valves, and the system may comprise a mass spectrometer in fluid communication with the fourth valve. The method may comprise operating in a first mode in which the third valve, the first valve, optionally the second
10 valve, and the at least one pump are controlled so as to pump a first portion of the sample, or a first sample, into the first fluid holding coil and to add a first agent thereto; and at a subsequent time controlling these valves and the fourth valve so that this first portion of the sample or the first sample is pumped to the mass spectrometer. Additionally, or alternatively, the method may comprise operating in a second, different mode in which the
15 third and fourth valves and the at least one pump are controlled so as to pump a different portion of the sample or a different sample to the fourth valve and then to the mass spectrometer whilst bypassing the first and second valves and the first holding coil.

In the second mode sample therefore does not pass to the first and second valves or into the first holding coil and does not have the first and second agents added to it.

20 The fourth valve may comprise at least 4 ports that are connected to respective fluid lines. For example, the fourth valve may comprise x ports that are connected to a respective x fluid lines, where $x \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 .

The fourth valve may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

25 The method may comprise: using a sensor to determine the concentration of a protein or polypeptide in a or the first portion of a sample, or in a or the first sample, passing through a fluid line between the third valve and the first valve, in the first mode, and produce a signal representative of the determined concentration; performing a third routine that comprises controlling the amount of a first agent introduced into the system via
30 a port of the first or second valve and transmitted to the first fluid holding coil for mixing with the sample based on said signal representative of the determined concentration, and/or controlling the amount of a second agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil for mixing with the sample based on said signal; and/or performing a fourth routine that comprises controlling the
35 amount of a first agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil so as to be a first pre-determined amount, and controlling the volume of the sample transmitted to the first fluid holding coil for mixing with the first agent based on said signal representative of the determined concentration and based on said first pre-determined amount.

40 The sensor may be a spectroscopic sensor such an ultra-violet spectroscope.

The system may comprise a user interface configured to enable a user to select which of the third or fourth routines the system is to operate. The method may comprise a

user selecting the third or fourth routine at the user interface, and the system automatically performing the selected third or fourth routine in response thereto.

5 The system may comprise a separation column, such as a purification column, between the third valve and the sensor for separating the protein or polypeptide from other components in the sample, and the method may comprise pumping sample through the separation column such that the protein or polypeptide arrives at the sensor separated from the other components.

10 A source of binding buffer may be connected to a port of the third valve, and the method may comprise preparing the separation column, prior to pumping sample to the separation column, by controlling the third valve and the one or more pump so as to pump the binding buffer from its source to the separation column.

15 A source of elution buffer may be connected to a port of the third valve, and the method may comprise causing sample to elute from the separation column to the sensor, after the sample has been supplied to the separation column, by controlling the third valve and the one or more pump so as to pump the elution buffer to the separation column.

20 The system may comprise a fifth valve (e.g. Valve 2) between the sensor and the first valve, the fifth valve having a port connected to the sensor by a fluid line, a port connected to a fluid line for supplying sample to the first valve, a port connected to the third valve by a fluid line that bypasses the sensor, ports connected to a first sample loop, and ports connected to a second sample loop. The first mode may comprise controlling the fifth valve and the at least one pump such that sample is pumped from the sensor into the first sample loop and such that, in a further mode, sample is pumped from the third valve to the fifth valve through the fluid line that bypasses the sensor and into the second sample loop.

25 When the system operates in the first mode then it may subsequently control the fifth valve and pump so as to pump sample from the first sample loop to the first valve. In contrast, when the system operates in the further mode then it may subsequently control the fifth valve and pump so as to pump sample from the second sample loop to the first valve.

30 The system may comprise a user interface configured to enable a user to select which of the first or further modes the system is to operate. A user may select the first or further mode at the user interface, and the system may perform the selected first or further mode in response thereto.

35 The fifth valve may comprises between 4 and 15 ports that are connected to respective fluid lines. For example, the fifth valve may comprise x ports that are connected to a respective x fluid lines, where $x \leq 15, \leq 14, \leq 13, \leq 12, \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 . Additionally, or alternatively, the fifth valve may have at least 5 ports, at least 6 ports, at least 7 ports or at least 8 ports that are connected to respective fluid lines.

The fifth valve may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

40 The method may control the valves and at least one pump such that the sample in the first sample loop is processed as described herein, i.e. by mixing with the first and second agents etc., and then the sample in the second sample loop is processed in the same manner or in a different manner.

The second mode may comprise pumping diluent into the fluid line between the third and fourth valves at the time that the sample is passing therethrough.

5 The system may comprise a sixth valve (e.g. Valve 7) having a port connected to the fourth valve for receiving sample from the fourth valve, ports connected to a first sample loop, ports connected to a second, different sample loop, and a port in fluid communication with a mass spectrometer by a fluid line. The method may comprise controlling the sixth valve and the at least one pump such that sample is pumped from the fourth valve into one of the first or second sample loop that is connected to the sixth valve.

10 The sixth valve may comprise between 4 and 15 ports that are connected to respective fluid lines. For example, the sixth valve may comprise x ports that are connected to a respective x fluid lines, where $x \leq 15, \leq 14, \leq 13, \leq 12, \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 . Additionally, or alternatively, the sixth valve may have at least 5 ports, at least 6 ports, at least 7 ports or at least 8 ports that are connected to respective fluid lines.

15 The sixth valve may or may not comprise additional ports that are sealed closed, i.e. additional ports that are not connected to fluid lines.

The first and second sample loops may have different sample receiving volumes, and the system may comprise a user interface configured to enable a user to select a method of processing or analysing the sample from a plurality of different methods. The method may comprise a user selecting a method of processing or analysing the sample at the user interface, and the system automatically supplying the sample to either the first or the second sample loop in response to the selected method.

20 Alternatively, the method may comprise controlling the fourth and sixth valves such that sample is supplied from the fourth valve into the first sample loop connected to the sixth valve whilst sample is pumped from the second sample loop connected to the sixth valve through the port in fluid communication with the mass spectrometer. Optionally, the method controls the fourth and sixth valves such that sample is supplied from the fourth valve into the second sample loop connected to the sixth valve whilst sample is pumped from the first sample loop connected to the sixth valve through the port in fluid communication with the mass spectrometer.

25 The system described herein may comprise a mass spectrometer for mass analysing the sample, and the control circuitry may be configured to control the valves and at least one pump so as to pump the sample to the mass spectrometer. The system may comprise a liquid chromatography separator upstream of the mass spectrometer.

30 Accordingly, the method may comprise separating sample using a liquid chromatography separator in the fluid line between the mass spectrometer and the port of the sixth valve that is in fluid communication with the mass spectrometer.

35 The system may comprise a seventh valve (e.g. Valve 0) having a plurality of ports, and the method may comprise connecting a plurality of sources of sample to the plurality of ports by a plurality of respective fluid lines, and controlling the seventh valve and the one or more pumps so as to select which source of sample to pump through the system.

40 The sources of sample may be bioreactors.

The seventh valve may comprise between 3 and 15 ports that are connected to respective fluid lines. For example, the seventh valve may comprise x ports that are

connected to a respective x fluid lines, where $x \leq 15, \leq 14, \leq 13, \leq 12, \leq 10, \leq 9, \leq 8, \leq 7, \leq 6$, or ≤ 5 . Additionally, or alternatively, the third valve may have at least 3 ports, at least 4 ports, at least 5 ports or at least 6 ports that are connected to respective fluid lines.

The seventh valve may or may not comprise additional ports that are sealed closed,
5 i.e. additional ports that are not connected to fluid lines.

BRIEF DESCRIPTION OF THE DRAWINGS

Various embodiments of the present invention will now be described, by way of
10 example only, and with reference to the accompanying drawings in which:

Fig. 1 shows a schematic of a sample preparation system according to an
embodiment of the present invention; and

Fig. 2 is a flow chart showing some the sample processing techniques that the
system may be configured to perform.

15

DETAILED DESCRIPTION

Fig. 1 shows a schematic of a sample preparation apparatus according to an
embodiment of the present invention. The apparatus comprises one or more sources of
20 sample to be analysed, such as one or more bioreactors 2 for a cell culture. The apparatus
also comprises a fluid line 3 and a pump 4, such as a peristaltic pump, for pumping sample
from the one or more sources to a holding coil 5.

If it is desired for the apparatus to process samples from more than one source of
sample 2, then a multi-port valve 1 (Valve 0) may be provided for selecting which sample is
25 to be pumped to the holding coil 5 and subsequently processed. More specifically, multiple
different fluid lines may be provided for connecting the multiple different sources of sample
to multiple respective inlet ports of the multi-port valve, and the inlet end of the fluid line 3
for supplying sample to the holding coil 5 is connected to the outlet of the multi-port valve
1. As such, the multi-port valve may be controlled so as to select which of the different
30 sources of sample is pumped to the holding coil by the pump. Each of the one or more
sources of sample may also include a waste line 6 and valve system 7 that is controllable
by the sample processing apparatus so as to divert sample from any given source to the
waste line rather than to the multi-port valve.

In the illustrated embodiment port 5 of Valve 0 is connected to a first source of
35 sample by a fluid line, port 4 is connected to a second source of sample by a fluid line, and
port 3 of Valve 0 is connected to a third source of sample by a fluid line. Port 8 of Valve 0
is connected to a source of NaOH for use in cleaning fluid lines downstream of Valve 0.
Although Valve 0 is illustrated as having 13 ports, it will be appreciated that the valve may
have a different number of ports, depending on the number of sample sources that are
40 required to be analysed. For example, in the illustrated embodiment ports 1, 2, 6, 7 and 9-
12 are redundant ports that are sealed with plugs so that fluid does not enter or leave
them.

Pumping samples from the one or more sources in the above-described manner is a non-limiting example of means of providing a sample into the system. For example, an automated sample manager device may be provided that is configured to automatically draw a sample from one or more sources of sample, such as from one or more vials containing samples, and then inject that sample into the fluid line 3 so that it passes to the holding coil 5.

The fluid line 3 and holding coil 5 are connected to each other at two ports of a T-junction 8. A degasser 9 and pinch valve 11 may be provided in the fluid line between the multi-port valve 1 and T-junction 8. The T-junction is connected to one end of the holding coil 5. The other end of the holding coil is connected to a valve system 12 that is controllable to eject liquid from the holding coil to a waste line and/or to allow gas such as air to be removed from the holding coil without removing liquid.

The apparatus comprises a further multi-port valve 10 (Valve 1) having an inlet port (port 4) that is connected to the third port of the T-junction 8 by a fluid line, such that sample may be supplied from the holding coil 5 to Valve 1. Valve 1 has a plurality of other inlet ports that are connected to fluid lines for receiving a plurality of different solutions such as a port for receiving a solution of NaOH (port 1), a port for receiving an elution buffer (port 11), a port for receiving a binding buffer (port 12), and a port for receiving a calibrant (port 8). The multi-port valve 10 also includes a plurality of outlet ports. For example, the valve may include an outlet port for outputting liquid received at the valve to waste (port 2). The valve may include an outlet port for allowing gas such as air to be removed from the system (port 3). An air sensor 13 may be provided on the fluid line between the holding coil 5 and Valve 1 for sensing if air is present in the line. If the air sensor detects the presence of such air then Valve 1 may be controlled so as to divert the sample to waste port 2 of the Valve 1.

Port 13 of Valve 1 is connected to one end of a further holding coil 14. The other end of the holding coil 14 is connected to a pump 15, such as a syringe pump or piston pump, for drawing fluid from Valve 1 into the holding coil or for urging fluid out of the holding coil and into Valve 1. A pressure sensor 16 may be provided in the fluid line between the pump 15 and the holding coil 14 for use in controlling the pump. Port 7 of Valve 1 is connected to a further multiport valve 60 (Valve 6) via fluid lines and a T-junction 17. A source of diluent is also connected to the T-junction 17, and a pump 18 and one way valves 19 are provided for pumping diluent to the T-junction 17. A degasser 21 may be provided in the line between the source of diluent and the T-junction 17.

