biochrom
a division of
Harvard Bioscience, Inc.

BIOCHROM BIO 30+
AMINO ACID ANALYSER
USER MANUAL

PLEASE READ THIS MANUAL CAREFULLY BEFORE USING THE BIO 30+ SERIES AMINO ACID ANALYSER.

5061-058-REV-12 – JAN 2023

Description of Version Changes

Date	Version #	List of changes
01/03/2010	1	• Initial version
17/08/2011	2	• Part number for finger tight fittings changed from 80-2104-45 to 80-6002-13 to reflect the use of the new high-pressure fittings. • Section 4.6.4: BAM software description and operations improved.
30/01/2012	3	• Section 4.6.5 updated to reflect the introduction of EZChrom Elite v3.3.2. • Introduction of new buffer names
21/05/2012	4	• New screenshot of Biosys v3.0.1 and Alias Manager v2.0 under Windows 7. • Revised installation process of Biosys and Alias Manager under Windows 7.
9/05/2013	5	• Added new part number for new GL-45 bottle cap in section 8.
28/10/2013	6	• Updated with OpenLAB CDS EZChrom Edition software. • Biosys/Alias and OpenLAB software installation and setup moved to the software suite installation guide v2.0
09/07/2014	7	• New columns part numbers added in section 8 and throughout the manual. • Reference to EZ Nin added. • Tidy up of section 7
11/09/2015	8	• User manual part number added. Biochrom address changed
25/08/2017	9	• New pumps reference added. New calibration standards reference added. New Biochrom header/footer design added.
06/08/2019	10	• Compatibility with Windows 10 added. New physiological calibration standard reference added.
06/05/2022	11	• Updated as per EU IVDR requirements and removed nonclinical applications. .
05/01/2023	12	• Removed abbreviation for CSF from section 00. • Updated Intended use under section 1.1. • Section 1.3 point 13 - Updated chemical shelf life of reagent from ‘1 month’ to ’14 days’. • Updated section 1.6 title as ‘European Authorised Representative’ • Updated section 2.5 to remove sample types other than blood. • Updated section 5.1.14 to remove sample preparation methods.

		• Updated section 6.2.1 to give reference of Reagent IFU. • Updated sections 8.2.1, 8.2.2, 8.2.5,and 8.2.6 to add part no. for products Ultrosolve Plus and Ultra Ninhydrin solution. • Updated reference in section 8.2.10 from 'ISO Guide 34' to 'ISO 17034'. • Removed section 9.6.Appendix F – Sample preparation.

General Index

0 Bio 30+ Part Numbers, Warranty and Liability.

Part Number & Description

Part Number	Description
80-6000-50	Bio 30+ System Physio Accel
80-6000-51	Bio 30+ Physio Accelerated
80-6000-52	Bio 30+ Physio Accel W/O A/S
80-6000-53	Bio 30+ System Physio HP
80-6000-53EZ	Bio 30+ Sys Physio HP EZ Nin
80-6000-54	Bio 30+ Physio High Performance
80-6000-55	Bio 30+ Physio W/O A/S HP
80-6000-56	Bio 30+ System Physio HR
80-6000-57	Bio 30+ Physiological HR
80-6000-58	Bio 30+ Physio W/O A/S HR

Warranty and Liability

Biochrom Ltd guarantee that the product supplied has been thoroughly tested to ensure that it meets its published specification. This warranty may only be valid if the product has been used within its specification and in all respects has been operated and maintained in a normal, proper manner in accordance with the Instruction Manual.

Chromatographic performance may only be guaranteed to our specification if the columns, chemicals and reagents used are provided or approved by Biochrom Ltd.

Use of non Biochrom approved columns, reagents, etc, may invalidate this warranty.

The Bio 30+ Amino Acid Analyser System hardware is guaranteed for a period of 12 months from date of installation or 15 months from date of dispatch. whichever is sooner. This warranty does not cover the analytical column or the pre-wash column.

Due to the susceptibility of the columns to poor sample preparation, these are only guaranteed for a period of 3 months from date of installation.

The general warranty conditions may not be valid if the defect is due to accident, unauthorised modification, unauthorised repair attempt, incorrect operation or transport damage.

Biochrom Ltd can accept no liability for loss or damage, however caused, arising from the faulty or incorrect use of this product.

Pictorials used in this manual

The following pictorials are used in this guide:

The danger sign warns about a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in injury or loss of life.

Do not proceed beyond a danger sign until the indicated conditions are fully understood and met.

The warning sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in severe injury or damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.

The caution sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in damage or destruction of parts or all of the equipment. Do not proceed beyond a cautions sign until the indicated conditions are fully understood and met.

The attention sign signals relevant information. Read this information, as it might be helpful.

The note sign signals additional information. It provides advice or a suggestion that may support you in using the equipment.

Abbreviation of Terms

Terms	Abbreviation
GHS	Globally Harmonized System
PTFE	Polytetrafluoroethylene
PEEK	Polyether Ether Ketone
PVC	Polyvinyl Chloride
CV	Coefficient of Variation
RSD	Relative Standard Deviation
PKU	Phenylketonuria
Phe	Phenylalanine
Tyr	Tyrosine
HP	High Performance
HR	High Resolution
SSA	Sulphosalicylic Acid
EDTA	Ethylenediaminetetraacetic Acid
HPLC	High-Performance Liquid Chromatography
HCl	Hydrochloric Acid
IPA	Isopropyl Alcohol/Isopropanol
ERNDIM	European Research Network for Evaluation and Improvement of Screening, Diagnosis, and Treatment of Inherited Disorders of Metabolism.
CLSI	Clinical Laboratory Standards Institute, previously known as National Committee for Clinical Laboratory Standards (NCCLS)
OD	Optical Density

1 SAFETY AND REGULATION

CONTENTS

1 SAFETY AND REGULATION

1.1 Intended Use

The Biochrom Bio 30+ Amino Acid Analyser is a fully automated laboratory instrument intended as an aid to the diagnosis of phenylketonuria by trained professionals. The Biochrom Bio 30+ Amino Acid Analyser is designed to provide quantitative analysis of phenylalanine and tyrosine present in physiological samples using ion-exchange chromatography in combination with post-column derivatization using ninhydrin. Physiological samples of human origin limited to de-proteinised blood plasma samples.

The Biochrom Bio 30+ Amino Acid Analyser is not intended to be used as a standalone diagnostic test for any patient condition or disease and is for in vitro use only.

The Biochrom Bio 30+ Amino Acid Analyser is intended to be used for the detection and quantification of amino acids in physiological samples. Where appropriate regulatory approval has been sought, this includes the quantitative analysis of phenylalanine and tyrosine as an aid in the diagnosis and monitoring of Phenylketonuria (PKU) in patients belonging to any age group including neonates.

Fig 1.1 Bio 30+ layout

1.2 Technical Specification

Detection Limit	Ninhydrin - 9 picomoles.
Reproducibility	Area: Better than 1.5% CV. Retention time: Better than 0.5%.
Buffers	Up to five buffers and one regeneration solution, all stored in graduated plastic-coated glass bottles under nitrogen pressure. Reservoir volume is 1.0 litre.

Ninhydrin	Stored under nitrogen pressure in a 2.0 litre graduated plastic-coated glass bottle.
Pumps	Two independently controllable pumps with integrated pressure transducer. Ceramic pump head, sapphire pistons and valves, adjustable flow rate and time controlled automatic piston flush.
Operating Pressures	Buffer pressure: maximum 145 bar (minimum 15 bars). Ninhydrin pressure: maximum 24 bar (minimum 6 bars). Coil pressure: maximum 12 bar (minimum 2 bar).
Analytical Column	High pressure PEEK column with Peltier heating/cooling system.
Resin	Ultropac 8 cation-exchange resin, sodium or lithium form.
Sample Injection	3 injection modes, 84 positions cooled autosampler. Sample volumes from 1 to 5000µL. 200µL loop supplied as standard. PC controlled through software interface.
Temperature	Column temperature variable between 20°C and 99°C. Reaction Coil temperature adjustable between 40°C and 145°C (135°C is default).
Control Software	BioSys v4.1.2 software controls all the instrument functions from a PC via the RS232 serial interface port on the instrument and on the autosampler.
Photometer	Single flow cell with optical beam splitter to provide detection at 440nm and 570nm using ninhydrin and tungsten halogen lamp. Outputs to Integrator System: 570nm, 440nm and Sum of the two channels. Flow cell volume 8µL, path length 15mm.
Data Handling	OpenLAB CDS EZChrom Edition 2 channels with 0.05 to 50Hz sampling. Connected to PC via RJ45 No limit on number of data points. Data storage every 10 seconds.
Weight	Fluidics cabinet: 50kg Autosampler: 21kg (cooled version) 19kg (ambient version)
Dimensions (WxDxH)	Fluidics Cabinet : 48 x 59 x 57cm Autosampler: 30 x 57.5 x 36cm/30 x 51 x 36cm (ambient version)
Safety Systems	Automatic shut-down in the event of: Photometer lamp failure Low ninhydrin, buffer, coil and nitrogen pressures High coil and column temperatures, high ninhydrin, buffer and coil pressures Buffer pump running in manual mode for more than 90min.
Personal Computer	If the computer is supplied locally, it must be of the following minimum specification: •Intel Dual Core, 3GHz (or equivalent processor) •4 Gb RAM •200Gb Hard Disk Drive •Two serial ports. (USB-serial converters not acceptable) •LAN card

•Mouse, Keyboard & Monitor
•Windows 7 Professional 64-bit.
•Microsoft .NET framework v3.5 or higher

Interference and Cross-reactivity

Unidentified substances.
In both normal and diseased clinical samples there have always been substances that have never been identified. These are minor peaks and generally do not interfere with the detection of major metabolites. There remains the possibility that they may do so: it is recommended that clinical diagnosis does not rely solely on Amino Acid Analysis by Ion-Exchange and that complementary techniques are used.

Chemical interactions.
Generally, and consistent with first principles, amino acids do not interfere with each other chemically. There are exceptions:

- Amino-acids with disulphide bonds or sulphydryl groups can disproportionate and re-combine into mixed [assymetric] disulphides. The positions of the more common ones are known.
- Amino-acids can ligate around metal ions, particularly transition metals. This can be avoided during sample preparation [e.g. by addition of EDTA or any similar substance which the system will tolerate].
- Cyclisations, anhydride formation, thermal decomposition, Schiff-base formation, oxidation, reduction and other rare chemical reactions may occur in unusual situations. To minimise such events Biochrom supply Lithium Loading Buffer which will minimise the likelihood of such reactions by stabilising pH, concentration and preventing redox.