Ports 9 and 10 of Valve 1 are connected to a further multiport valve 20 (Valve 2) via a fluid line. A separation column 22 (e.g. Pro-A column), pre-column filter 23, and protein concentration sensor 24 such as an ultra-violet detector are provided in the fluid line between Valve 1 and Valve 2.

Although Valve 1 is illustrated as having 13 ports, it will be appreciated that the valve may have a different number of ports, depending on the sample processing functions that the apparatus is required to be configured to perform. For example, in the illustrated embodiment ports 5 and 6 are redundant ports that are sealed with plugs so that fluid does not enter or leave them.

Inlet ports 4 and 7 of Valve 2 are connected to outlet ports of 10 and 9 of Valve 1. The ends of a first sample loop 25 are connected to ports 2 and 5 of Valve 2, and the ends of a second sample loop 26 are connected to ports 7 and 10 of Valve 2. Ports 3 and 9 of Valve 2 are connected to waste lines. Port 1 of Valve 2 is connected to an air line. Port 6 of Valve 2 is connected to port 7 of a further multiport valve 50 (Valve 5) by a fluid line.

Valve 5 has a port 3 that is connected to a source of a quenching buffer, port 8 that is connected to a source of a neutralising buffer, port 9 that is connected to a source of denaturing agent and port 12 that is connected to a waste line. Port 4 of Valve 5 is connected to one end of a fluid line having a degasser 27 therein, and the other end may be connected to a waste line and/or air line. One end of a further holding coil 28 is connected to port 5 of Valve 5 and the other end is connected to a waste line and/or air line. One end of a reaction coil 29 is connected to port 6 of Valve 5 and the other end is connected to a waste line and/or air line. Port 13 of Valve 5 is connected to a further multiport valve 30 (Valve 3) by a fluid line and port 1 of Valve 5 is connected to a further multiport valve 60 (Valve 6) by a fluid line.

Although Valve 5 is illustrated as having 13 ports, it will be appreciated that the valve may have a different number of ports, depending on the sample processing functions that the apparatus is required to be configured to perform. For example, in the illustrated embodiment ports 2, 10 and 11 are redundant ports that are sealed with plugs so that fluid does not enter or leave them.

Valve 3 has a port 1 that is connected to a source of a dilution buffer, port 4 that is connected to a source of NaOH, port 5 that is connected to a waste line, port 7 that is connected to a reducing agent such as TCEP, port 8 that is connected to an alkylating agent such as iodoacetamide (IAM), port 9 that is connected to a digestion agent such as Trypsin, and port 12 that is connected to a digestion and/or desalting buffer. Port 3 may be connected to an air line. Port 6 may be connected to a vessel for holding a sample after it has been processed by the system. Ports 10 and 11 are connected to ports of a further multiport valve 40 (Valve 4). Valve 3 and at least some of the sources of liquid that are supplied into its ports are located in a refrigerated compartment 31. For example, the test sample, and the sources of the reducing agent, alkylating agent, and digestion agent may be located in the refrigerated compartment. These sources are preferably connected to Valve 3 by relatively short fluid lines. The source of calibrant supplied to port 8 of Valve 1 may also be located in the refrigerated compartment. The source of diluent and the source of digestion and/or desalting buffer may be located outside of the refrigerated compartment. Port 13 of Valve 3 is connected to one end of a further holding coil 32. The other end of the holding coil is connected to a pump 33, such as a syringe pump or piston pump, for drawing fluid from Valve 3 into the holding coil 32 or for urging fluid out of the holding coil 32 and into Valve 3. A pressure sensor 34 may be provided in the fluid line between pump 33 and the holding coil 34 for use in controlling the pump 33.

As mentioned above, ports 10 and 11 of Valve 3 are connected to Valve 4 by fluid lines. More specifically, port 10 of Valve 3 is connected to port 4 of Valve 4 by a fluid line, and port 11 of Valve 3 is connected to port 2 of Valve 4 by a fluid line. A desalting column 35 may be provided in the fluid line between port 11 of Valve 3 and port 2 of Valve 4. A

sample loop 36 is connected to ports 3 and 6 of Valve 4. A waste line may be connected to port 1 of Valve 4 and an air line may be connected to port 5 of Valve 4.

As mentioned above, port 1 of Valve 6 is connected to port 1 of Valve 5, and port 3 of Valve 6 is connected to port 7 of Valve 1. Port 2 of Valve 6 may be connected to a sample fraction collector vessel. Ports 4 and 8 of Valve 6 may be connected to a further valve 70 (Valve 7) by fluid lines. Although Valve 6 is illustrated as having 8 ports, it will be appreciated that the valve may have a different number of ports, depending on the sample processing functions that the apparatus is required to be configured to perform. For example, in the illustrated embodiment ports 5, 6 and 7 are redundant ports that are sealed with plugs so that fluid does not enter or leave them.

Port 4 of Valve 6 is connected to port 4 of Valve 7, and port 8 of Valve 6 is connected to port 8 of Valve 7. Port 1 of Valve 7 is connected to a liquid chromatograph column, which in turn is connected to an ionisation source of a mass spectrometer. Port 6 of Valve 7 is connected to a source of one or mobile phases for the liquid chromatograph column and a pump for pumping the mobile phase(es) into port 6. Ports 2 and 5 of Valve 7 are connected to a first sample loop 37, and ports 7 and 10 of Valve 7 are connected to a second sample loop 38. Port 3 of Valve 7 is connected to a waste line.

The system has control circuitry that is configured to automatically control the fluid paths through the multiport valves and to control the pumps so as to prepare the sample for mass analysis by adding the various agents to the sample, as will be described below.

The sample from any given one of the one or more sources of sample 2 may be processed by the apparatus in a number of different ways, as will be described in more detail below. However, each method begins by loading sample from one of the sources of sample into holding coil 5 that is upstream of Valve 1. This may be performed by controlling Valve 0 so as to connect the source of sample that is desired to be processed with the outlet port 13 of Valve 0. The pump 4 is then operated so as to urge sample from that sample source to holding coil 5. Alternatively, as described above, an automated sample manager device may be used to deliver the sample to the holding coil 5. The sample may pass through a degasser 9 and pinch valve 11, if one or both of these components are present, on the way to the holding coil 5. The waste valve 12 at the end of the holding coil may be maintained open while filling the holding coil with sample so that any fluid in the holding coil that is displaced by the sample can pass to waste. The waste valve may then be closed after the coil is filled with the sample. Additionally, or alternatively, the air valve 12 at the end of the holding coil may be maintained open while filling the holding coil with sample so that any air in the holding coil that is displaced by the sample, or that is present in the sample being supplied to the coil, can pass through the air valve whilst retaining the sample in the coil. The holding coil may have a volume of, for example, 2 mL.

The apparatus is configured to send the sample from holding coil 5 to holding coil 14. However, the apparatus may be configured to detect if there is air in the fluid line and then expel any such air prior to transferring the sample from holding coil 5 to holding coil 14. Accordingly, as described above, the apparatus may comprise an air sensor 13 configured to monitor the fluid line that connects holding coil 5 to port 4 of Valve 1 so as to

determine if air is present in the fluid line. If the sensor detects the presence of air in sample being transmitted along the fluid line then it may send a signal to a valve control system having circuitry that, in response thereto, controls Valve 1 such that port 4 is in communication with port 2 so as to expel the portion of the sample containing the detected
5 air from the fluid line and out of Valve 1. In contrast, if the air sensor does not detect air in the fluid line then the valve control system controls Valve 1 such that its port 4 is in communication with a different port, such as port 13 so that the sample is sent to holding coil 1.

10 Various different examples of how the sample may be processed will now be described.

The apparatus may be configured to send the sample from the source of sample to the LC-MS analyser, without first adding agents to the sample other than a diluent.

In order to do this, the valve control system controls Valve 1 so that the input port 4 is in communication with port 13. Pump 15 then draws an aliquot of the sample into
15 holding coil 14. Pump 15 is a pump that is able to urge fluid in either direction, such as a syringe pump or piston pump. A pressure sensor 16 may be provided to sense the pressure in the fluid line between pump 15 and holding coil 14, for determining the pressure in the fluid line such that the apparatus can control pump 15 based on the sensed pressure. The valve control system then controls Valve 1 so that its ports 7 and 13 are in
20 communication, and pump 15 is then controlled so as to pump the sample out of holding coil 14, into port 13 of Valve 1, out of port 7 of Valve 1, through the fluid line extending between port 7 of Valve 1 and T-junction 17 and into the fluid line extending between T-junction 17 and Valve 6.

The apparatus is configured to supply diluent into the fluid line extending between
25 T-junction 17 and Valve 6 at the same time that the sample is supplied into this fluid line, so as to mix with and dilute the sample. The remaining port of T-junction 17 may be connected to a first port of a further T-junction 19 by a fluid line, and that further T-junction 19 may be connected at a second of its ports to a pump 18 via a fluid line. The further T-junction 19 is connected at a third of its ports to a source of the diluent via a fluid line. The
30 diluent is supplied to the sample by pump 18 drawing diluent through the line from the source of diluent to the further T-junction 19. The diluent may pass through a degasser 21 on the way. Sample is prevented from being drawn by the pump by a valve between T-junction 17 and the further T-junction 19. The diluent that is drawn by the pump is drawn into the fluid line between the further T-junction 19 and pump 18. Pump 18 is then
35 controlled so as to urge the diluent through the further T-junction 19 and T-junction 17 so as to mix with the sample in the fluid line between T-junction 17 and Valve 6. A valve in the fluid line between the source of diluent and the further T-junction 19 prevents the diluent from being pumped back towards the source of diluent. The diluted sample is therefore pumped through the fluid line between T-junction 17 and Valve 6, and into port 3
40 of Valve 6.

The valve control system controls Valve 6 so that port 3 is in communication with another of its ports so that Valve 6 sends the diluted sample to Valve 7. Valve 7 may have a first sample loop 37 connected to two of its ports and a second sample loop 38

connected to two of its ports. The two sample loops may have different volumes, and the valve system controller may configure the valves to send the diluted sample to one sample loop or the other, depending on a user input to the apparatus that is indicative of the size of the diluted sample that is desired to be analysed. For example, the control circuitry in the valve system controller may control Valve 6 such that its ports 3 and 8 are in communication, such that the diluted sample is pumped into port 3 of Valve 6, out of port 8 of Valve 6, into port 8 of Valve 7, and then into sample loop 38. Alternatively, the valve system controller may control Valve 6 such that its ports 3 and 4 are in communication, such that the diluted sample is pumped into port 3 of Valve 6, out of port 4 of Valve 6, and into port 4 of Valve 7, where Valve 7 is controlled such that its port 4 is in communication with port 5 such that the diluted sample is pumped into first sample loop 37.

The valve control system then controls Valve 7 so that the diluted sample in either sample loop 37 or sample loop 38 is output to the liquid chromatography (LC) device. For example, if the diluted sample is located in sample loop 37 then Valve 7 is controlled such that ports 1 and 2 are in communication, and ports 5 and 6 are in communication. An LC pump is then controlled to pump one or more solvents into port 6 of Valve 7 so as to pump the sample through sample loop 37, out of port 1 of Valve 7 and into the LC device. The one or more solvents is continued to be pumped by the LC pump so as to cause the sample to be chromatographically separated in the LC device and analysed, such as by being ionised and the resulting ions subjected to mass spectrometry. Alternatively, if the diluted sample is located in sample loop 38 then Valve 7 is controlled such that ports 1 and 10 are in communication, and ports 6 and 7 are in communication. The LC pump is then controlled to pump one or more solvents into port 6 of Valve 7 so as to pump the sample through sample loop WB, out of port 1 of Valve 7 and into the LC device. The one or more solvents is continued to be pumped by the LC pump so as to cause the sample to be chromatographically separated in the LC device and analysed, such as by being ionised and the resulting ions subjected to mass spectrometry.