Drug interferences.
Many drugs are amino-acids or aliphatic amines. In addition, the metabolic breakdown products may also be detectable by our system and cause interference.
The elution position of some of the more important ones are known and set out in the table below.

Medication	Elution position
Penicillamine	between Methionine and Isoleucine
Augmentin	between NorLeucine and Tyrosine
Paracetamol	between Tyrosine and b-alanine
Cefotaxamine	between β-Alanine and Phenylalanine
Trimethoprim	between Phenylalanine and Aminoisobutyric Acid
Metranidazole	between γ- Aminobutyric Acid and Ethanolamine
Vigabatrim	between Ammonia and Hydroxylysine
Cephradin	between Ammonia and Ornithine
Cephalexin	between Tryptophan and 1-Methyl histidine

1.3 Analytical Performance Characteristics

1) The system time of analyses:

- Lithium Accelerated analysis: Maximum time to the Arginine peak is 110 min
- Lithium High Performance analysis: Less than 172 min injection to injection
- Lithium High Resolution analysis: Less than 265 min injection to injection

2) Limits of Detection: For selected amino acids the lower detection limit is not less than 15 picomoles. The upper detection limit [without resort to serial dilution] is not less than 10 nanomoles.
3) Limit of Quantification: For selected amino acids the lower quantification limit [using the recognised standard x5 multiplier] is not less than 75 picomoles.
4) Limit of Blank: No formal claim as the measure is subjective. Measured to be <5 picomoles.
5) Precision: This should be within the range <1% [570nm selected primary amino-acids] to <5% [440nm, selected secondary amino-acids].
6) Quantification Reproducibility: The Relative Standard Deviation [RSD or CV] between samples is better than 1.5% [Conditional upon the injected sample volume being >10µL].
7) Retention Time Reproducibility: better than 0.5% run-to-run.
8) Linearity: Tested over the range 12picomoles/20µL to 25nanomoles/20µL the regression coefficient [R2] shall be >0.998. Detector saturation [high-dose hook effect] is observed at 100 nanomoles.
9) Instrument-to-Instrument Reproducibility: Data from different instruments is comparable using visual inspection of chromatographic data. [Measured area variability <10%, and Peak Position variability <1.1min].
10) Carry over: Guidance on measuring carry-over shall be provided. Suitable blank liquid [Lithium Loading Buffer] will be provided. Advice on electronic compensation for carry-over [by data-subtraction] made available through provision of an Application Note.
11) Chemicals Effect on Reproducibility: Retention time differences occurring as a result of changing the chromatographic buffers does not exceed ± 1min between successively manufactured lot numbers. The detection reagents may not be mixed and no claim as to comparability between them is made. No warranty exists over materials not manufactured by Biochrom.
12) Chemical Shelf Life – Unopened: The chemicals are guaranteed to perform correctly for a period not less than three years if unopened and stored in the manner described on the labels.
13) Chemical Shelf Life – In-use: The reagent prepared from Ultranin and Ultrosolve Plus is usable for a period of up to 14 days when on the instrument provided they are kept out of direct sunlight.
14) Chemical Transportation - Effects of Normal Temperature Ranges: The chemical buffers and EZNin reagent performance is maintained when transported at normal temperatures.
15) Chemical Transportation - Effects of Exception Temperature Ranges (freeze thaw): The chemical buffers and EZNin reagent performance is maintained when exposed to freezing and thawing.

1.4 Clinical Performance Characteristics

Phenylketonuria (PKU). The device resolves chromatographically, and quantify by comparison with an external reference standard, Phenylalanine at clinically diagnostic levels [> 0.6mM] and at the upper reference range for the condition [>1.2mM] in blood plasma. Simultaneous confirmation of low levels [or undetectable levels] of Tyrosine is also required. [Phe and Tyr must be chromatographically resolved].

1.5 Manufacturer's Details

Any technical assistance regarding the Bio 30+, its accessories and consumables should be addressed to:

Biochrom Limited

Unit 7, Enterprise Zone

3970 Cambridge Research Park

Beach Drive, Waterbeach

Cambridge, United Kingdom, CB25 9PE

E-mail : support@biochrom.co.uk

Tel: +44 (0)1223 423723

1.6 European Authorised Representative

Medical Device Safety Service GmbH (MDSS)

Schiffgraben 41, Hannover 30175

Germany

1.7 Electrical Safety

The instrument's electrical system is built to very high safety standards. The mains supply must be earthed.

The instrument should be positioned so the main electrical panel on the left-hand side can be easily reachable in order to disconnect the mains supply.

WARNING: User servicing of the electronic unit is forbidden; only the instrument and autosampler fuses on the mains switch panel being user replaceable.

1.7.1 Electrical Supply and Fuse Rating

The voltage range within the instrument can be used is 110-240VAC 50/60Hz.

The fuse rating for the mains input are:

6.3A H T for operation at 110-240VAC

The fuse rating for the autosampler is:

2.5 AT for operation at 230VAC

5.0 AT for operation at 115VAC

If an electrical fault occurs, a service engineer must be called.

1.7.2 Input and Output Connections

The picture below shows the rear inlet panel that contains all the connections of the instrument.

Fig. 1.2 Mains Switch Panel

1.8 Installation Requirement

The Bio 30+ comprises three modules:

- Fluidics cabinet
- Autosampler unit
- Computer and monitor

When assembled, the Bio 30+ requires bench space of approximately 180 cm wide by 70 cm deep, including the computer.

Two mains power outlets are needed to supply

the instrument and the autosampler. Extra mains power outlets are needed for the computer, printer and accessories.

The equipment consumes Nitrogen, which vents into the laboratory. Users should abide by local regulations with respect to ventilating the work area when using inert gases.

CAUTION: The Bio 30+ must be installed by a Biochrom Approved Engineer. The customer should not attempt to install the instrument on its own.

1.8.1 Environmental Consideration

Required Services	Oxygen free nitrogen gas (99.99%), regulated to 5 bar. Drainage facility.
Ambient Operating Temperature	15°C to 25°C
Maximum Humidity	80% at 25°C

1.8.2 Special Considerations

The amino acid analyser must be used under analytical laboratory conditions with additional environmental considerations. Although normal clean laboratory conditions are usually quite satisfactory, it is useful to consider some special points to which attention should be paid: Atmospheric conditions should be relatively free of ammonia and carbon dioxide to avoid interference with measurement of amino acids. This is very important when working at high sensitivity.

1.8.3 Ventilation

Ensure that adequate ventilation is provided around the units. The separation between the autosampler and the instrument must be at least 5 cm to allow air to enter the ventilation slots on the instrument right hand side panel.

1.8.4 Lifting and Carrying

The instrument weight is approximately 50 kg, and the autosampler is 21kg. No lifting aids are supplied. If the instrument is moved from its location it must be carefully lifted from the bottom edges at either side. Never lift the instrument from the front as the door may come off the hinges.

Disconnect the autosampler mains cable, pipes and control cables before moving the instrument.

WARNING: Do not attempt to move the equipment on your own. Injuries may occur.

1.9 Replaceable Parts

Please see Section 8 Chemical kit and spare parts for more details.

1.10 Warning Signs Used on The Instrument

Hazard

This sign is located on the rear inlet panel next to the mains input. DO NOT PROCEED BEYOND THIS SIGN.

Hot surface

This sign is located on hot surfaces such as the reaction coil, the lamp cover and the column oven.

1.11 Chemicals Hazard

The Bio 30+ is designed to enable amino acid analyses to be performed safely and consistently. However, the chemicals used in amino acid analysis must be handled with care.

Specific chemical warnings are given below:

Product #	Description	GHS label element	Hazard statement	Prevention
80-2038-10	Lithium Loading Buffer		H319: Causes serious eye irritation	P264: Wash hands thoroughly after handling P280: Wear protective gloves/protective clothing/eye protection/face protection.
80-2038-20	Lithium Regeneration Buffer 6			
80-2117-64	Ultra Ninhydrin solution		H361: Suspected of damaging fertility or the unborn child	P201: Obtain special instructions before use P202: Do not handle until all safety precautions have been read and understood P281: Use personal protective equipment as required.
80-2117-65	Ultrosolve Plus		H302: Harmful if swallowed	P264: Wash hands thoroughly after handling. P270: Do not eat, drink or smoke when using this product.
80-6000-12	EZ Nin Reagent		H361: Suspected of damaging fertility or the unborn child	P201: Obtain special instructions before use P202: Do not handle until all safety precautions have been read and understood P281: Use personal protective equipment as required.

WARNING: Wear suitable protective clothing, gloves and eye/face protection when manipulating these chemicals.

1.11.1 First Aid

After contact with skin, wash immediately with plenty of water. Seek medical advice if irritation persists.

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

If any chemical is swallowed, seek medical advice immediately and show the container or label.

Please also refer to the Material Safety Data Sheets produced for each chemical. These can be accessed via http://www.biochrom.co.uk/msdssearch.asp

1.12 Device and Waste Disposal

The waste container must be located below the level of the instrument. It should be labelled according to local regulations. The waste produced by the Amino Acid Analyser is classified as harmful.

WARNING: Do not empty chemicals waste into drains, dispose of this material at hazardous or special waste collection point, according to local, state or federal regulations.

Device must be taken to separate collection at the end of their working life. Do not dispose of these products as unsorted municipal waste: take them for recycling. For info on your nearest recycling point, check with your local waste authority.

1.13 Cleaning and General Care

Use a damp cloth for cleaning external surfaces.

CAUTION: Do not use solvents on the external surfaces as this may damage the paintwork.

Wash any buffer or ninhydrin spillages immediately with distilled water.

1.14 Serious Incidents

Serious incident that has occurred in relation to the device shall be reported to the manufacturer/authorised representative and the competent authority of the Member State in which the user and/or the patient is established.

1.15 Basic Operating Principles

The sample containing a mixture of amino acids is loaded onto a column of cation-exchange resin. Buffers of varying pH and ionic strength are then pumped through the column to separate the various amino acids. The analytical column temperature is accurately controlled and can be varied, as necessary, to produce the required separation.

The column eluent is mixed with the ninhydrin reagent, this mixture being passed through the high temperature reaction coil. In the reaction coil, ninhydrin reacts with the amino acids present in the eluate to form coloured compounds. The amount of coloured compound produced is directly proportional to the quantity of amino acid present in the eluate.