In either case, the valves may be controlled so that the diluted sample is not transmitted to the LC device for a predetermined period of time after both the sample and diluent are being supplied into T-junction 17. This time may be selected to ensure that sample is only supplied to the LC device after a stable flow of diluted sample has been established. Prior to this, the valve system may be controlled so as to supply the sample and/or diluent to a valve port that sends it to waste, such as port 3 or port 9 of Valve 7.

Only a portion of the sample from holding coil 14 may be sent to the LC device. The valve control system may control the valves such that the remainder of the sample is either pumped through to waste, e.g. via port 3 or port 9 of Valve 7, or to a fraction collector, e.g. via port 2 on Valve 6.

After the sample has been processed in the above manner, all of the fluid lines and valves that the sample has passed through on the way to Valve 7 may be washed out by pumping a solvent, such as NaOH, through these fluid lines to waste. For example NaOH may be supplied into port 8 of Valve 0 for cleaning the fluid lines between Valve 0 and Valve 1. NaOH may be supplied into port 1 of Valve 1 for cleaning Valve 1, the fluid lines

between Valve 1 and Valve 6, for cleaning Valve 6, for cleaning the fluid lines between Valve 6 and Valve 7, and for cleaning Valve 7.

5 A cell culture media may be provided for feeding the cell culture from which the sample has been taken. The cell culture media becomes spent after having been added to the cell culture, and it may be desirable to analyse the spent cell culture media. For instance, it may be desirable to analyse the spent cell culture media to determine which metabolites have been produced by the cell culture, e.g. which could be inhibiting cell protein production, to identify the presence of an amino acid or vitamin in the cell culture, or to determine whether more cell culture media should be added to the cell culture etc. The apparatus may also be controlled so as to analyse the spent cell culture media from the source of sample. This may be performed in the same manner as described above, except that pumps 15 and 18 may be controlled such that the spent media is diluted by a different dilution factor by the diluent than the sample is diluted.

10 In the above method, Valve 1 is controlled such that the sample bypasses Valves 2, 3 and 5 and the sample processing steps associated therewith. However, the apparatus is also configured to operate in an alternative mode in which Valve 1 is controlled so as to transmit the sample to these valves so that agents may be added to the sample prior to its analysis. The apparatus is therefore configured to automatically prepare the sample for a protein analysis, such as a peptide multi-attribute method (MAM) analysis.

20 In such an analysis, the apparatus is configured to control the valve and pump system such that a sample from a sample source is loaded into holding coil 5, in the manner as described further above.

The apparatus is also configured to automatically prepare the purification column 22 (Pro-A column) for use. The apparatus does this by automatically loading an elution buffer into holding coil 14. More specifically, the valve control system is configured to control Valve 1 such that port 11 is in fluid communication with port 13, and pump 15 is then controlled so as to draw elution buffer into port 11, out of port 13 and into holding coil 14. The apparatus is configured to then automatically rinse the column with the elution buffer by pumping the elution buffer from holding coil 14 through the column. In other words, the valve control system is configured to control Valve 1 such that port 13 is in fluid communication with port 10, and pump 15 is then controlled so as to pump elution buffer out of holding coil 14, into port 13, out of port 10 and through the column 22. The column may be a protein A column for purifying the sample.

35 The apparatus is configured to then automatically rinse the column with a binding buffer. More specifically, the valve control system is configured to automatically control Valve 1 such that port 12 is in fluid communication with port 13, and pump 1 is then controlled so as to draw binding buffer into port 12, out of port 13 and into holding coil 14. The apparatus is configured to then rinse the column with the binding buffer by pumping the binding buffer from holding coil 14 through the column 22. More specifically, the valve control system is configured to control Valve 1 such that port 13 is in fluid communication with port 10, and pump 15 is then controlled so as to pump binding buffer out of holding coil 14, into port 13, out of port 10 and into the column.

The apparatus is configured to then automatically load the holding coil 14 with both the sample and binding buffer such that the sample and binding buffer are loaded in the holding coil at the same time. Either the sample may be loaded into the holding coil before the binding buffer is added to it, or vice versa. The binding buffer is loaded into the holding coil in the same manner as described above, i.e. the valve control system is configured to control Valve 1 such that port 12 is in fluid communication with port 13, and pump 15 is then controlled so as to draw binding buffer into port 12, out of port 13 and into holding coil 14. The valve control system is configured to load the sample into the holding coil by controlling control Valve 1 such that port 4 is in fluid communication with port 13, and pump 15 is then controlled so as to draw the sample into port 4, out of port 13 and into holding coil 14.

The apparatus is configured to automatically load the stacked sample and binding buffer from holding coil 14 to the column 22 such that the sample binds to the column. The valve control system is configured to do this by controlling control Valve 1 such that port 13 is in fluid communication with port 10, and pump 15 is then controlled so as to pump the stacked sample and binding buffer into port 13, out of port 10 and into the column.

The apparatus may be configured to then automatically supply further binding buffer to the column in order to rinse off excess sample that has not bound to the column. More specifically, the valve control system is configured to control Valve 1 such that port 12 is in fluid communication with port 13, and pump 15 is then controlled so as to pump binding buffer into port 12, out of port 13 and into holding coil 14. The valve control system is configured to then control Valve 1 such that port 13 is in fluid communication with port 10, and pump 15 is then controlled so as to pump binding buffer out of holding coil 14, into port 13, out of port 10 and into the column.

The apparatus is configured to then automatically supply elution buffer, and optionally also binding buffer, to the column in order to cause sample that has bound to the column to elute such that the sample passes to the ultra-violet (UV) detector 24.

The apparatus is configured to do this by loading the holding coil 14 with the elution buffer, and optionally also binding buffer. Both the elution and binding buffers may be loaded into the holding coil such that they reside in the holding coil at the same time. Either the elution buffer may be loaded into the holding coil before the binding buffer is added to it, or vice versa. The elution buffer is loaded into the holding coil in the same manner as described above, i.e. the valve control system is configured to control Valve 1 such that port 11 is in fluid communication with port 13, and pump 15 is then controlled so as to draw elution buffer into port 11, out of port 13 and into holding coil 14. The binding buffer is loaded into the holding coil in the same manner as described above, i.e. the valve control system is configured to control Valve 1 such that port 12 is in fluid communication with port 13, and pump 15 is then controlled so as to draw binding buffer into port 12, out of port 13 and into holding coil 14. The apparatus is configured to then automatically supply the elution buffer and binding buffer to the column by the valve control system automatically controlling Valve 1 such that port 13 is in fluid communication with port 10, and pump 15 is then controlled so as to pump the elution buffer and binding buffer into port 13, out of port 10 and to the column.

The UV detector 24 performs spectroscopy on the sample passing thereby so as to obtain a signal that is representative of the concentration of the compound of interest that is present in the sample, such as a protein of interest. In other words, the UV detector automatically obtains a signal representing the titer value for the protein of interest. This
5 titer value may be automatically used by the apparatus to determine the amount of each of one or more agents that are required to be added to the sample in order to process it. The apparatus may be configured to automatically control the valve and pump system, in response to determined signal for the titer value, so as to add said amount of each of the one or more agents to the sample. For example, the signal representative of the titer value
10 may be used to control the quantity of one or all of a denaturing agent, a neutralising agent, a reduction agent, and a digestion agent that may be added to the sample, as will be described further below. Alternatively, the amount of each of the one or more agents that are to be added to the sample may be pre-selected and constant, and the apparatus may be configured to dilute the sample based on the detected signal for the titer value, so that
15 the protein of interest in the sample is diluted to a pre-selected concentration. A user interface on the apparatus may be configured to allow the user to select which of the above techniques the apparatus uses in response to the titer value. Alternatively, the apparatus may be configured to automatically perform one or other of the techniques.

The valve control system may control Valve 2 such that its port 4 is in
20 communication with one of the ports that an end of sample loop 25 is connected to, such that the sample that elutes from the column 22 and passes through the UV detector 24 passes into port 4 of Valve 2 and is captured in sample loop 25. Prior to capturing the sample in sample loop 25, the valve control system may control Valve 2 so that port 4 is in communication with port 3, so that the buffers that are pumped through the column are
25 sent to waste via port 3 of Valve 2.

If the protein of interest in the sample is required to be diluted to a pre-selected concentration based on the titer value determined, the apparatus may be configured to automatically supply a diluent to the sample so as to dilute the sample to the pre-selected concentration. The apparatus is configured to calculate the amount of diluent to supply to
30 the sample based on the titer value determined, and then to control the apparatus to supply this calculated amount to the sample.

The system may be configured to operate in a further mode, in which the titer value of the sample is not determined. In this mode the valve control system may automatically control the valves and pump such that sample is pumped from port 9 of Valve 1 into port 8
35 of Valve 2 and then into sample loop 26. The sample in sample loop 26 may also be processed in the same manner as will be described herein in relation to the sample in sample loop 25, or in a different manner.

The sample from sample loop 25 is sent to holding coil 32, along with one or more other agents such as a denaturing agent for denaturing the protein of interest, and a
40 neutralising buffer for neutralising the sample. The apparatus is configured to automatically perform these steps by controlling Valves 2, 3 and 5 such that sample loop 25 is in fluid communication with holding coil 32, and then controlling pump 33 to draw the sample from the sample loop to holding coil 32. More specifically, the valve control system

controls Valve 2 such that the sample loop is in communication with port 6 of Valve 2, such that ports 7 and 13 of Valve 5 are in communication with each other, such that port 13 of Valve 5 is in communication with port 2 of Valve 3, and such that ports 2 and 13 of Valve 3 are in communication with each other. Holding coil 32 is connected to port 13 of Valve 3 and so receives the sample.

The neutralising buffer may be added to holding coil 32 by the valve control system controlling Valve 5 such its ports 8 and 13 are in communication with each other, controlling Valve 3 such its ports 2 and 13 are in communication with each other, and then using pump 33 to draw the neutralising buffer into port 8 of Valve 5, out of port 13 of Valve 5, into port 2 of Valve 3, out of port 13 of Valve 3 and into holding coil 32. Similarly, the denaturing agent may be added to holding coil 32 by the valve control system controlling Valve 5 such its ports 9 and 13 are in communication with each other, controlling Valve 3 such its ports 2 and 13 are in communication with each other, and then using pump 33 to draw the denaturing agent into port 9 of Valve 5, out of port 13 of Valve 5, into port 2 of Valve 3, out of port 13 of Valve 3 and into holding coil 32.

The sample, neutralising buffer and denaturing agent are loaded into the holding coil 32 one after the other, such that they are present in the holding coil at the same time. They may be loaded into the holding coil in any order. However, in order to assist in mixing the sample agent with the neutralising buffer and denaturing agent, it may be desired to introduce the sample into the holding coil, then introduce neutralising buffer and denaturing agent (in any order), and then introduce more of the sample into the holding coil. The second instance of introducing the sample into the holding coil may be performed in the same manner described above for the first instance that the sample is introduced into the holding coil, i.e. by controlling Valves 2, 5 and 3 and pump 33 such that sample is supplied from the sample loop to the holding coil. Alternatively, after the sample has been transferred to holding coil 32, but before the neutralising buffer and denaturing agent are loaded into holding coil 32, a portion (such of half) of the sample may be removed from holding coil 32 and reintroduced after the neutralising buffer and denaturing agent have been loaded into holding coil 32. For example, the portion of the sample may be removed from holding coil 32 by the valve control system automatically controlling Valve 3 such that its ports 2 and 13 are in communication with each other, controlling Valve 5 such that its ports 4 and 13 are in communication with each other, and the controlling pump 33 so as to pump the portion of the sample from holding coil 32, into port 13 of Valve 3, out of port 2 of Valve 3, into port 13 of Valve 5, and out of port 4 of Valve 5 to the degasser 27. As a further alternative technique, the portion of the sample may be sent to the degasser 27 from sample loop 25, via Valve 5, without having first being passed to holding coil 32.