From the reaction coil, the eluate/ninhydrin mixture is fed to the photometer unit where the amount of each coloured compound is determined by measuring the amount of light absorbed. The light absorption is measured at two wavelengths, 570nm and 440nm. This is because secondary amines such as Proline and Hydroxyproline produce coloured compounds which absorb light with a wavelength of 440nm, whereas other amino acid coloured compounds absorb light at 570nm.

The photometer output is connected to a PC controlled integration system which plots the amino acid concentrations as a series of peaks to form a chromatogram. The retention time of the peak on the chromatogram identifies the amino acid and the area under the peak indicates the quantity of amino acid present. As an amino acid analyser is a comparative instrument, a standardisation analysis must be performed before commencing a series of analyses, to produce a standard chromatogram for comparison purposes.

After each sample analysis, the column is regenerated by pumping a strong base through the column followed by the lowest pH buffer which equilibrates the column prior to the next analysis.

Operation of the Bio 30+ Amino Acid Analyser is completely automatic, all functions of the analyser being controlled by the Biosys software. Various analytical standard methods are supplied with the instrument. These methods, also known as programmes contain all the information required to perform the analysis. This information includes:

The selection of the various buffers by controlling the buffer solenoid valves

The control of the buffer and ninhydrin pumps

The loading of samples

The reaction coil and column temperatures

The time of each operation

The control of the data handling system

Various safety interlocks are present in the electronic unit to protect the instrument.

2 FUNCTIONAL DESCRIPTION

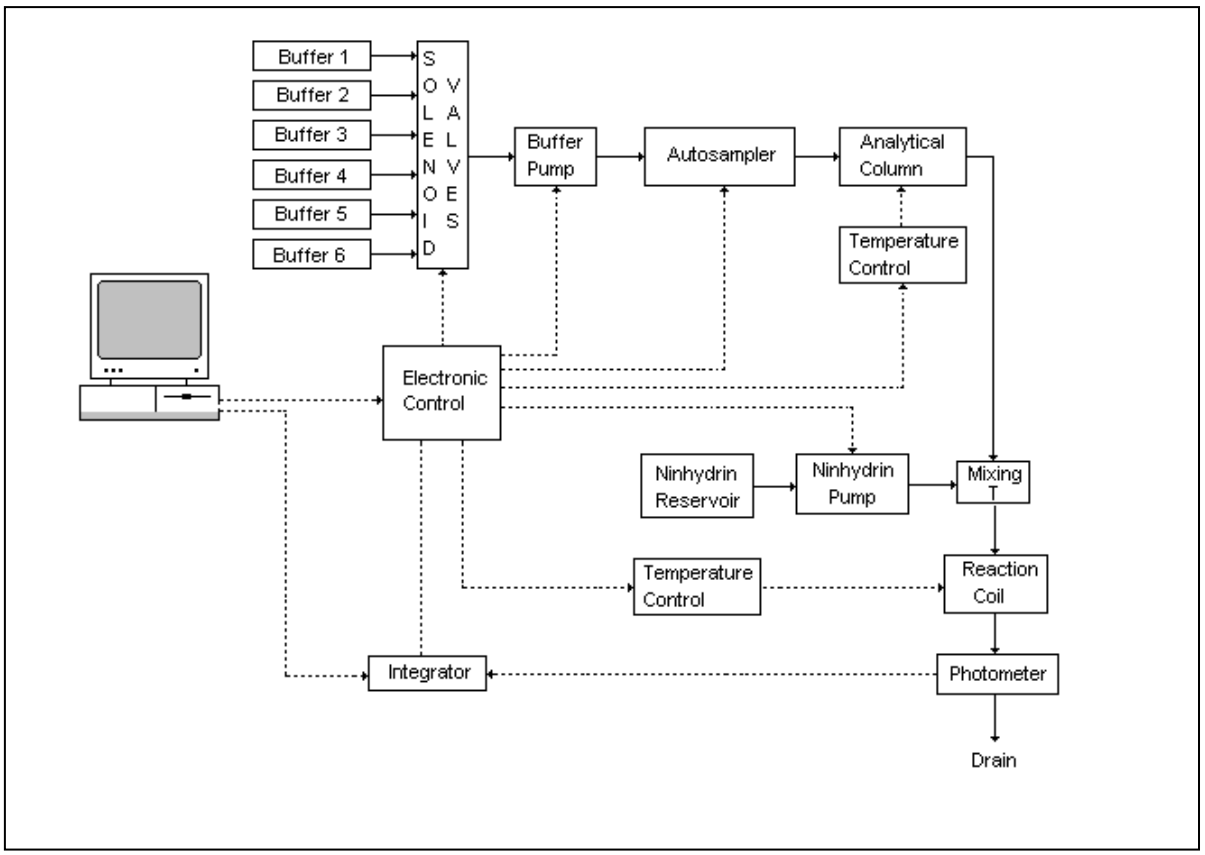
CONTENTS

2 FUNCTIONAL DESCRIPTION

2.1 Introduction

This section describes the control system and the fluidic system used in the Bio 30+ Amino Acid Analyser.

2.2 Control System



The control system is as shown in Fig 2.1. Five buffer solutions and the regeneration solution are fed via six solenoid valves to the buffer pump. These solenoid valves and the pump are controlled from the computer via the electronics unit which energises any one of the six solenoid valves and switches on the pump. The output from the buffer pump is connected to the prewash column and then to the Autosampler which loads the samples into the buffer flow path. A directional control valve (injection valve) in the autosampler switches the buffer either to bypass the sample loop or to flow through the sample loop. When the buffer flows through the sample loop, the sample is introduced into the buffer stream and is deposited onto the analytical column.

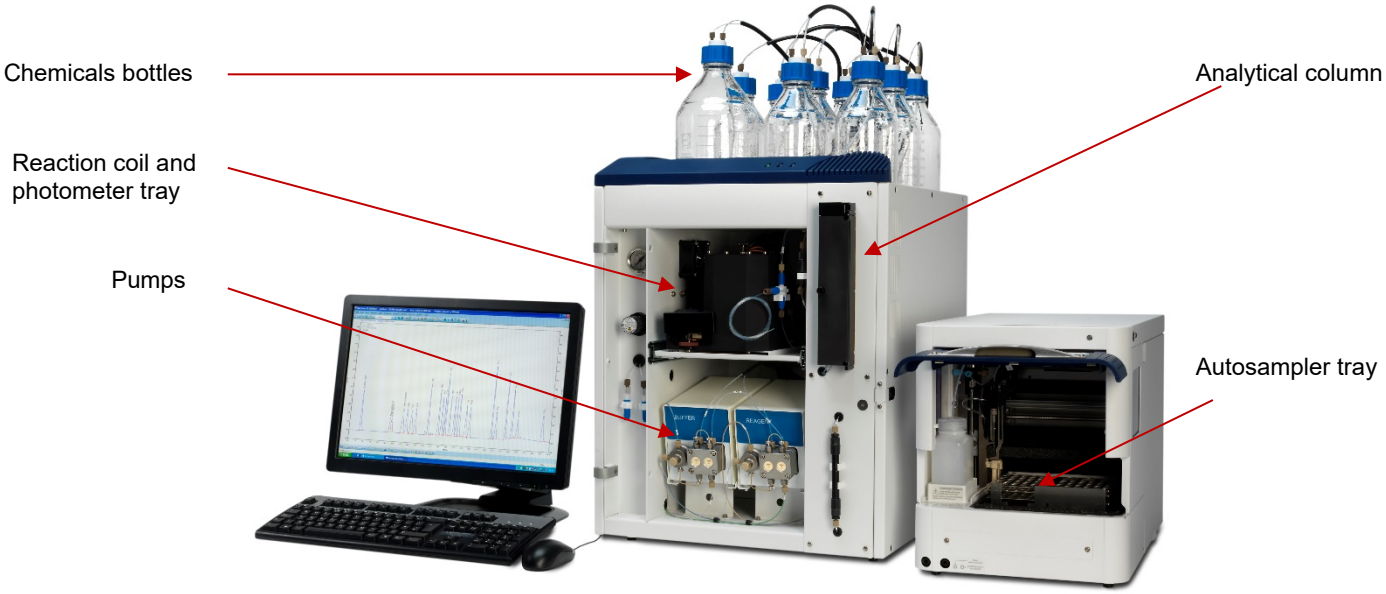


Figure 2.2 Bio 30+ general description

The temperature of the analytical column is regulated by two Peltier units controlled by the electronic system. When the sample has been loaded onto the column, the buffer solenoid valves are energised sequentially to deliver a series of successively higher pH buffer solutions. These buffers elute the amino acids from the cation-exchange resin, the eluate being fed to the mixing manifold. The second input to the mixing manifold is the ninhydrin supply which is delivered by the ninhydrin pump, as required by the program.

In the mixing manifold the eluate and ninhydrin are mixed. However, little reaction occurs between the ninhydrin and amino acids until they pass through the reaction coil. The reaction coil is contained in a solid state heating jacket, which is controlled by the electronic system and maintains the reaction coil at the selected temperature. In the reaction coil the ninhydrin and the amino acids in the eluate react, forming coloured compounds.

The coloured compounds from the reaction coil are fed to the photometer unit where the light absorption of these compounds is measured at two wavelengths, 440nm and 570nm. Electrical signals produced by the photometer are processed by the electronic system and sent to the data handling system. The photometer signals can also be sent to a chart recorder as a linear outputs. A two level 'fold-back' facility is provided by the amplifier to enable peak amplitudes of up to three times the chart width to be recorded. Separate linear outputs from 570nm and 440nm channels are supplied to the integration system. The liquid from the photometer unit is then taken to the waste container.

2.3 Fluidics System

2.3.1 Buffer Supply

The buffer storage area contains six bottles for buffers, the ninhydrin reagent and two extra bottles, one for the coil flush device, the other for the piston flush system. The bottles are supplied with low pressure nitrogen from the regulated nitrogen supply. A pressure regulator valve is used to decrease the main nitrogen pressure from 5 bar to the low pressure required by the bottles. Each of the buffer feed pipes is connected to a solenoid valve which, in the de-energised state prevents buffer flow. To select a particular buffer, the corresponding solenoid valve is energised. The solenoid valve outlets are connected through a manifold to the buffer pump inlet filter, which also removes air bubbles from the buffers.