The apparatus is configured to cause the sample, neutralising buffer and denaturing agent to mix by using a pump to urge these liquids back and forth within a holding coil, within another fluid line in the system or between multiple holding coils. For example, the valve control system may control Valves 3 and 5 such that holding coil 32 is in fluid communication with the holding coil 28 connected to port 5 of Valve 5. More specifically, the valve control system controls Valve 3 so that its ports 2 and 13 are in communication with each other and controls Valve 5 so that its ports 5 and 13 are in communication with

each other. The apparatus is then configured to control pump 33 so as to urge the sample, neutralising buffer and denaturing agent from holding coil 32 to the holding coil 28 connected to port 5 of Valve 5, and then back to holding coil 32. This transfer from holding coil 32 to the other holding coil 28 and back again may be performed one or more times, and provides a particularly efficient way of mixing the sample with the agents within the fluid lines of the apparatus. A degasser may be provided in a fluid line that the liquids pass through for removing air bubbles that might otherwise inhibit the mixing.

Although the neutralising buffer and denaturing agent have been described as being added to the sample together and then mixed, it is contemplated that one of these may be added to the sample and mixed in the above-described manner, and then the other may be added to the sample and mixed in the above-described manner.

After mixing, the apparatus is configured to cause the mixture to be automatically transferred from holding coil 32 to a reaction coil 29 connected to port 6 of Valve 5. More specifically, the valve control system controls Valve 3 so that its ports 2 and 13 are in communication with each other and controls Valve 5 so that its ports 6 and 13 are in communication with each other. The apparatus is then configured to control pump 33 so as to urge the mixture from holding coil 32 to the reaction coil. The reaction coil may be pre-heated, for example to a temperature between 37 and 65 degrees Celsius, such that when the mixture is received in the reaction coil it is heated. After a pre-selected period of time of the mixture being in the reaction coil, the heating of the reaction coil is turned off or otherwise reduced so as to allow the mixture to cool in the reaction coil.

The apparatus is configured to then automatically transfer the mixture from the reaction coil to holding coil 32. More specifically, the valve control system maintains ports 6 and 13 of Valve 5 in communication with each other and ports 2 and 13 of Valve 3 in communication with each other, and then controls pump 2 so as to urge the mixture from reaction coil to holding coil 32.

Optionally, the apparatus is configured to mix an alkylating agent with the sample, as will be described below. However, it is contemplated that this step may be omitted. An alkylating agent may be added to prevent reformation of the disulphide bonds between fragments of peptides in the sample. However, instead, the sample may be processed sufficiently quickly that a significant number of disulphide bonds do not have time to reform. If an alkylating agent is desired to be added to the sample, then the apparatus is configured to automatically transfer a portion of the sample mixture, such as half the sample mixture, from holding coil 32 to a degasser. More specifically, the valve control system controls Valve 3 so that its ports 2 and 13 are in communication with each other, controls Valve 5 such that its ports 4 and 13 are in communication with each other, and then pump 33 is controlled so as to pump the portion of the sample from holding coil 32, into port 13 of Valve 3, out of port 2 of Valve 3, into port 13 of Valve 5, and out of port 4 of Valve 5 to the degasser 27.

The apparatus is configured to then automatically transfer an alkylating agent such as iodoacetamide into holding coil 32 such that it resides in the holding coil with the portion of the sample that has not been transferred to the degasser. More specifically, the valve control system controls Valve 3 so that its ports 8 and 13 are in communication with each

other, and the apparatus then controls pump 33 so as to urge the alkylating agent into port 8, out of port 13 and into holding coil 32. The portion of the sample that was transferred to the degasser is then returned to the holding coil, in which the remainder of the sample and alkylating agent are located. The apparatus is configured to automatically perform this by
5 the valve control system being configured to control Valve 3 such that its ports 2 and 13 are in communication with each other, control Valve 5 such that its ports 4 and 13 are in communication with each other, and then pump 33 is controlled so as to draw the portion of the sample from the degasser into port 4 of Valve 5, out of port 13 of Valve 5, into port 2 of Valve 3, out of port 13 of Valve 3, and into holding coil 32. As such, the alkylating agent is
10 located between two portions of the sample in holding coil 32.

The apparatus is configured to cause the portions of sample and alkylating agent to mix by using a pump to urge these liquids back and forth within a holding coil, within another fluid line in the system or between multiple holding coils. For example, the valve control system may control Valves 3 and 5 such that holding coil 32 is in fluid
15 communication with the holding coil 28 connected to port 5 of Valve 5. More specifically, the valve control system controls Valve 3 so that its ports 2 and 13 are in communication with each other and controls Valve 5 so that its ports 5 and 13 are in communication with each other. The apparatus is then configured to control pump 33 so as to urge the portions of sample and alkylating agent from holding coil 32 to the holding coil 28 connected to port
20 5 of Valve 5, and then back to holding coil 32. This transfer from holding coil 32 to the other holding coil 28 and back again may be performed one or more times, and provides a particularly efficient way of mixing the sample with the alkylating agent within the fluid lines of the apparatus.

The apparatus may then be configured to automatically transfer a portion of the
25 sample, such as half the mixture, to the degasser 27 connected to port 4 of Valve 5. This may be performed in the same manner as has been described above. The apparatus is configured to then automatically transfer a reducing agent such as tris (2-carboxyethyl) phosphine hydrochloride (TCEP) into holding coil 32 such that it resides in the holding coil with the portion of the sample that has not been transferred to the degasser. More
30 specifically, the valve control system controls Valve 3 so that its ports 7 and 13 are in communication with each other, and the apparatus then controls pump 33 so as to urge the reducing agent into port 7, out of port 13 and into holding coil 32. The portion of the sample that was transferred to the degasser is then returned to the holding coil, in which the remainder of the sample and reducing agent are located. The apparatus is configured
35 to automatically perform this in the same manner as has been described above. As such, the reducing agent is located between two portions of the sample in holding coil 32.

The apparatus is configured to cause the portions of sample and reducing agent to mix by using a pump to urge these liquids back and forth within a holding coil, within another fluid line in the system or between multiple holding coils. For example, the valve
40 control system may automatically control Valves 3 and 5 such that holding coil 32 is in fluid communication with the holding coil 28 connected to port 5 of Valve 5. More specifically, the valve control system controls Valve 3 so that its ports 2 and 13 are in communication with each other and controls Valve 5 so that its ports 5 and 13 are in communication with

each other. The apparatus is then configured to control pump 33 so as to urge the portions of sample and reducing agent from holding coil 32 to the holding coil 28 connected to port 5 of Valve 5 so that the reducing agent mixes with the sample. If it is desired to desalt the sample then the mixed sample may be held in the holding coil 28 connected to port 5 of Valve 5 while the desalting column is prepared, as described below. The desalting column may be a porous graphitic carbon (PGC) column.

The desalting column 35 may be prepared by washing it through with desalting buffer and optionally also a solvent solution such as NaOH. The apparatus is configured to automatically load the desalting buffer into the desalting column by the valve control system controlling Valve 3 such that its ports 12 and 13 are in communication with each other, and by controlling pump 33 so as to draw the desalting buffer into port 12, out of port 13 and into holding coil 32. The valve control system is configured to then control Valve 3 such that its ports 11 and 13 are in communication with each other, and pump 33 is controlled so as to pump the desalting buffer from holding coil 32, into port 13, out of port 11 and into the desalting column 35. If the desalting column is desired to also be washed with NaOH then Valve 3 may be controlled in a corresponding manner in order to load NaOH from port 4 of Valve 3 into holding column 32, and then to urge the NaOH from holding coil 32 to the desalting column.

The sample that is being held in the holding coil 28 connected to port 5 of Valve 5 is then returned to holding coil 32. This is performed in the same manner as previously described, i.e. by the valve control system controlling Valve 3 so that its ports 2 and 13 are in communication with each other and controlling Valve 5 so that its ports 5 and 13 are in communication with each other, and controlling pump 33 so as to urge the sample from the holding coil 28 connected to port 5 of valve back to holding coil 32.

The apparatus is configured to then automatically move the sample from holding coil 32 into the desalting column 35. In order to do this, the valve control system is configured to control Valve 3 such that its ports 11 and 13 are in communication with each other and to control Valve 4 such that its port 2 is in communication with its port 3 or 6. Pump 33 is then controlled so as to pump the sample from holding coil 32 into port 13 of Valve 3, out of port 11 of Valve 3, and into the desalting column.

After the sample has been loaded into the desalting column, the apparatus is configured to flush the desalting column with the desalting buffer. The apparatus is configured to automatically do this by loading the desalting buffer into holding coil 32 and then subsequently moving the desalting buffer to the desalting column. As such, the valve control system is configured to control Valve 3 such that its ports 12 and 13 are in communication with each other, and by controlling pump 33 so as to pump the desalting buffer into port 12, out of port 13 and into holding coil 32. The valve control system is configured to then control Valve 3 such that its ports 11 and 13 are in communication with each other, and pump 33 is controlled so as to pump the desalting buffer from holding coil 32, into port 13, out of port 11 and into and through the desalting column. Whilst this flushing is occurring, the valve control system controls Valve 4 such that one end of the sample loop 36 connected to Valve 4 is in communication with port 2 so as to enable the sample loop to receive the sample from the desalting column, and the other end of the

sample loop may be in communication with port 1 so that excess material can be sent to waste. The flushing of the desalting column with the desalting buffer therefore washes the desalted sample into the sample loop and the excess material to waste.

5 The apparatus is configured to then move the desalted sample from the sample
loop 36 connected to Valve 4 to holding coil 32. In order to do this, the valve control
system is configured to control Valve 4 such that one end of the sample loop is in
communication with port 4 of Valve 4 and to control Valve 3 such that its ports 10 and 13
are in communication with each other. Pump 33 is then controlled so as to draw the
sample from the sample loop 36 through port 4 of Valve 4, into port 10 of Valve 3, out of
10 port 13 of Valve 3 and into holding coil 32.

The apparatus is configured to then transfer a portion of the sample, such as half
the mixture, to the degasser 27 in the same manner as has been described above, i.e. the
valve control system controls Valves 3 and 5 so as to move the sample to the degasser 27
connected to port 4 of Valve 5.

15 The apparatus is configured to then automatically transfer an enzyme such as
trypsin into holding coil 32 for digesting the protein in the sample. The previously
described desalting step is preferably performed before this, as salts can inhibit the
digestion of the protein by the enzyme. However, the desalting step could be omitted
and/or the sample may be diluted instead so as to reduce the concentration of the salts.
20 Such a dilution may be performed by controlling Valve 3 such that ports 1 and 13 are in
communication, pumping the dilution buffer to holding coil 32 using pump 33, and then
mixing the sample and dilution buffer, e.g. by moving these fluids to the holding coil 28
connected to port 5 of Valve 5 and then back to holding coil 32. In either case, in order to
mix the enzyme with the sample, the apparatus is configured to transfer a portion of the
25 sample in holding coil 32, such as half the sample, to the degasser 27 in the same manner
as has been described above. The apparatus is configured to then transfer the enzyme
such that it resides in holding coil 32 with the portion of the sample that has not been
transferred to the degasser. More specifically, the valve control system controls Valve 3 so
that its ports 9 and 13 are in communication with each other, and the apparatus then
30 controls pump 33 so as to urge the enzyme into port 9, out of port 13 and into holding coil
32. The portion of the sample that was transferred to the degasser is then returned to
holding coil 32, in which the remainder of the sample and enzyme are located. The
apparatus is configured to perform this in the same manner as has been described above.
As such, the enzyme is located between two portions of the sample in holding coil 32.

35 The apparatus is configured to cause the portions of sample and enzyme to mix by
using a pump to urge these liquids back and forth within a holding coil, within another fluid
line in the system or between multiple holding coils. For example, the valve control system
may control Valves 3 and 5 such that holding coil 32 is in fluid communication with the
holding coil 28 connected to port 5 of Valve 5. More specifically, the valve control system
40 controls Valve 3 so that its ports 2 and 13 are in communication with each other and
controls Valve 5 so that its ports 5 and 13 are in communication with each other. The
apparatus is then configured to control pump 33 so as to urge the portions of sample and
enzyme from holding coil 32 to the holding coil 28 connected to port 5 of Valve 5, and then

back to holding coil 32. This transfer from holding coil 32 to the other holding coil 28 and back again may be performed one or more times.

After mixing, the apparatus is configured to cause the mixture to be transferred from holding coil 32 to the reaction coil 29 connected to port 6 of Valve 5. More specifically, the valve control system controls Valve 3 so that its ports 2 and 13 are in communication with each other and controls Valve 5 so that its ports 6 and 13 are in communication with each other. The apparatus is then configured to control pump 33 so as to urge the sample from holding coil 32 to the reaction coil. The reaction coil may be pre-heated, for example to a temperature of about 37 degrees Celsius, such that when the sample is received in the reaction coil it is heated. This may assist in the digestion of the protein in the sample, especially as the enzyme may have been stored in refrigeration prior to being added to the sample. The apparatus is configured such that after a preselected duration in the reaction coil, the sample is then transferred back to holding coil 32. More specifically, the valve control system maintains ports 6 and 13 of Valve 5 in communication with each other and ports 2 and 13 of Valve 3 in communication with each other, and then controls pump 33 so as to urge the sample from reaction coil to holding coil 32.

Optionally, the apparatus is configured to perform a quenching step by supplying a quenching buffer, such as an acid, to the sample. The apparatus may automatically perform this by being configured to transfer a portion of the sample, such as half the mixture, from holding coil 32 to the degasser 27 in a corresponding manner to that which has been described above. The apparatus is configured to then transfer the quenching buffer into holding coil 32 such that it resides in the holding coil with the portion of the sample that has not been transferred to the degasser. More specifically, the quenching buffer may be added to holding coil 32 by the valve control system controlling Valve 5 such its ports 3 and 13 are in communication with each other, controlling Valve 3 such its ports 2 and 13 are in communication with each other, and then using pump 33 to draw the quenching buffer into port 3 of Valve 5, out of port 13 of Valve 5, into port 2 of Valve 3, out of port 13 of Valve 3 and into holding coil 32.

The portion of the sample that was transferred to the degasser 27 is then returned to holding coil 32, in which the remainder of the sample and the quenching buffer are located. The apparatus is configured to automatically perform this in the same manner as has been described above. As such, the quenching buffer is located between two portions of the sample in holding coil 32.

The apparatus is configured to cause the portions of sample and quenching buffer to mix by using a pump to urge these liquids back and forth within a holding coil, within another fluid line in the system or between multiple holding coils. For example, the valve control system may control Valves 3 and 5 such that holding coil 32 is in fluid communication with the holding coil 28 connected to port 5 of Valve 5. More specifically, the valve control system controls Valve 3 so that its ports 2 and 13 are in communication with each other and controls Valve 5 so that its ports 5 and 13 are in communication with each other. The apparatus is then configured to control pump 33 so as to urge the portions of sample and quenching buffer from holding coil 32 to the holding coil 28 connected to

port 5 of Valve 5, and then back to holding coil 32. This transfer from holding coil 32 to the other holding coil and back again may be performed one or more times.

The apparatus is configured to then automatically send the sample to the LC device for LC-MS analysis. In order to do this, the valve control system is configured to control Valve 3 such that its ports 2 and 13 are in communication with each other, control Valve 5 such that its ports 1 and 13 are in communication with each other, control Valve 6 such that its ports 2 and 4 are in communication with each other, and control Valve 7 such that its ports 1 and 4 are in communication with each other. Pump 33 is then able to push the sample from holding coil 32 into port 13 of Valve 3, out of port 2 of Valve 3, into port 13 of Valve 5, out of port 1 of Valve 5, into port 1 of Valve 6, out of port 4 of Valve 6, into port 4 of Valve 7, out of port 1 of Valve 7, and into the LC device. The valve control system may then be configured to control Valve 7 such that its ports 1 and 6 are in communication with each other such that a pump is able to pump solvent into port 6 of Valve 7, out of port 1 of Valve 7 and into the LC device so as to cause the sample to elute from the LC device to an ionisation source of a mass spectrometer. The ionisation source is arranged and configured to ionise the eluent and the resulting ions are then mass analysed in the mass spectrometer.

After the sample has been processed in the above manner, all of the fluid lines and valves that the sample has passed through on the way to Valve 7 may be washed out by pumping a solvent, such as NaOH, through these fluid lines to waste. For example NaOH may be supplied into port 8 of Valve 0 for cleaning the fluid lines between Valve 0 and Valve 1. NaOH may be supplied into port 1 of Valve 1 for cleaning Valve 1, Valve 2, the fluid lines between Valves 1 and 2, the sample loops connected to Valve 2, and the fluid line between Valves 2 and 5. NaOH may be supplied into port 4 of Valve 3 for cleaning the remaining fluid lines and valves that the sample has passed through, such as Valves 5, 6 and 7.

Although methods have been described above in which the system pumps the agents from their own respective individual sources into the system and adds them to the sample separately it is contemplated that, additionally or alternatively, multiple ones of these agents may be provided as a mixture and the system is configured to be able to pump this mixture of agents from a source of the mixture to the sample. Additionally, or alternatively, the system may be configured to pump multiple agents from their own respective sources into the system and mix the agents together within the system prior to adding these agents to the sample.

For example, the denaturing agent, reducing agent and neutralising buffer may be provided as a mixture in the same solution, which is connected to a port of one of the valves, such as port 8 of Valve 5. The mixture may be added to holding coil 32 by the valve control system controlling Valve 5 such its ports 8 and 13 are in communication with each other, controlling Valve 3 such its ports 2 and 13 are in communication with each other, and then using pump 33 to draw the mixture into port 8 of Valve 5, out of port 13 of Valve 5, into port 2 of Valve 3, out of port 13 of Valve 3 and into holding coil 32. In order to assist in mixing the sample with the mixture of agents, a portion of the sample may be transferred out of the holding coil before the mixture of agents is transferred into the

holding coil, that portion of the sample may then be returned to the holding coil such that the mixture of agents is sandwiched between portions of the sample, and a pump may move these liquids so as to cause them to mix together. For instance, this may be performed by the liquids being pumped by pump 33 to the holding coil 28 connected to port 5 of Valve 5 and then back to holding coil 32.

Alternatively, the denaturing agent, reducing agent and neutralising buffer may be pumped into the system from their own respective sources and mixed together within the system prior to any of these agents being supplied to the sample. This may be performed, for example, by sequentially loading the agents into holding coil 32 and mixing them together in a corresponding manner to the mixing procedure described above. The mixture of agents may then be combined with the sample, e.g. in holding coil 32, and mixed therewith in a corresponding manner to the mixing procedure described above.

Fig. 2 is a flow chart showing some the sample processing techniques that the apparatus described herein may be configured to perform. At step 200 the system pumps the sample into the sample processing system. At step 202 the system determines the titer value for a protein of interest in the sample. As described above, the system may automatically control either the volume of the sample or the concentration of the sample (by the system automatically diluting the sample) that is subsequently processed, based on the titer value determined. The system may have a user interface configured for a user to input which of these two options the system should perform in response to the titer value determined, and the system automatically performs that routine in response to the selection.

The user interface is also configured to enable the user to select the manner in which the sample is to be processed, such as by enabling the user to select the agents that are to be added to the sample to perform such sample processing and/or to select whether agents that are to be added to the sample should be added to the sample simultaneously or separately. At step 204 the system determines which inputs have been made at the user interface and proceeds to one of steps 206, 214 or 216 based on this determination. For example, the system may proceed to step 206, in which it adds a mixture of a denaturing agent and a neutralising agent to the sample. Alternatively, the denaturing agent and neutralising agent may be added to the sample in separate steps, such as in steps 206 and 208. If step 204 involved selecting that a reduction agent is to be added to the sample then the reduction agent is added at step 210. Similarly, if step 204 involved selecting that an alkylation agent is to be added to the sample then the alkylation agent is added at step 212.

Alternatively, the denaturing agent, neutralising agent and reduction agent may be provided to the system pre-mixed. If the system is connected to a source containing such a mixture then the user input at step 204 may cause the system to perform step 214, in which the mixture is supplied to the sample.

Alternatively, the user input at step 204 may enable the user to select the agents that are to be mixed prior to being supplied to the sample. The system then proceeds to step 216 in which the system pumps the agents from their respective separate sources and mixes them, prior to supplying the resulting mixture to the sample.

As described above, after the agents have been mixed with the sample and prior to digestion, the system may be configured to desalt the sample or to dilute the sample so as to lower the concentration of the salts therein. The user interface is configured to enable a user to input which of these two routines the system should perform. At step 218, the system determines which of the two routines has been input by the user and controls the system to perform the dilution routine at step 220 or the desalting routine at step 222.

The system is then controlled to perform step 224, in which it adds the digestion agent to the sample. The system may then be controlled to perform optional step 226, in which a different digestion agent is added to the sample.

As described above, the system may be configured to add a quenching agent to the sample. The user interface is configured to enable a user to input if this is desired. At step 227, the system determines whether the user has input that a quenching step should be performed. If there is no input indicating that quenching should be performed then the system proceeds to perform step 228, in which the sample is sent to the LC column and then to the mass spectrometer for mass analysis. Alternatively, if the system determines that there has been a user input that a quenching step should be performed, then the system may perform step 230 in which the quenching agent is added to the sample.

After the sample has been mass analysed in step 228, the system proceeds to perform step 232 in which the solvent for cleaning the fluid lines and valves is pumped through the system (e.g. NaOH).

Although the present invention has been described with reference to various embodiments, it will be understood by those skilled in the art that various changes in form and detail may be made without departing from the scope of the invention as set forth in the accompanying claims.

For example, although embodiments have been described in which Valve 4 is directly connected to Valve 3 by two fluid lines so that the sample is able to be desalted in desalting column 35 between Valve 3 and Valve 4, it is contemplated that Valve 4 may instead be directly connected to Valve 5 by two corresponding fluid lines so that the sample is able to be desalted in desalting column 35 between Valve 5 and Valve 4. In such embodiments, one of the ports of Valve 5 may be connected to the source of desalting buffer instead of one of the ports of Valve 3. In such embodiments the desalting process is performed in a corresponding manner to that which has been described above, except that the sample is transmitted to the desalting column and Valve 4 from Valve 5 instead of from Valve 3.

Although an example has been described above in which the sample is prepared for a peptide MAM analysis, it will be appreciated that the systems and methods described herein may be configured and used to perform other forms of analysis. For example, after purifying the sample in column 22, the valves and pumps may be controlled so that the sample is sent to the LC-mass spectrometer so as to mass analyse the intact proteins in the sample, i.e. without them having been digested etc.

Embodiments have been described in which the system automatically obtains a titer or dilution value for the protein of interest based on the response of a protein concentration sensor 24, but the system may also be used to determine the calibration relationship that

relates the response of the sensor 24 to the concentration determined for the protein in the sample. When a sample having an unknown protein concentration is then analysed the response of the sensor and the calibration relationship may be used to determine the concentration of the protein in the sample, and the titer or dilution value may be selected based on this determined concentration.