From the filter, the buffer feed line is connected to the buffer pump inlet valve. The buffer pump is a high pressure, low volume, unit which provides buffer solution at a flow rate of up to 132ml/hr. An interlock system prevents the pump from being operated without first energising a buffer solenoid valve. A pressure transducer is integrated into the pump assembly which monitors buffer pressure. The pump assembly also contains a bleed valve. This valve is manually operated and directs the buffer either to the pre-wash column, or to the drain output. From the pump outlet, the buffer passes into the pre-wash column which removes ammonia present in the buffers. From the pre-wash column outlet, the buffer goes into the analytical column via the autosampler.

Sample loading is performed by placing the vial under the needle assembly then operating the needle to fill the sample loop with the required volume. The injection valve then operates to divert the buffer flow through the sample loop to deliver the sample to the analytical column.

When the sample has been loaded onto the analytical column, the buffer solenoid valves are operated sequentially to pump the buffers through the sample in the analytical column to effect the desired separation. The output from the column is connected to the mixing manifold.

2.3.2 Ninhydrin Supply

The ninhydrin supply to the pump is direct from the ninhydrin reservoir. A filter is fitted to the end of the ninhydrin feed line to prevent any dust being drawn into the pump. The ninhydrin reservoir is connected to the ninhydrin pump inlet filter, which also removes air bubbles from the ninhydrin solution.The ninhydrin pump is regulated to provide ninhydrin at a flow rate of up to 50ml/hr. The pump contains a bleed valve. This valve is manually operated and directs the ninhydrin either to the mixing manifold or to the drain. A pressure transducer is integrated into the pump to monitor the ninhydrin pressure. From the pump, the ninhydrin feed line is connected to a 3.5 bar back-pressure valve. This creates the back-pressure in the ninhydrin system. The ninhydrin feed line is connected directly from the back-pressure valve to the mixing manifold.

2.3.3 Reaction Coil

In the mixing manifold the ninhydrin and buffer eluate are mixed and then fed to the reaction coil. The reaction coil comprises a coil of PTFE tubing contained in a temperature controlled vessel maintained at the selected reaction temperature. In this coil the ninhydrin reacts with any amino acids present in the eluate to form coloured compounds, which are then fed to the photometer unit. The colour absorption is measured by the photometer unit, the pressure being maintained at approximately 3.5 bar by the back-pressure valve. The output from the reaction coil back-pressure valve is connected to the waste container.

2.4 Nitrogen Supply

The nitrogen supply from the cylinder is regulated to 5 bar at the cylinder and fed to the instrument. At a pressure of 5 bar, the nitrogen is used to operate the coil flush device. A pressure regulator valve produces 0.1 to 0.2 bar of gas for delivery to the buffer bottles, the coil flush bottle and the ninhydrin reagent bottle.

2.5 Use Of Buffer System

Physiological fluids are analysed using a lithium citrate based buffer system.Physiological fluids are referred to as samples containing most of the 40-50 compounds normally found in blood. A buffer system using 5 lithium citrate buffers is required to achieve a satisfactory separation.

3 FLUIDICS SYSTEMS

CONTENTS

3 FLUIDICS SYSTEM

3.1 Introduction

This section describes in detail the individual modules that comprise the fluidics system of the Bio 30+. To facilitate this, the instrument is divided into seven major areas and the modules in these areas described individually.

3.2 Buffer Storage Area

The buffer storage area is at the top of the fluidics cabinet, holding eight 1 litre graduated plastic coated glass bottles. Five of these bottles are designed to contain buffer solutions, one bottle is for the regenerating solution. The remaining two bottles are used for the coil flush device and for the automatic piston wash. The coil flush bottle generally contains a mixture of Isopropanol/Water 50/50 v/v. The piston wash bottle must contain only distilled water.

Nitrogen at a pressure of about 0.2 bar/3 psi is fed to all the bottles through their caps.

3.3 Ninhydrin Reservoir

The ninhydrin is stored in a plastic coated 2 litre glass bottle which is located at the top of the fluidics cabinet. This bottle is supplied with nitrogen, via the bottle cap, the ninhydrin feedline also passing through this cap. A filter cartridge attached to the end of the feedline rests on the bottom of the ninhydrin bottle.

3.4 Autosampler

3.4.1 General Description

The autosampler unit is located on the right hand side of the fluidics cabinet. A maximum of 84 sample vials can be loaded on the refrigerated sample tray. To load the sample, a vial is automatically placed under the needle assembly, the needle is inserted into the vial, the loop fills with the required volume then the injection valve switches to the inject position. The sample loop becomes part of the high pressure buffer line and the sample is transferred to the analytical column (see the autosampler manual for more information).

The injection valve is a high pressure switching valve. This valve controls the flow of buffer, directing it either through the loop or directly to the analytical column.

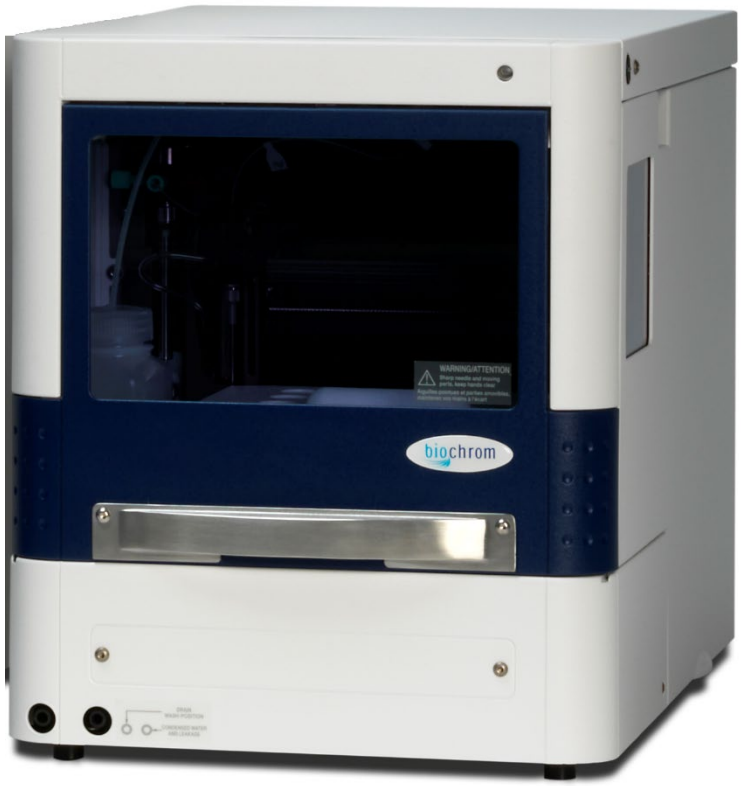


Fig 3.1 Biochrom Alias Autosampler

3.4.2 Injection Modes

To enable a series of analyses to be performed without operator intervention, the samples are introduced into the analytical system using the sample loop.

3 injection modes are available: Flushed loop, partial loop and microlitre pickup.

- Partial mode: A minimum of 1µl to a maximum of 100µl of sample may be loaded using the 200µl loop supplied.
- Microlitre pick-up mode: A minimum of 1µl to a maximum of 70µL can be loaded
- Flushed loop mode: the full loop volume can be injected (see autosampler manual for further information).

3.5 Analytical Column

The analytical column is mounted vertically on the front of the instrument, as shown below.

This high pressure, PEEK column is supplied as required for your individual analytical requirements. Cation exchange resin is packed into the column. The resin is retained within the column by PEEK frits.

The complete column is contained within a temperature stabilised aluminium block, the temperature is controlled by the programme. Peltier units provide both heating and cooling of the aluminium block. The cooling is assisted by a fan unit.

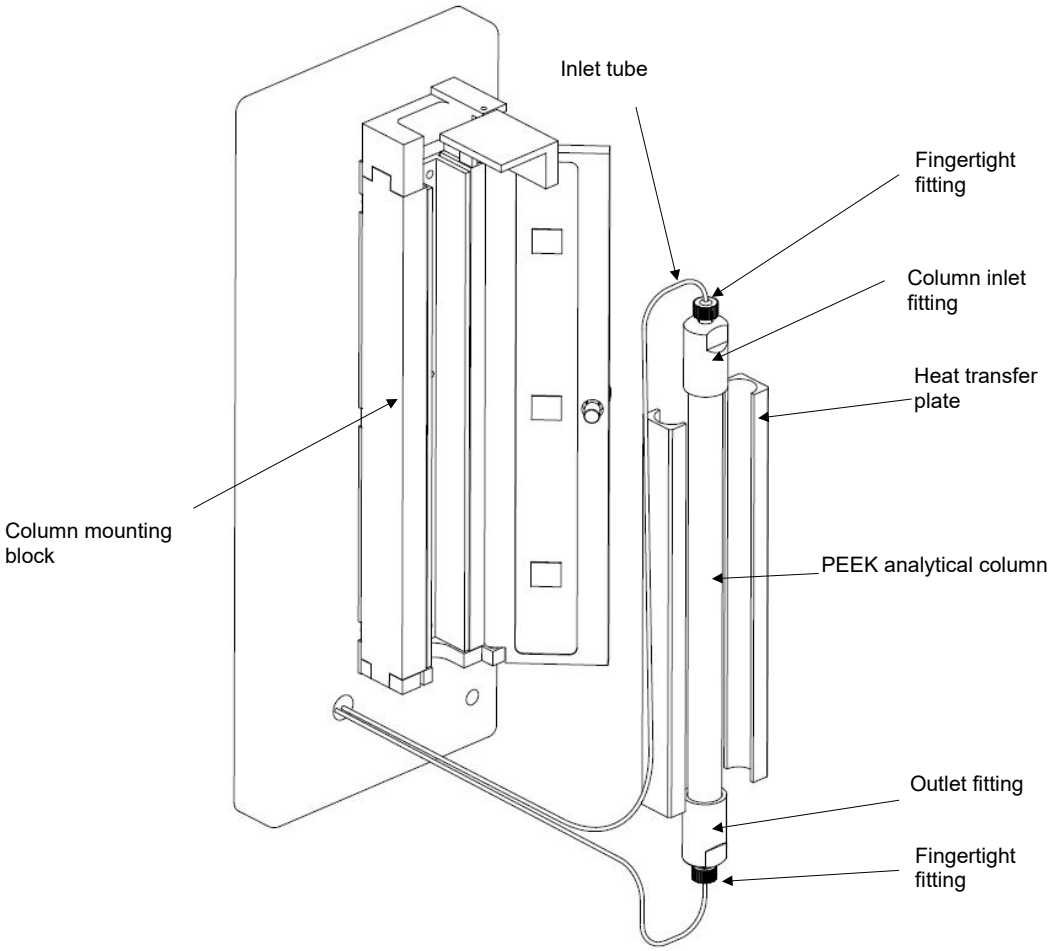


Fig 3.2 Analytical Column

3.6 Pump Assembly

3.6.1 Dual Piston Pumps

The Bio 30+ is equipped with a high pressure, low volume pump unit which comprises two Knauer Azura P4.1S pumps. One pump provides the buffer supply, the other supplies ninhydrin. The flow rate of each pump is controlled via the operating software, these flow rates being set as required for the particular application.

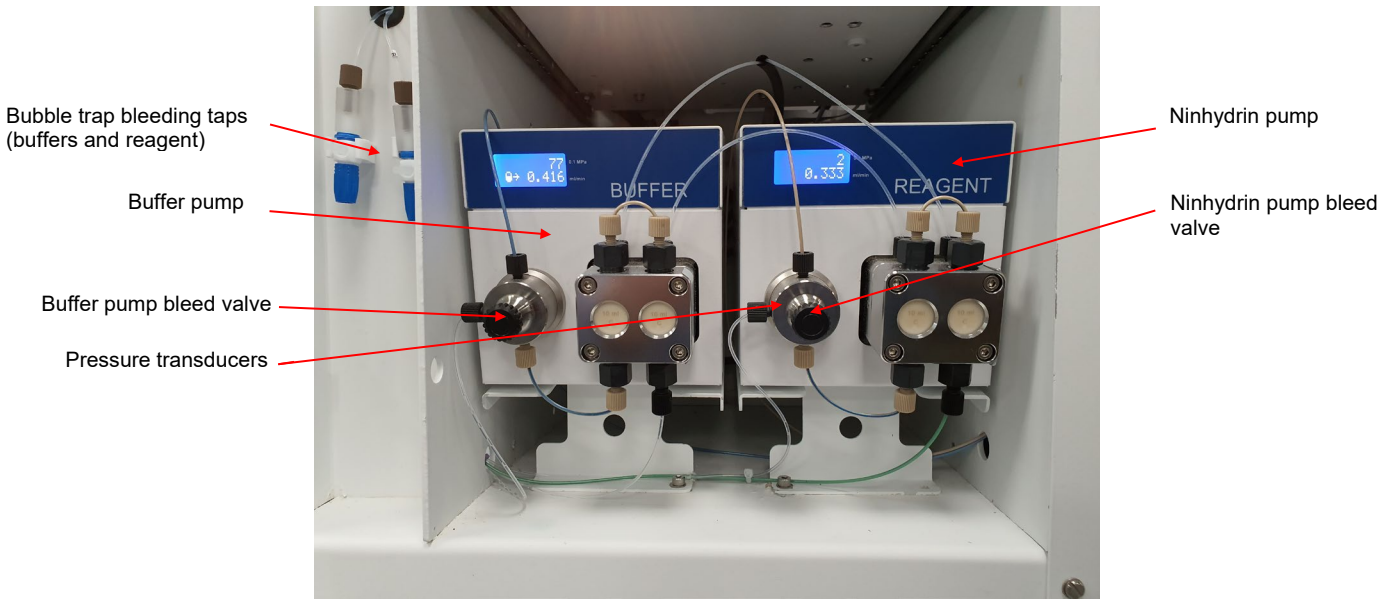


Fig 3.3 Pump Unit (Azura P4.1S)

Each pump assembly comprises a DC motor, where the speed can be varied via the programming, and a twin piston head reciprocating pump. The flow rate is dependent on the speed of the motor. This pump is designed to provide a pulse-free delivery of liquid at accurately controlled flow rates.

3.6.2 Buffer and Ninhydrin Filters

Filters are connected to the inlet of the buffer and Ninhydrin pumps and are mounted on the left hand side of the main chassis. Each filter cartridge is fitted to the base section which forms the filter inlet. The filter unit cover is held onto the base section by a screw through the cover into a slot in the base. The inlet is at the base section. A bleed tap is connected to the outlet from the cover. The Buffer and the Ninhydrin bleed taps are mounted on the front of the chassis, on the left hand side. They can be used to remove air or flush chemicals through the line.

3.6.3 Automatic Piston Wash System

The pumps have an automatic piston flushing system attached which constantly flushes the back of the pistons with deionised water to prevent any build up of salts. The automatic piston flushing system is controlled via a solenoid valve mounted on the left hand side of the fluidic cabinet. The solenoid valve opens at regular intervals only when the buffer pump is running. When the valve opens, water from the pressurised piston wash bottle flows through the back of the pumps. The system is set up so that a full 1L water bottle should last for about 10 days.

3.7 Buffer Solenoid Valves

The flow of buffers through the Bio 30+ is controlled by the six buffer solenoid valves which are mounted on a horizontal PEEK manifold on the low pressure side of the fluidics cabinet (see Fig. 3.4). These valves are controlled by the BioSys software.

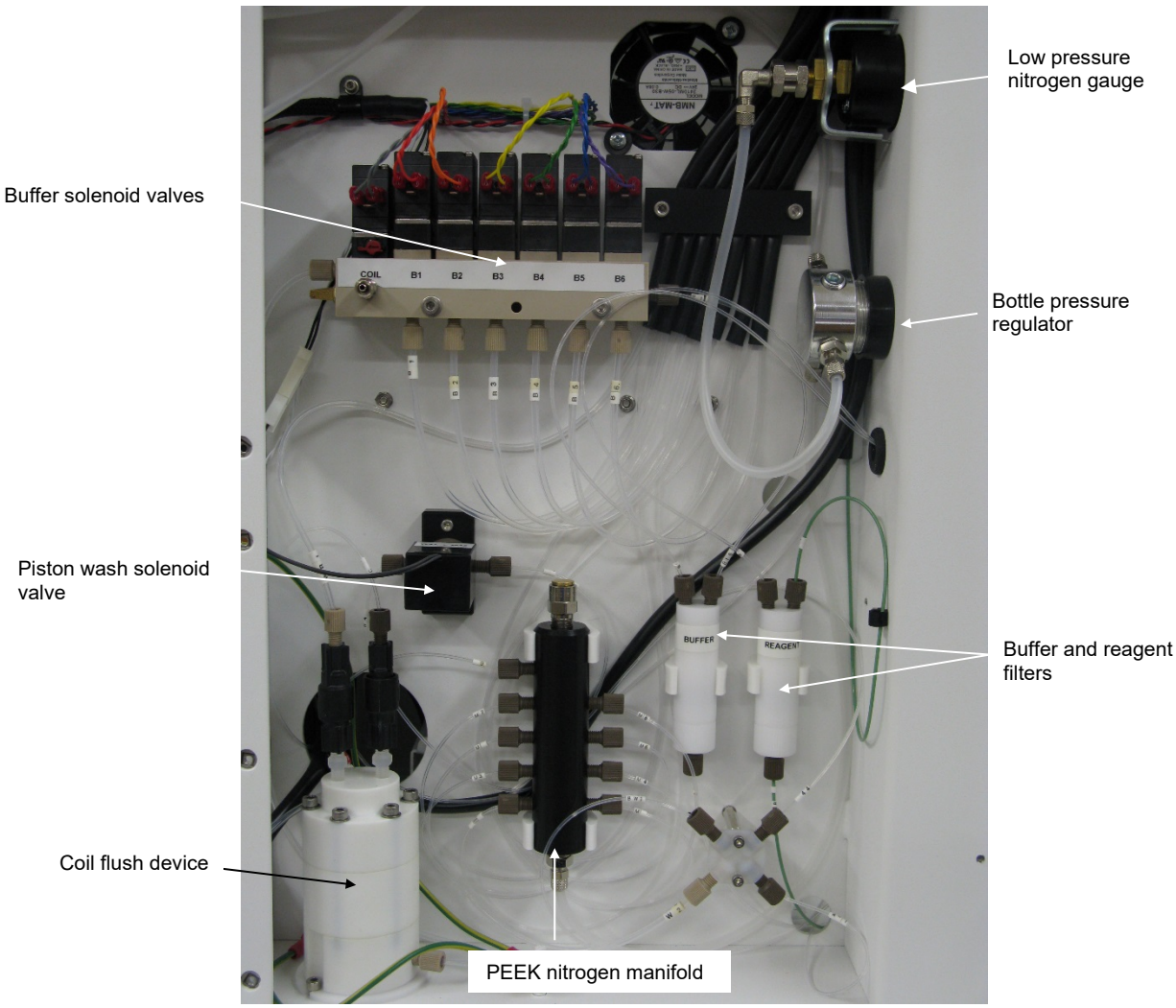


Fig 3.4 Low Pressure Side Panel

3.7.1 Pre-wash Column

The PEEK pre-wash column is mounted on the front of the instrument below the analytical column and is connected on the outlet (high pressure) side of the buffer pump. Ammonia present in all buffers is retained by the pre-wash column and thus prevented from being fed to the analytical column. At the end of each analysis, regeneration solution (buffer 6) is pumped through the pre-wash column to remove the accumulated ammonia from the resin.

3.7.2 Pressure Transducers

The buffer and ninhydrin pressure transducers are integrated into the pump assemblies.

3.7.3 Back Pressure Valve

The ninhydrin back pressure valve is mounted on the right hand side of the reaction coil area, maintaining the ninhydrin feed at the required pressure. This valve is factory set to 3.5 bar.

3.7.4 Coil Flush Device

The coil flush device is mounted on the low pressure panel area (figure 3.4) and is connected via a one way valve to the seventh bottle in the storage area, the bottle contains a mixture of Isopropanol/Water 50/50 v/v. When the system is switched on and operating, the coil flush fills with the liquid from the bottle. If the buffer pump is then switched off or the power fails then nitrogen from the cylinder forces the coil flush piston to expel the liquid via a second one way valve through the reaction coil. This ensures that no reagent remains in the coil or flowcell when the unit is shut down.

3.8 Photometer and Reaction Coil Unit

The photometer unit comprises the mixing manifold, reaction coil, back pressure valve, pressure transducer, photometer and flow cell.

3.8.1 Reaction Coil

The colour development by the reaction between ninhydrin and the amino acids is faster at high temperatures. A high temperature reaction coil is used to provide this reaction temperature. This coil is formed from PTFE tubing which is contained in a heated, temperature controlled jacket. The temperature can be programmed from 40°C to 145°C (default is set to 135°C). Safety interlocks are provided to monitor the temperature of the reaction coil and the back pressure.