In order to generate the calibration relationship, the system may be configured to sequentially generate and analyse a plurality of calibration samples that have different known concentrations of the protein therein. The stock solution for making up these calibration samples may be stored in refrigerated compartment 31 and/or may be the calibrant connected to port 8 of Valve 1. The user interface of the system is configured for the user to input the concentration of the protein that is in the stock solution, which enables the system to automatically prepare the plurality of calibration samples having the different known concentrations of the protein therein. In order to analyse these calibration samples the apparatus is configured to automatically prepare the purification column 22 in the manner that has been described above, i.e. by automatically rinsing the column with an elution buffer, and then automatically rinsing the column with a binding buffer. The apparatus is configured to then automatically prepare a first of the calibration samples having a known concentration of the protein therein, e.g. by loading the holding coil 14 with a first known volume of the stock solution, and also loading binding buffer at the same time. The calibration sample and buffer are then pumped the column 22 such that the sample binds to the column. Further binding buffer may be supplied to the column in order to rinse off excess sample that has not bound to the column so that it is flushed to waste. The apparatus is configured to then automatically supply elution buffer, and optionally also binding buffer, to the column in order to cause sample that has bound to the column to elute such that the calibration sample passes to the detector 24. The detector 24 obtain a response that is representative of the known concentration of the protein of interest that is present in the sample. The apparatus is configured to then automatically prepare and analyse the next calibration sample in a corresponding manner to that described above, where the next calibration sample has a second, different known concentration of the protein therein, so as to obtain a detector response that is representative of the second known concentration. The apparatus repeats this process until all of the plurality of calibration samples have been prepared and analysed by detector 24, preferably in a manner such that each subsequent sample is prepared with a higher concentration of the protein than the previous sample. The known concentration and the detector response for each calibration sample are then used to form the calibration relationship.

Embodiments have been described in which the processed sample is sent to an LC mass spectrometer for analysis. The system may be configured to determine a calibration relationship that relates the response of the mass spectrometer to the concentration of a compound of interest in the sample. For example, when the spent cell culture media is being analysed the compound of interest may be an amino acid, vitamin, or metabolite etc. When a sample is then analysed the response of the mass spectrometer and the calibration relationship may be used to determine the quantity or concentration of the compound of interest in the sample. In order to generate the calibration relationship, the

system may be configured to sequentially generate and analyse a plurality of calibration samples that have different known concentrations of the compound of interest therein. The stock solution for making up these calibration samples may be stored in refrigerated compartment 31 and/or may be connected to a port of Valve 1. The user interface of the system is configured for the user to input the concentration of the compound of interest that is in the stock solution, which enables the system to automatically prepare the plurality of calibration samples having the different known concentrations of the compound of interest therein. The apparatus is configured to automatically prepare a first of the calibration samples having a known concentration of the compound of interest therein by loading the holding coil 14 with a first known volume of the stock solution. The calibration sample is then pumped to the mass spectrometer and mass analysed so as to obtain a response that is representative of the known concentration of the compound of interest that is present in the sample. The apparatus is configured to then automatically prepare and mass analyse the next calibration sample in a corresponding manner to that described above, where the next calibration sample has a second, different known concentration of the compound of interest therein, so as to obtain a mass spectrometer response that is representative of the second known concentration. The apparatus repeats this process until all of the plurality of calibration samples have been prepared and analysed by the mass spectrometer. The known concentration and the mass spectrometer response for each calibration sample are then used to form the calibration relationship.

The system described herein may be used in a size exclusion chromatography experiment. The user may input into the system user interface a target protein concentration to analyse. The system may then purify the sample in column 22 in a corresponding manner to that described above until the target protein concentration has eluted to sample loop 25. The valves and pumps may then be controlled so that the sample from sample loop 25 is sent to the LC-mass spectrometer so as to mass analyse the intact proteins in the sample, i.e. without then having been digested etc.

The systems and methods described herein may be configured and used to perform a mAb analysis.

The systems and methods described herein may be configured and used to perform a glycan analysis. A glycan analysis may be performed by performing at least some of the steps described above, such as obtaining a titer value for the sample, denaturing and neutralising the sample, reducing the sample, digesting the sample and optionally diluting the sample. The steps of the analysis may be performed in a corresponding manner to the analysis described above, except optionally using different and/or additional reagents. For instance, Rapigest may be used to denature the sample in order to enhance the digestion of the sample by the enzyme (e.g. PNGase F). The Rapigest may be introduced to the sample and mixed therewith in a corresponding manner to that described above for the other reagents, e.g. by introducing it into the holding coil 32 via an input port of Valve 3. After digestion, a labelling reagent such as Rapifluor may be used to label components of the sample with a fluorophore or ionisation enhancer, i.e. so that it can be detected by fluorescence detection or mass analysis. The sample may then be mixed with a dilution reagent in a corresponding manner to that described above for the

other reagents, e.g. by arranging the sample and dilution reagent in holding coil 32 and then mixing them by passing them to holding coil 28 and back to holding coil 32. In the glycan analysis the desalting column 35 may be replaced with a porous graphitic carbon (PGC) column or a hydrophilic interaction liquid chromatography (HILIC) column. This column may be washed with a glycan elution buffer (e.g. water) and a glycan binding buffer (e.g. acetonitrile), e.g. by pumping these buffers into the holding coil 32 and then to the column in a corresponding manner to that in which preparation of the desalting column has been described. The sample and glycan buffer may then be loaded into holding coil 32 and pumped to the PGC/HILIC column. A glycan elution buffer is then loaded into holding coil 32 and pumped to the PGC/HILIC column so as to capture the material eluting from the column in sample loop 36. The sample from sample loop may then be diluted, e.g. by being passed to holding coil 32 along with dilution buffer, passing the resulting mixture to holding coil 28 and back to holding coil 32. The diluted sample may then be sent to the LC-mass spectrometer for analysis.

Although examples have been described above of how a single aliquot (i.e. portion) of the sample in holding coil 5 may be drawn into holding coil 14 and then processed and mass analysed, it is contemplated that one or more further aliquot (i.e. portion) of the same sample in holding coil 5 may be drawn into holding coil 14 and then processed and mass analysed. The one or more further aliquot may be processed in the same manner that the preceding aliquot was, or in a different manner. For instance, an embodiment has been described above in which the aliquot is prepared for a peptide MAM analysis, but one or more other aliquots may be prepared for a different analysis such as by adding agents for a glycan analysis.

The further aliquot may be processed during or after the processing of the preceding aliquot. By way of example, whilst a first aliquot of a sample is being processed by having agents added to it when it is in holding coil 32, a further aliquot of the sample may be being processed upstream, such as by being pumped from holding coil 14 through the separation column 22 (e.g. Pro-A column) and protein concentration sensor 24 so as to determine the titer value.

Alternatively, rather than the system processing multiple aliquots (i.e. portions) of the same sample during overlapping timescales, the system may process samples from different sources of sample during overlapping timescales. For example, after a first sample has been moved from holding coil 5 to holding coil 14 and whilst it is being processed, a second different sample may be moved to holding coil 5. The second sample may then be processed in the same manner that the preceding aliquot was, or in a different manner.

In embodiments that process multiple portions of a sample or multiple different samples in overlapping timescales, one or more additional reaction coil may be provided so that the different portions or different samples can be sent to different reaction coils. Additionally, or alternatively, one or more additional pump may be provided so that the different portions or different samples can be pumped, e.g. to their respective reaction coils.

Although embodiments have been described herein in which each source of agent is only supplied to a single port of one of the valves, it is contemplated that each of one or more sources of agents may be connected to multiple ports of the same valve and/or to ports of multiple valves.

Claims:

1. An automated sample processing system for preparing a sample comprising polypeptides for analysis, comprising:
 - a first valve having a plurality of ports connected to a plurality of respective fluid lines, and configured such that a fluid path through the first valve is switchable between different pairs of its ports;
 - a second valve having a plurality of ports connected to a plurality of respective fluid lines, and configured such that a fluid path through the second valve is switchable between different pairs of its ports, where a port of the first valve is in fluid communication with a port of the second valve;
 - a third valve having a plurality of ports connected to a plurality of respective fluid lines, and configured such that a fluid path through the third valve is switchable between different pairs of its ports, where a port of the third valve is in fluid communication with a port of the first valve or a port of the second valve;
 - a first fluid holding coil connected to a port of the second valve;
 - a second fluid holding coil connected to a port of the third valve; and
 - at least one pump for pumping fluid through the system.
2. The system of claim 1, comprising a liquid chromatography separator for separating a sample processed by the system and/or a mass spectrometer for mass analysing a sample processed by the system.
3. The system of claim 1 or 2, comprising control circuitry configured to automatically control the first, second and third valves and the at least one pump so as to: pump a first portion of a first sample comprising polypeptides into the system and into the first fluid holding coil; introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil; and pump a second portion of the first sample into the second fluid holding coil, whilst the first portion of the sample is in the system.
4. The system of claim 1 or 2, comprising control circuitry configured to automatically control the first, second and third valves and the at least one pump so as to: pump a first sample comprising polypeptides into the system and into the first fluid holding coil; introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil; and pump a second sample comprising polypeptides into the system and into the second fluid holding coil, whilst the first sample is in the system.
5. The system of claim 1 or 2, comprising control circuitry configured to automatically control the first, second and third valves and the at least one pump so as to: pump a first sample, or a first portion of a first sample, comprising polypeptides into the system and into

the first fluid holding coil; and introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil.

- 5 6. The system of claim 3, 4 or 5, wherein the control circuitry is configured to automatically control the valves and the at least one pump so as to introduce a second, different agent for preparing the first sample for analysis, or first portion of the first sample, into the system, and transmit it to the first fluid holding coil.
- 10 7. The system of any one of claims 3-6, wherein the control circuitry is configured to automatically control the valves and the at least one pump so as to either:
- 15 (i) pump the first portion of the first sample out of the first fluid holding coil; then to pump the second portion of the first sample from the second fluid holding coil to the first fluid holding coil; and then to introduce at least one agent for preparing the second portion of the sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil to meet the second portion of the sample; or
- 20 (ii) pump the first sample out of the first fluid holding coil; then to pump the second sample from the second fluid holding coil to the first fluid holding coil; and then to introduce at least one agent for preparing the second sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil to meet the second sample.
- 25 8. The system of any one of claims 3-7, wherein the control circuitry is configured to automatically control the first and/or second valves and the at least one pump so as to:
- 30 i) load the first agent and first portion of the sample, or first sample, into the first fluid holding coil such that the first agent is sandwiched between volumes of sample in the first fluid holding coil, or such that the sample is sandwiched between volumes of the first agent, and then control the at least one pump so as to move the sandwiched first agent and sample along one or more fluid lines within the system so as to cause the first agent and sample to mix together; and/or
- 35 ii) load the second agent and first portion of the sample, or first sample, into the first fluid holding coil such that the second agent is sandwiched between volumes of sample in the first fluid holding coil, or such that the sample is sandwiched between volumes of the second agent, and then control the at least one pump so as to move the sandwiched second agent and sample along one or more fluid lines within the system so as to cause the second agent and sample to mix together.
- 40 9. The system of claim 8, wherein the control circuitry is configured to automatically control the at least one pump and the first and/or second valve so as to:
- (i) move the sandwiched first agent and sample from the first fluid holding coil to another fluid holding coil in the system so as to cause the first agent and sample to mix together; and optionally to then return the sample to the first fluid holding coil; and/or

(ii) move the sandwiched second agent and sample from the first fluid holding coil to another fluid holding coil in the system so as to cause the second agent and sample to mix together; and optionally to then return the first sample to the first fluid holding coil.

- 5 10. The system of claim 8 or 9, wherein said one or more fluid lines comprises a degasser configured to remove air from the sandwiched first agent and sample, and/or from the sandwiched second agent and sample, as it is transmitted along said one or more fluid lines so as to cause the agent and sample to mix together.
- 10 11. The system of any preceding claim, wherein a source of a, or the, first agent is connected to a port of the first and/or second valve by a fluid line, and the source of the first agent is a source of denaturing agent for denaturing the polypeptides.
- 15 12. The system of any claim 11, wherein a source of a, or the, second agent is connected to a port of the first and/or second valve by a fluid line, and the source of the second agent is a digestion agent, such as an enzyme, for digesting the polypeptides.
- 20 13. The system of claim 12, wherein a source of a quenching agent for inhibiting a digestion agent from digesting the polypeptides is connected to a port of the first and/or second valve by a fluid line; and the control circuitry is configured to automatically control the first and/or second valves and the at least one pump so as to pump the quenching agent from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of a sample or first sample is located in the first fluid holding coil.
- 25 14. The system of any one of claims 11-13, wherein a source of a neutralising agent for neutralising the pH of a sample is connected to a port of the first and/or second valve by a fluid line; and the control circuitry is configured to automatically control the first and/or second valve and the at least one pump so as to pump the neutralising agent from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of a sample or first sample is located in the first fluid holding coil.
- 30 15. The system of any preceding claim, wherein a source of a reducing agent for breaking disulphide bonds of the polypeptides is connected to a port of the first and/or second valve by a fluid line; and the control circuitry is configured to automatically control the first and/or second valves and the at least one pump so as to pump the reducing agent from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of a sample or the first sample is located in the first fluid holding coil.
- 40 16. The system of claim 15, wherein a source of an alkylating agent for preventing disulphide bonds of the polypeptides reforming is connected to a port of the first and/or

second valve by a fluid line; and the control circuitry is configured to automatically control the first and/or second valves and the at least one pump so as to pump the alkylating agent from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of a sample or first sample is located in the first fluid holding coil.

17. The system of any preceding claim, wherein a source containing a mixture of a neutralising agent for neutralising the pH of a sample and a reducing agent for breaking disulphide bonds of the polypeptides and/or a denaturing agent for denaturing the polypeptides is connected to a port of the first and/or second valve by a fluid line; and the control circuitry is configured to automatically control the first and/or second valves and the at least one pump so as to pump the mixture from its source into the port of the valve and, optionally, to a first portion of a sample or a first sample while the first portion of the sample or first sample is located in the first fluid holding coil.

18. The system of any preceding claim, comprising a reaction coil having a heater coupled thereto, connected via a fluid line to a port of one of the first or second valves; wherein the control circuitry is configured to automatically control the heater to heat the reaction coil and control the valves and the at least one pump so as to pump the sample to the heated reaction coil.

19. The system of any preceding claim, comprising a desalting column connected, via a fluid line, to a port of one of the first or second valves; wherein the control circuitry is configured to perform a first routine in which it automatically controls the valves and the at least one pump so as to pump the sample through the desalting column so as to desalt the sample; and/or

comprising a source of diluent for diluting the sample; wherein the control circuitry is configured to perform a second routine in which it automatically controls the valves and the at least one pump so as to pump the source of diluent to the sample so as to dilute salts in the sample.

20. The system of claim 19, wherein the system comprises a user interface configured to enable a user to select which of the first or second routines the system is to operate; and wherein the system is configured to perform the selected first or second routine in response thereto.

21. The system of any preceding claim, comprising a refrigeration compartment, and a liquid chromatography separator for separating a sample processed by the system and/or a mass spectrometer for mass analysing a sample processed by the system;

wherein the system is configured to pump a portion of the sample processed by the system to the liquid chromatography separator and/or a mass spectrometer, and to pump at least part of the remainder of the sample processed by the system to the refrigeration compartment.

22. The system of any preceding claim, wherein one of the first and second valves is located in a refrigeration compartment; and optionally wherein the other of the first or second valves is located outside of the refrigeration compartment.

23. The system of claim 22, wherein the valve located in the refrigeration compartment comprises at least one port connected to at least one respective fluid line for transmitting fluid from at least one source of fluid in the refrigerated compartment into said at least one port; wherein each of said at least one fluid lines has a length of ≤ 20 cm.

24. The system of claim 23, wherein the at least one source of fluid in the refrigerated compartment is at least one of: a reducing agent for breaking disulphide bonds of the polypeptides; an alkylating agent for preventing disulphide bonds of the polypeptides combining; and a digestion agent, such as an enzyme, for digesting the polypeptides.

25. The system of claim 22, 23 or 24, wherein one or more sources of the following agents are connected to a port of said other of the first and second valves: a source of denaturing agent for denaturing the polypeptides; a neutralising agent for neutralising the pH of the sample; and a quenching agent for inhibiting a digestion agent from digesting the polypeptides; and wherein this one or more sources are located outside of the refrigerated compartment.

26. The system of any preceding claim, wherein the third valve is in fluid communication with a port of the first valve, and the system comprises a fourth valve in fluid communication with a different port of the first valve, wherein the third and fourth valves are in fluid communication in a manner such that fluid can pass from the third valve to the fourth valve whilst bypassing the first and second valves, and comprising a mass spectrometer in fluid communication with the fourth valve;

wherein the control system is configured to operate in a first mode in which it automatically controls the third valve, the first valve, optionally the second valve, and the at least one pump so as to pump a first portion of the sample, or a first sample, into the first fluid holding coil for a first agent to be added thereto; and at a subsequent time to control these valves and the fourth valve so that this first portion of the sample or the first sample is pumped to the mass spectrometer; and/or

wherein the control system is configured to operate in a second, different mode in which it automatically controls the third and fourth valves and the at least one pump so as to pump a different portion of the sample or a different sample to the fourth valve and then to the mass spectrometer whilst bypassing the first and second valves and the first holding coil.

27. The system of claim 26, comprising a sensor for determining the concentration of a protein or polypeptide in the first portion of the sample, or the first sample, passing through

a fluid line between the third valve and the first valve, in the first mode, and producing a signal representative of the determined concentration;

wherein the control system is configured to automatically perform a third routine that controls the amount of a first agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil for mixing with the sample based on said signal representative of the determined concentration, and/or control the amount of a second agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil for mixing with the sample based on said signal; and/or

wherein the control system is configured to perform a fourth routine that controls the amount of a first agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil so as to be a first pre-determined amount, and controls the volume of the sample transmitted to the first fluid holding coil for mixing with the first agent based on said signal representative of the determined concentration and based on said first pre-determined amount.

28. The system of claim 27, comprising a user interface configured to enable a user to select which of the third or fourth routines the system is to operate; and wherein the system is configured to perform the selected third or fourth routine in response thereto.

29. The system of claim 27 or 28, comprising a separation column, such as a purification column, between the third valve and the sensor for separating the protein or polypeptide from other components in the sample such that the protein or polypeptide arrives at the sensor separated from the other components.

30. The system of claim 29, wherein a source of binding buffer is connected to a port of the third valve, wherein the system is configured to automatically prepare the separation column, prior to pumping sample to the separation column, by controlling the third valve and the one or more pump so as to pump the binding buffer from its source to the separation column.

31. The system of claim 29 or 30, wherein a source of elution buffer is connected to a port of the third valve, wherein the control circuitry is configured to cause the sample to elute from the separation column to the sensor, after the sample has been supplied to the separation column, by controlling the third valve and the one or more pump so as to pump the elution buffer to the separation column.

32. The system of any one of claims 27-31, comprising a fifth valve between the sensor and the first valve, the fifth valve having a port connected to the sensor by a fluid line, a port connected to a fluid line for supplying sample to the first valve, a port connected to the third valve by a fluid line that bypasses the sensor, ports connected to a first sample loop, and ports connected to a second sample loop; wherein the control circuitry is configured, in the first mode, to control the fifth valve and the at least one pump such that sample is

pumped from the sensor into the first sample loop and such that, in a further mode, sample is pumped from the third valve into the second sample loop through the fluid line that bypasses the sensor.

- 5 33. The system of claim 32, wherein the system comprises a user interface configured to enable a user to select which of the first or further modes the system is to operate; and wherein the system is configured to perform the selected first or further mode in response thereto.
- 10 34. The system of any one of claims 26-33, comprising a source of diluent connected to the fluid line between the third and fourth valves and a pump for pumping the diluent; wherein the control circuitry is configured, in the second mode, to control the pump so as to pump diluent into the fluid line between the third and fourth valves at the time that the sample is passing therethrough.
- 15 35. The system of any one of claims 26-34, comprising a sixth valve having a port connected to the fourth valve for receiving sample from the fourth valve, ports connected to a first sample loop, ports connected to a second, different sample loop, and a port in fluid communication with a mass spectrometer by a fluid line; wherein the control circuitry is
- 20 configured to control the sixth valve and the at least one pump such that sample is pumped from the fourth valve into one of the first or second sample loop that is connected to the sixth valve.
- 25 36. The system of claim 35, wherein the first and second sample loops have different sample receiving volumes, wherein the system comprises a user interface configured to enable a user to select a method of processing or analysing the sample from a plurality of different methods, and wherein the control system is configured to selectively supply the sample to either the first or the second sample loop in response to the selected method.
- 30 37. The system of claim 35, wherein the system is configured to control the fourth and sixth valves such that sample is supplied from the fourth valve into the first sample loop connected to the sixth valve whilst sample is pumped from the second sample loop connected to the sixth valve through the port in fluid communication with the mass spectrometer; and
- 35 optionally wherein the system is configured to control the fourth and sixth valves such that sample is supplied from the fourth valve into the second sample loop connected to the sixth valve whilst sample is pumped from the first sample loop connected to the sixth valve through the port in fluid communication with the mass spectrometer.
- 40 38. The system of claim 35, 36 or 37, wherein a liquid chromatography separator is provided in the fluid line between the mass spectrometer and the port of the sixth valve that is in fluid communication with the mass spectrometer, for separating the sample being provided to the mass spectrometer.

39. The system of any preceding claim, comprising a seventh valve having a plurality of ports, wherein a plurality of sources of sample are connected to the plurality of ports by a plurality of respective fluid lines; and wherein the control circuitry is configured to control the seventh valve and the one or more pumps so as to select which source of sample to pump through the system.
40. The system of claim 39, wherein the sources of sample are bioreactors.
41. A method of preparing a sample comprising polypeptides for analysis, comprising:
providing a sample preparation system comprising: a first valve having a plurality of ports connected to a plurality of respective fluid lines; a second valve having a plurality of ports connected to a plurality of respective fluid lines, where a port of the first valve is in fluid communication with a port of the second valve; a third valve having a plurality of ports connected to a plurality of respective fluid lines, where a port of the third valve is in fluid communication with a port of the first valve or a port of the second valve; a first fluid holding coil connected to a port of the second valve; a second fluid holding coil connected to a port of the third valve; and at least one pump for pumping fluid through the system; and
controlling the ports in each of the first, second and third valves that are connected to each other and the at least one pump so as to pump the sample through the valves to at least one of the fluid holding coils.
42. The method of claim 41, comprising pumping the sample to a liquid chromatography separator and/or mass spectrometer.
43. The method of claim 42, comprising pumping the sample through a chromatography separator so as to separate it prior to mass analysing the sample in the mass spectrometer.
44. The method of claim 41, 42 or 43, comprising controlling the first, second and third valves and the at least one pump so as to: pump a first portion of a first sample comprising polypeptides into the first fluid holding coil; introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil; and pump a second portion of the first sample into the second fluid holding coil, whilst the first portion of the sample is in the system.
45. The method of claim 41, 42 or 43, comprising controlling the first, second and third valves and the at least one pump so as to: pump a first sample comprising polypeptides into the system and into the first fluid holding coil; introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil; and pump a second different sample comprising

polypeptides into the system and into the second fluid holding coil, whilst the first sample is in the system.