Note: the ninhydrin pump will not operate if the reaction coil temperature is below 100°C.

3.8.2 Mixing Manifold

The mixing manifold is a specialised T connection into which are fed the eluate from the analytical column and the ninhydrin reagent. This mixture is then fed to the reaction coil.

3.8.3 Back Pressure Valve

A back pressure valve is connected to the photometer outlet and maintains the liquid in the reaction coil under pressure. This prevents the buffer from boiling at reaction temperatures over 100°C. This valve is factory set to 3.5 bar.

3.8.4 Photometer

The optical density in the ninhydrin/eluate mixture is measured using a flowcell. A 20W tungsten halogen lamp is the light source for the photometer, the lamp being fixed to an adjustable mount which allows the lamp to be focused. Light from this lamp is focused by a lens before passing through the flowcell. The flowcell has a volume of 8µl with a path length of 15mm. The light from the flowcell passes to the beam splitter which then feeds the light to two photodetectors. Filters are fitted in the light path to these photocells so that one cell receives light with a wavelength of 440nm, the second cell at 570nm.

3.8.5 Reaction Coil Pressure Transducer

The pressure transducer is connected into the feed line to the reaction coil to measure the back pressure. The output from the transducer is connected to the electronic unit and the coil pressure is displayed on the Pressures Display in the manual control box or by pointing at the relevant pressure meter in the graphical display area.

3.9 Tubing and Connectors

The tubing used in the Bio 30+ is manufactured from PEEK, PTFE, or PVC as listed below:

Tube	Material
Low pressure ninhydrin lines	PEEK
Low pressure buffer lines	PEEK
Nitrogen gas lines	PVC
High pressure ninhydrin lines	PEEK
High pressure buffer lines	PEEK
Reaction coil	PTFE
Column to coil line	PEEK
Waste lines	PTFE

Various types of connector are used, fingertight and flangeless couplings. These connectors are described on Section 6, paragraph 6.2.8.

4 INSTRUMENT CONTROLS

CONTENTS

4 INSTRUMENT CONTROLS

When a complete system has been ordered, the instrument is supplied with a computer. In this instance, the complete system will have been set up and tested at Biochrom and should be ready to use after installation.

However, if the computer was obtained locally, then the software will need to be installed and set up. **Please refer to the *Bio30+ Software suite installation and setup guide***

Note: Biochrom only supports the use of the following Operating Systems:

- **Windows 10 Professional or Enterprise Edition 64-bit**
- **Windows 7 Professional or Enterprise Edition 64-bit.**

Unauthorized use of Bio 30+ is forbidden; the instrument and BioSys control software can be used only by authorized and trained individuals.

Do not connect the PC to the Internet to protect from malware attacks.

4.1 Overview Of System Operation

Bio 30+ chromatography is performed by running programs. Each program is a sequence of steps specifically designed to control the flow of buffers and reagent through the system to complete an analysis.

Each step allows the column temperature, buffer, buffer flow rate and ninhydrin pump to be controlled, as well as controlling the optional chart recorder and performing a load or baseline reset when required. Each step is maintained for the period defined in the time field. One program completes a single analysis and also regenerates and equilibrates the analytical column ready for the next analysis.

The Ninhydrin pump rate can be set once for each program. Normally either one or no sample load is performed per program. This is usually done at the start of each program.

The program can also contain a title and a comments field, which can be used to identify the program.

A number of analyses are run using programs linked together in a sample list.

The operation of the OpenLAB CDS EZChrom Edition chromatography system is under the control of the BioSys software.

4.1.1 BioSys Control and Data Handling

The BioSys Control software is designed to run all operations required for the control of the Bio 30+ amino acid analyser. It can therefore be run in parallel with any suitable data handling system.

For OpenLAB there are special functions to integrate BioSys operation with the data handling system.

A special link enables the interfacing of BioSys and the OpenLAB data handling package. This link allows BioSys to automatically open an OpenLAB method and starting data collection upon an injection step in the instrument program.

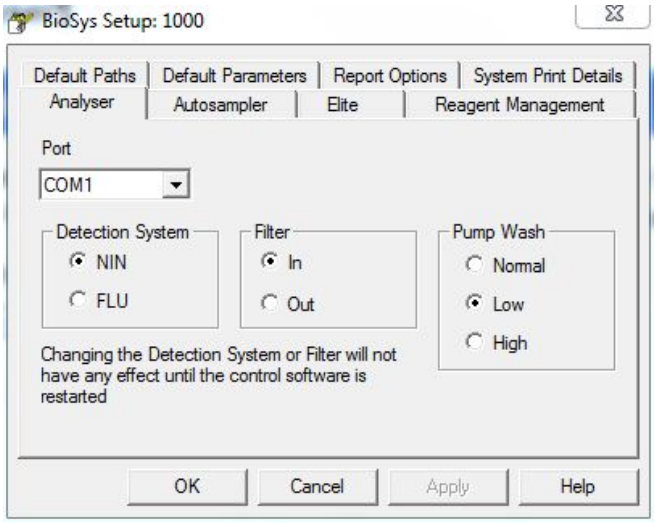
4.1.2 Running BioSys Setup

- **The Setup window**

  The BioSys Setup program can be found in the BioSys directory. This is where a new system is set up by selecting the different parameters required for operation.

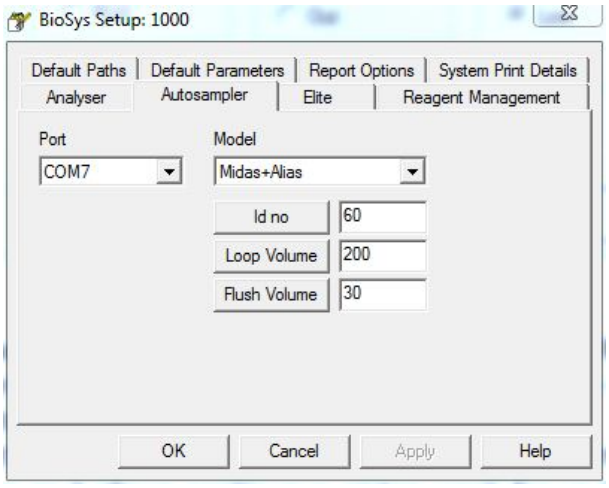
  These settings can be selected from each of the seven tabs in the Setup window.

  - **Analyser**



  The analyser serial port can be defined here (default is COM1). Also the Detection system, Ninhydrin or Fluorimeter, can be set. The Filter In/Out selection affects the recorder outputs only.
  Select the pump speed for the piston wash system. **Low** is the prefered setting as it allows continuous piston wash for up to a week without topping up the water bottle.

  - **Autosampler**



Click on the Autosampler tab and select a proper communications port (default is COM 7). This COM port must match the Biosys Virtual Port set in the Alias Manager (see section 4.6.2.2). Set the the Autosampler type to Midas+Alias.

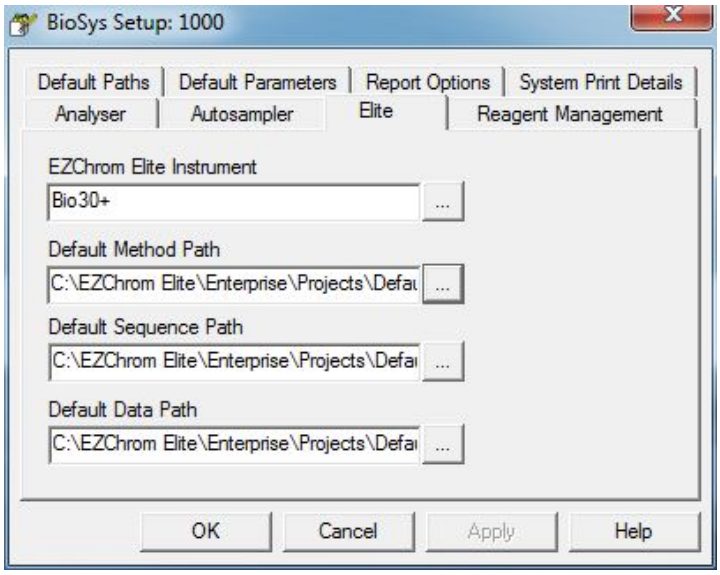
The Id No. should be set to 60.

A different loop volume can be set if the default loop of 200µL is replaced.

The flush volume is the volume of standard or sample needed to prime the sample needle. The default value is 30µL.

- **Elite**

Note: Please perform the following setting after OpenLAB has been fully installed.

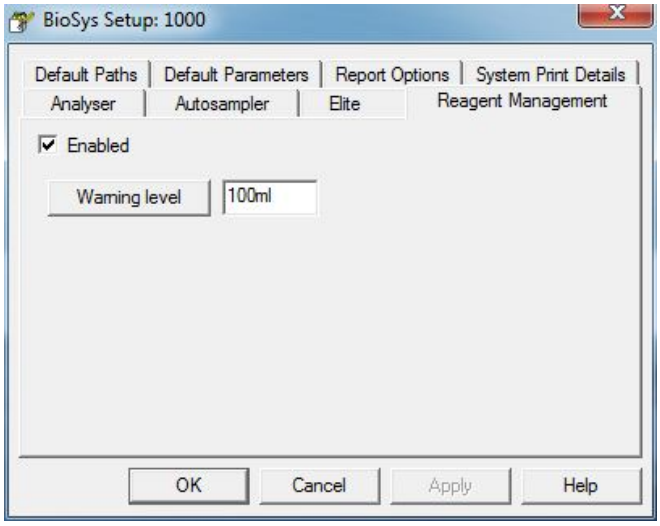


To enable the OpenLAB-BioSys link, click on the button next to the blank box and select the Bio 30+ instrument from the list.

Set the Method, Data and Sequence paths by clicking on the buttons for each path.