46. The method of claim 41, 42 or 43, comprising controlling the first, second and third valves and the at least one pump so as to: pump a first sample, or a first portion of a first sample, comprising polypeptides into the system and into the first fluid holding coil; and introduce at least a first agent for preparing the first sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil.
47. The method of claim 44, 45 or 46, comprising controlling the valves and the at least one pump so as to introduce into the system a second, different agent for preparing the first sample, or first portion of the first sample, for analysis, and transmit the second agent to the first fluid holding coil.
48. The method of any one of claims 44-47, comprising controlling the valves and the at least one pump so as to either:
- (i) pump the first portion of the first sample out of the first fluid holding coil; then to pump the second portion of the first sample from the second fluid holding coil to the first fluid holding coil; and then to introduce at least one agent for preparing the second portion of the sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil to meet the second portion of the sample; or
 - (ii) pump the first sample out of the first fluid holding coil; then to pump the second sample from the second fluid holding coil to the first fluid holding coil; and then to introduce at least one agent for preparing the second sample for analysis into the system via a port of the first or second valve, and transmit it to the first fluid holding coil to meet the second sample.
49. The method of any one of claims 44-48, comprising controlling the first and/or second valves and the at least one pump so as to:
- i) load the first agent and first portion of the sample, or first sample, into the first fluid holding coil such that the first agent is sandwiched between volumes of sample in the first fluid holding coil, or such that the sample is sandwiched between volumes of the first agent, and then control the at least one pump so as to move the sandwiched first agent and sample along one or more fluid lines within the system so as to cause the first agent and sample to mix together; and/or
 - ii) load the second agent and first portion of the sample, or first sample, into the first fluid holding coil such that the second agent is sandwiched between volumes of sample in the first fluid holding coil, or such that the sample is sandwiched between volumes of the second agent, and then control the at least one pump so as to move the sandwiched second agent and sample along one or more fluid lines within the system so as to cause the second agent and sample to mix together.

50. The method of claim 49, comprising controlling the at least one pump and the first and/or second valve so as to:

(i) move the sandwiched first agent and sample from the first fluid holding coil to another fluid holding coil in the system so as to cause the first agent and sample to mix together; and optionally to then return the sample to the first fluid holding coil; and/or

(ii) move the sandwiched second agent and sample from the first fluid holding coil to another fluid holding coil in the system so as to cause the second agent and sample to mix together; and optionally to then return the first sample to the first fluid holding coil.

51. The method of claim 49 or 50, wherein said one or more fluid lines comprises a degasser that removes air from the sandwiched first agent and sample, and/or from the sandwiched second agent and sample, as it is transmitted along said one or more fluid lines so as to cause the agent and sample to mix together.

52. The method of any one of claims 41-51, wherein a source of a, or the, first agent is connected to a port of the first and/or second valve by a fluid line, and the source of the first agent is a source of denaturing agent for denaturing the polypeptides; optionally wherein the method comprises controlling the first and/or second valves and the at least one pump so as to pump the first agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

53. The method of any one of claims 41-52, wherein a source of a, or the, second agent is connected to a port of the first and/or second valve by a fluid line, and the source of the second agent is a digestion agent, such as an enzyme, for digesting the polypeptides; optionally wherein the method comprises controlling the first and/or second valves and the at least one pump so as to pump the second agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

54. The method of claim 53, wherein a source of a quenching agent for inhibiting a digestion agent from digesting the polypeptides is connected to a port of the first and/or second valve by a fluid line; and the method comprises controlling the first and/or second valves and the at least one pump so as to pump the quenching agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

55. The method of any one of claims 52-54, wherein a source of a neutralising agent for neutralising the pH of a sample is connected to a port of the first and/or second valve by a fluid line; and the method comprises controlling the first and/or second valve and the at least one pump so as to pump the neutralising agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

56. The method of any one of claims 41-55, wherein a source of a reducing agent for breaking disulphide bonds of the polypeptides is connected to a port of the first and/or second valve by a fluid line; and the method comprises controlling the first and/or second valves and the at least one pump so as to pump the reducing agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

57. The method of claim 56, wherein a source of an alkylating agent for preventing disulphide bonds of the polypeptides reforming is connected to a port of the first and/or second valve by a fluid line; and the method comprises controlling the first and/or second valves and the at least one pump so as to pump the alkylating agent from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

58. The method of any one of claims 41-57, wherein a source containing a mixture of a neutralising agent for neutralising the pH of a sample and a reducing agent for breaking disulphide bonds of the polypeptides and/or a denaturing agent for denaturing the polypeptides is connected to a port of the first and/or second valve by a fluid line; and the method comprises controlling the first and/or second valves and the at least one pump so as to pump the mixture from its source into the port of the valve and, optionally, to a or the first portion or first sample while the first portion or first sample is located in the first fluid holding coil.

59. The method of any one of claims 41-58, wherein the system comprises a reaction coil having a heater coupled thereto, connected via a fluid line to a port of one of the first or second valves; wherein the heater is heated so as to heat the reaction coil, and wherein the valves and the at least one pump are controlled so as to pump a or the first portion of sample, or first sample, to the heated reaction coil after an agent for preparing the sample has been added to it.

60. The method of any one of claims 41-59, wherein the system comprises a desalting column connected, via a fluid line, to a port of one of the first or second valves; wherein the method comprises performing a first routine in which the valves and the at least one pump are controlled so as to pump the sample through the desalting column so as to desalt the sample; and/or

wherein the method comprises performing a second routine in which the valves and the at least one pump are controlled so as to pump diluent to the sample so as to dilute salts in the sample.

61. The method of claim 60, wherein the system comprises a user interface configured to enable a user to select which of the first or second routines the system is to operate;

wherein a user selects the first or second routine at the user interface, and wherein the system performs the selected first or second routine in response thereto.

- 5 62. The method of any one of claims 41-61, comprising pumping a portion of the sample processed by the system to a liquid chromatography separator and/or mass spectrometer, and pumping at least part of the remainder of the sample processed by the system to a refrigeration compartment.
- 10 63. The method of any one of claims 41-62, wherein one of the first and second valves is located in a refrigeration compartment; and optionally wherein the other of the first or second valves is located outside of the refrigeration compartment.
- 15 64. The method of claim 63, comprise transmitting fluid from at least one source of fluid located in the refrigerated compartment into at least one port of the valve located in the refrigeration compartment via at least one respective fluid lines; wherein the at least one source of fluid in the refrigerated compartment is at least one of: a reducing agent for breaking disulphide bonds of the polypeptides; an alkylating agent for preventing disulphide bonds of the polypeptides combining; and a digestion agent, such as an enzyme, for digesting the polypeptides.
- 20 65. The method of claim 62, 63 or 64, wherein one or more sources of the following agents are connected to a port of said other of the first and second valves: a source of denaturing agent for denaturing the polypeptides; a neutralising agent for neutralising the pH of the sample; and a quenching agent for inhibiting a digestion agent from digesting the polypeptides; and wherein this one or more sources are located outside of the refrigerated compartment.
- 25 66. The method of any one of claims 41-65, wherein the third valve is in fluid communication with a port of the first valve, and the system comprises a fourth valve in fluid communication with a different port of the first valve, wherein the third and fourth valves are in fluid communication in a manner such that fluid can pass from the third valve to the fourth valve whilst bypassing the first and second valves, and comprising a mass spectrometer in fluid communication with the fourth valve;
- 30 wherein the method comprises operating in a first mode in which the third valve, the first valve, optionally the second valve, and the at least one pump are controlled so as to pump a first portion of the sample, or a first sample, into the first fluid holding coil and to add a first agent thereto; and at a subsequent time controlling these valves and the fourth valve so that this first portion of the sample or the first sample is pumped to the mass spectrometer; and/or
- 35 wherein the method comprises operating in a second, different mode in which the third and fourth valves and the at least one pump are controlled so as to pump a different portion of the sample or a different sample to the fourth valve and then to the mass spectrometer whilst bypassing the first and second valves and the first holding coil.
- 40

67. The method of claim 66, comprising:
using a sensor to determine the concentration of a protein or polypeptide in a or the first portion of a sample, or in a or the first sample, passing through a fluid line between the third valve and the first valve, in the first mode, and produce a signal representative of the determined concentration;
performing a third routine that comprises controlling the amount of a first agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil for mixing with the sample based on said signal representative of the determined concentration, and/or controlling the amount of a second agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil for mixing with the sample based on said signal; and/or
performing a fourth routine that comprises controlling the amount of a first agent introduced into the system via a port of the first or second valve and transmitted to the first fluid holding coil so as to be a first pre-determined amount, and controlling the volume of the sample transmitted to the first fluid holding coil for mixing with the first agent based on said signal representative of the determined concentration and based on said first pre-determined amount.
68. The method of claim 67, wherein the system comprises a user interface configured to enable a user to select which of the third or fourth routines the system is to operate; wherein a user selects the third or fourth routine at the user interface, and wherein the system performs the selected third or fourth routine in response thereto.
69. The method of claim 67 or 68, wherein the system comprises a separation column, such as a purification column, between the third valve and the sensor for separating the protein or polypeptide from other components in the sample, and wherein the method comprises pumping sample through the separation column such that the protein or polypeptide arrives at the sensor separated from the other components.
70. The method of claim 69, wherein a source of binding buffer is connected to a port of the third valve, wherein the method comprises preparing the separation column, prior to pumping sample to the separation column, by controlling the third valve and the one or more pump so as to pump the binding buffer from its source to the separation column.
71. The method of claim 69 or 70, wherein a source of elution buffer is connected to a port of the third valve, wherein the method comprises causing sample to elute from the separation column to the sensor, after the sample has been supplied to the separation column, by controlling the third valve and the one or more pump so as to pump the elution buffer to the separation column.
72. The method of any one of claims 67-71, wherein the system comprises a fifth valve between the sensor and the first valve, the fifth valve having a port connected to the sensor

by a fluid line, a port connected to a fluid line for supplying sample to the first valve, a port connected to the third valve by a fluid line that bypasses the sensor, ports connected to a first sample loop, and ports connected to a second sample loop; wherein the first mode comprises controlling the fifth valve and the at least one pump such that sample is pumped
5 from the sensor into the first sample loop and such that, in a further mode, sample is pumped from the third valve to the fifth valve through the fluid line that bypasses the sensor and into the second sample loop.

73. The method of claim 72, wherein the system comprises a user interface configured
10 to enable a user to select which of the first or further modes the system is to operate; wherein a user selects the first or further mode at the user interface, and wherein the system performs the selected first or further mode in response thereto.

74. The method of any one of claims 66-73, wherein the second mode comprises
15 pumping diluent into the fluid line between the third and fourth valves at the time that the sample is passing therethrough.

75. The method of any one of claims 66-74, wherein the system comprises a sixth
20 valve having a port connected to the fourth valve for receiving sample from the fourth valve, ports connected to a first sample loop, ports connected to a second, different sample loop, and a port in fluid communication with a mass spectrometer by a fluid line; wherein the method comprises controlling the sixth valve and the at least one pump such that sample is pumped from the fourth valve into one of the first or second sample loop that is connected to the sixth valve.

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76. The method of claim 75, wherein the first and second sample loops have different sample receiving volumes, wherein the system comprises a user interface configured to enable a user to select a method of processing or analysing the sample from a plurality of different methods, wherein a user selects a method of processing or analysing the sample
30 at the user interface, and wherein the system supplies the sample to either the first or the second sample loop in response to the selected method.

77. The method of claim 75, comprising controlling the fourth and sixth valves such that
35 sample is supplied from the fourth valve into the first sample loop connected to the sixth valve whilst sample is pumped from the second sample loop connected to the sixth valve through the port in fluid communication with the mass spectrometer; and
optionally wherein the method controls the fourth and sixth valves such that sample is supplied from the fourth valve into the second sample loop connected to the sixth valve whilst sample is pumped from the first sample loop connected to the sixth valve through the
40 port in fluid communication with the mass spectrometer.

78. The method of claim 75, 76 or 77, comprising separating sample using a liquid chromatography separator in the fluid line between the mass spectrometer and the port of the sixth valve that is in fluid communication with the mass spectrometer.

5 79. The method of any one of claims 41-78, wherein the system comprises a seventh valve having a plurality of ports, and wherein the method comprises connecting a plurality of sources of sample to the plurality of ports by a plurality of respective fluid lines, and controlling the seventh valve and the one or more pumps so as to select which source of sample to pump through the system.

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80. The method of claim 79, wherein the sources of sample are bioreactors.

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