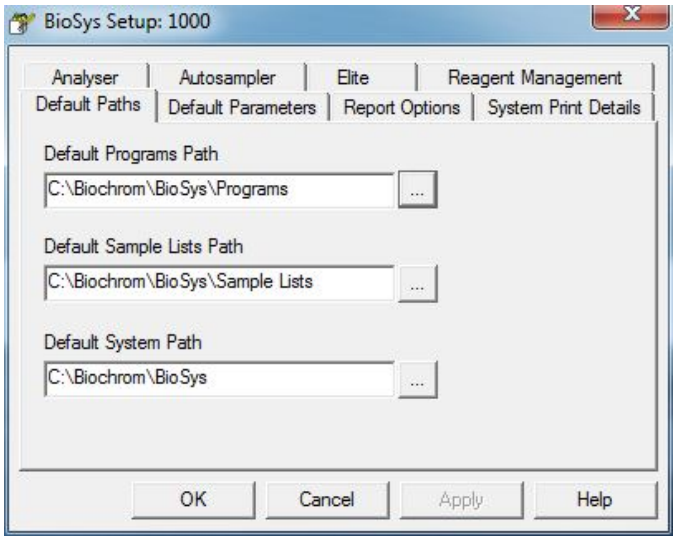
Note: BioSys will be able to start the integration system every time a programmed injection takes place. If the BioSys-OpenLAB link is disabled, the integration software can be run independently from BioSys.

- **Reagent Management**



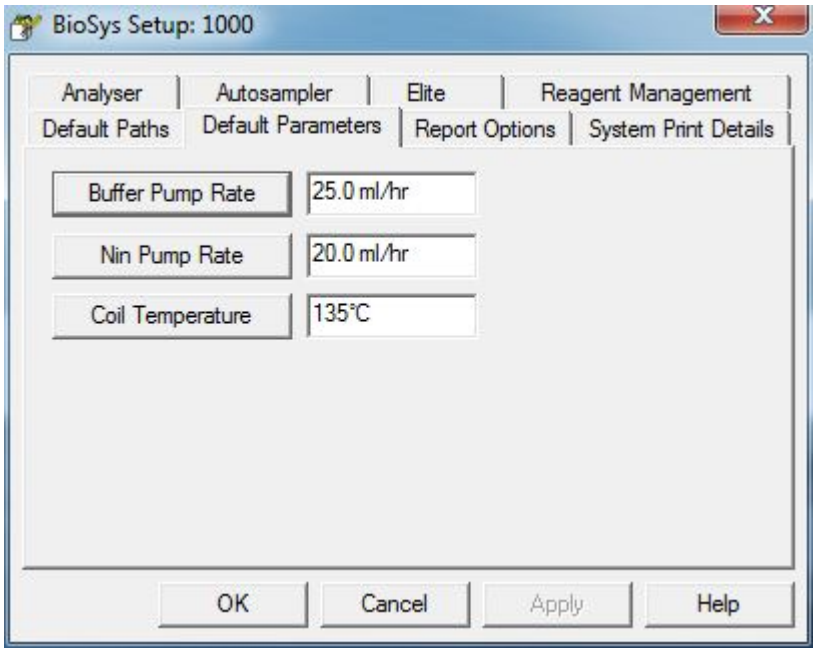
The reagent calculator can be enabled and the minimum warning level can also be set here. An error message will be displayed if any bottle level reaches the level set up on this tab. This error message is a warning and will not stop the instrument.

- **Default Paths**



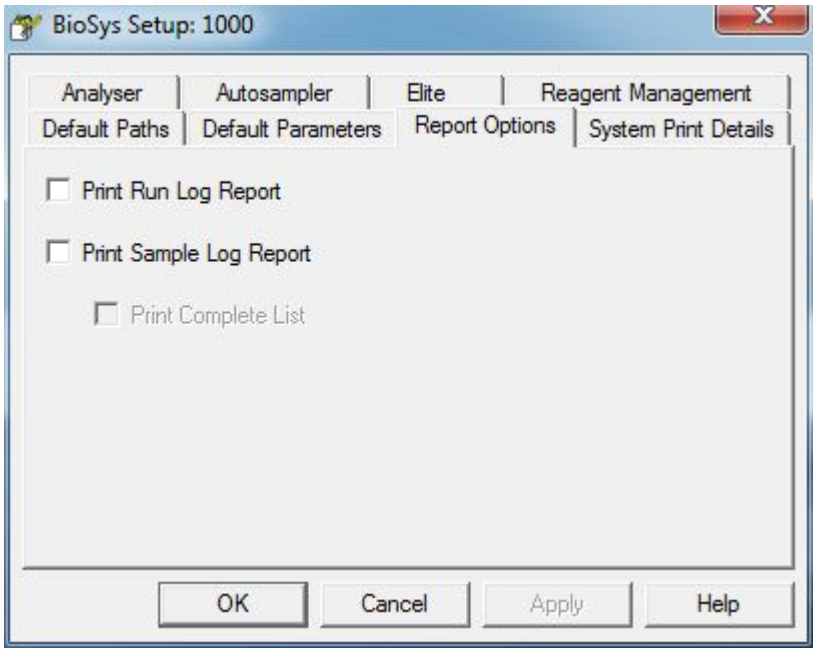
These settings tell the Control program where the sample lists, the programs and the main program directory are saved in the computer hard drive.

- **Default parameters**



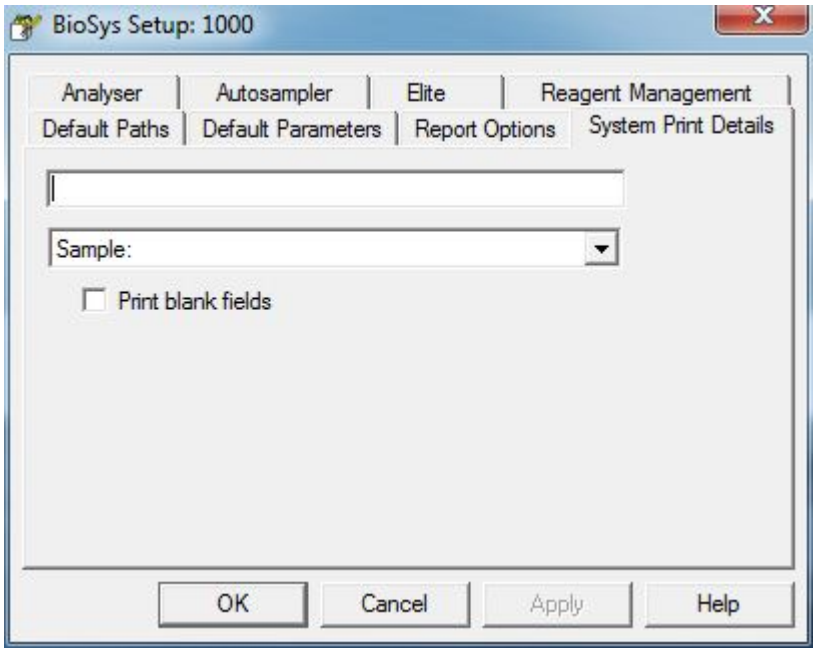
The buffer and ninhydrin pump flow rates and the reaction coil default temperature can be set here.

- **Report Options**



The Sample and Run reports can be enabled here so that they are printed at the end of a sample list. The print option can be disabled if required.

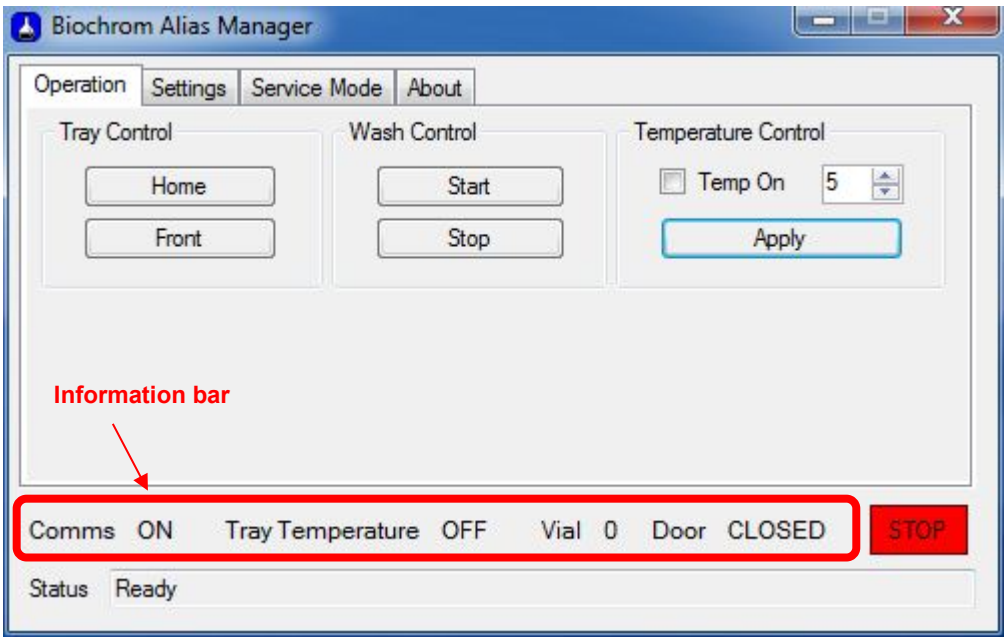
- **System Print Details**



The header for the printed programs can be customised here.

4.2 Alias Manager Software Description

4.2.1 Operation tab



Tray control:

- Click on **Front** to move the sample tray forward. This allows the access to the samples and enables the removal of the tray from the compartment.
- Click on **Home** in order to move the tray back in its default position

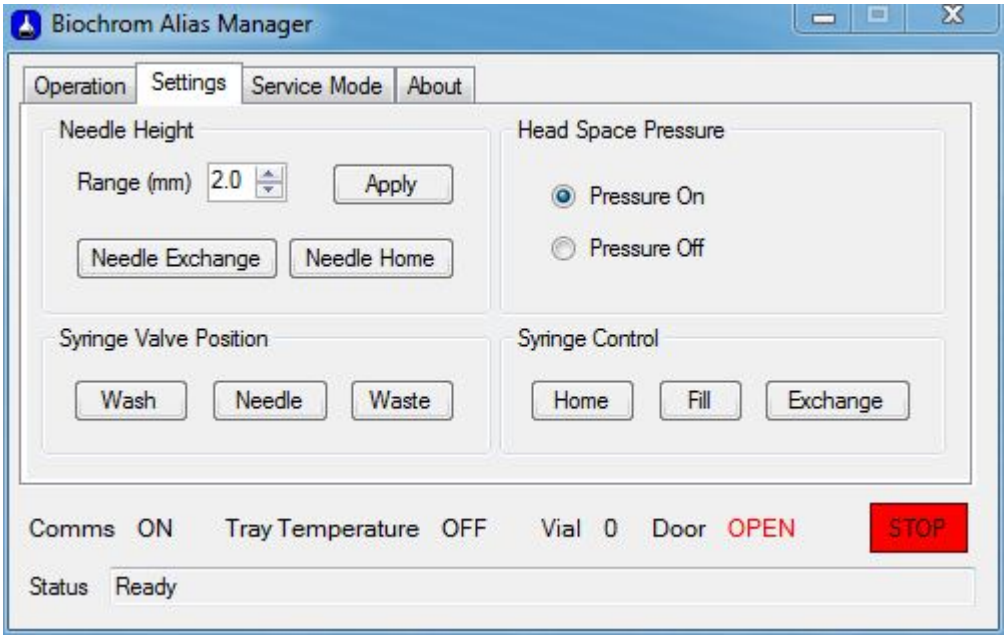
Wash control:

- Click on **Start** to perform a wash sequence of the needle and the syringe
- Click on **Stop** if you wish to stop the wash sequence before the end of a full cycle.

Temperature control:

- Set the temperature required in the temperature box
- Tick the **Temp ON** box and click **apply** to enable the cooling system to start.
- Once the cooling system has started, the actual temperature tray will be displayed in the information bar. If the temperature is in red, it means the actual temperature does not match the required temperature. Once the required temperature has been reached, the tray temperature will be displayed in black.

4.2.2 Settings tab



Needle height:

- Range: Select the distance in mm between the bottom of the vial and the needle. Click apply to record the change.
- Needle Exchange: click on this button to position the needle in the exchange position.
- Needle Home: Once the needle has been changed, click on this button to reinitialise the needle position.

Head space pressure:

- Pressure On: select this option when vials with septa are used.
- Pressure Off select this option when no caps are used on the vials.

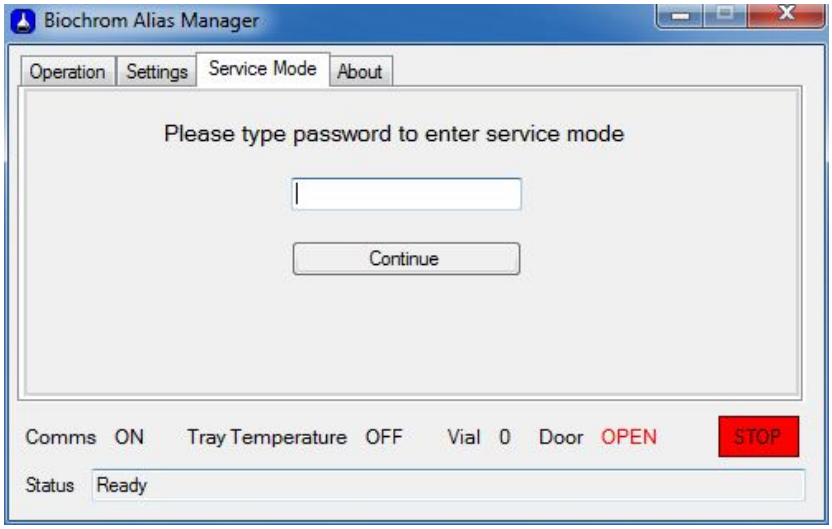
Syringe valve position

- Wash: move the valve in the wash position
- Needle: move the valve to divert the flow to the needle
- Waste: move the valve to divert the flow to the waste

Syringe control:

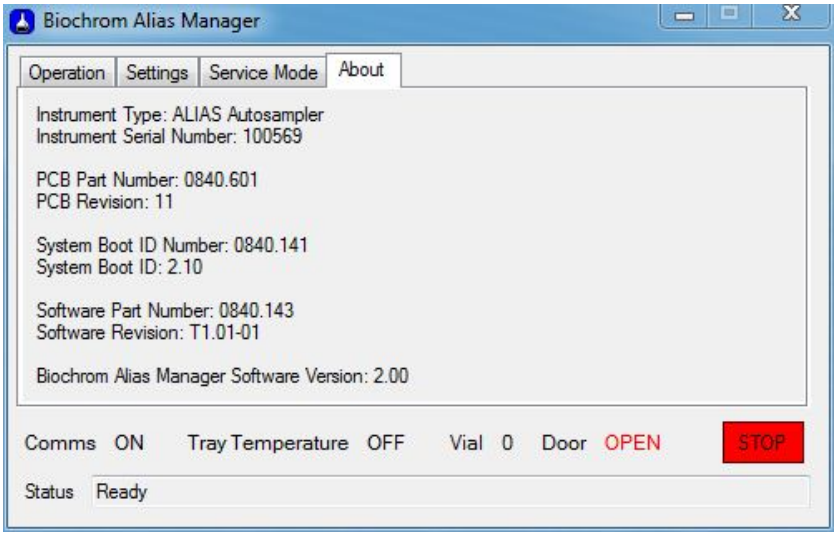
- Home: position the syringe in the home position
- Fill: fill the syringe with the washing liquid
- Exchange: move the syringe in the exchange position

4.2.3 Service mode tab



This tab is only accessible for service engineers with a suitable password.

4.2.4 About tab



This tab shows the instrument type, serial number and software version details.

4.2.5 Minimized window

This window is triggered when the minimize button is clicked on the main Alias Manager window. It contains all the basic information along with main operations buttons. To recover the full window of the Alias Manager, click on **Maximize**.

4.3 Biosys Control Software Description

The BioSys program consists of five main modules, these are accessed either via the following path: *Start\All Programs\Biochrom\BioSys* or by clicking on the **View** menu in any of the BioSys windows.

BioSys Manual: This is the module where the instrument can be operated manually, to check flow rates, temperatures, pressures, etc. The instrument can be prepared here for runs.

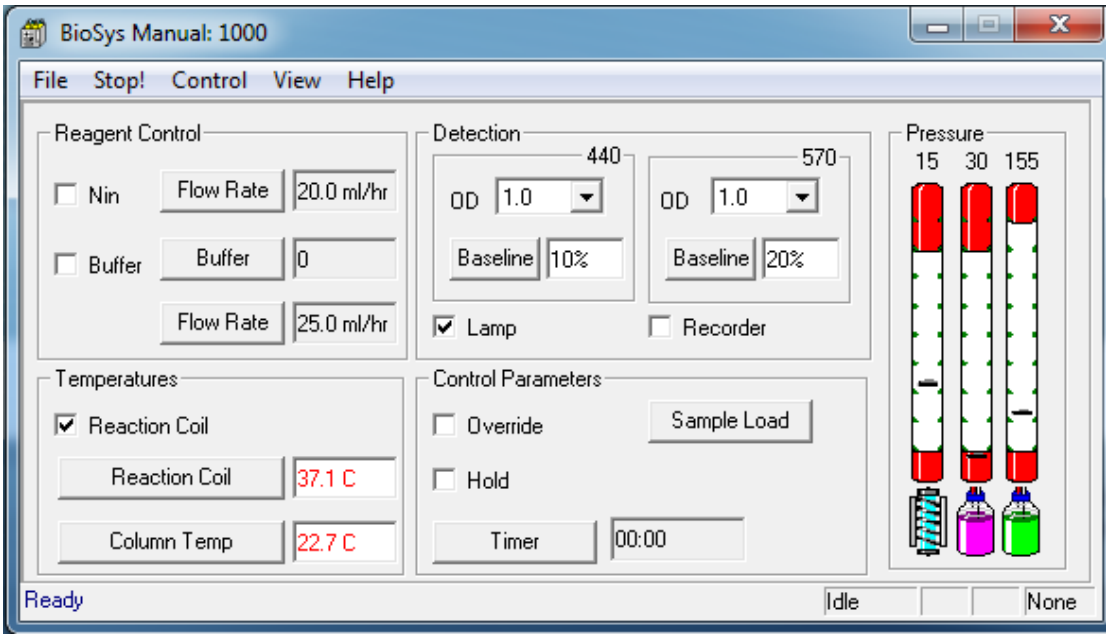
BioSys Programmer: This is where the sample list is created to run a number of analyses.

BioSys Editor: This is where analytical programs can be created or edited.

BioSys Fluidics: This is a graphical display of the instrument fluidics system, it is a real time active window that can be used to manually operate the instrument. The fluidics window functionality is disabled when running a sample list, it just shows the instrument activity when opened.

BioSys Setup: This module is used to set up the instrument at the factory, to select the communication ports, program paths, reagent management, autosampler, etc.

4.3.1 The Manual Operation window



The Bio 30+ can be manually operated and be prepared for running from this window.

Click on the **Reaction Coil** checkbox to switch the heater on.

Click on the **Column Temperature** button to select a column temperature.

Click on the **Lamp** checkbox to turn the lamp off/on when replacing the photometer lamp.

Click on the **Recorder** checkbox to start the paper drive, this is only valid for recorders that have a remote paper drive control.

Click on the **Override** checkbox to bypass the error detection system. This allows access to operate the instrument in case a non fatal fault has shut it down.

Note: do not run the instrument with the override on.

Click on the **Timer** button to enter a time to operate the instrument manually. The time counts down and when it reaches zero the instrument stops. Click on the **Hold** checkbox to hold any time running.

Click on the **Reagent Control** box to select any buffer and to pump ninhydrin when the coil temperature is above 100°C. Selecting buffer zero stops the buffer pump and closes the buffer valve. If the ninhydrin pump is operating it also stops. The buffer pump can be stopped by clicking on the **Buffer** checkbox, this action stops the buffer pump but keeps the buffer solenoid valve open.

Placing the cursor on any of the pressure gauges displays the current pressure of the Reaction Coil, the Ninhydrin or the Buffer system.

The detection system parameters can be set on the **Detection** box. The O.D. settings affect the recorder signals only. The baseline position settings affect both the recorder and the integrator outputs. Default positions are 10% for the 570 nm and for the 440 nm channels when a recorder is not used.

If a chart recorder is used the settings should be set at 10% for the 440nm channel and 20% for the 570nm channel.

Click on the **Sample Load** button to make an injection from any vial position, injecting any volume using any of the three injection modes. This function can be used to check the autosampler operation. This button is disabled when the Sample List is running.

Menu commands:

File\Setup… This is where the instrument operational parameters are set up. These parameters should be already set when purchasing a full system.

File\Shutdown This where the BioSys program can be closed, the Shutdown command closes the program and all communications between the computer and the instrument and the autosampler.

Note: Always use this command to shutdown BioSys

File\Exit Closes the window only, the communications will still be active.

Stop! This command stops anything that is operating manually.

Control\Flow Rates… This commands opens a dialogue window where the buffer and ninhydrin flowrates can be measured and the pumps can be calibrated.

Control\Assign Buffers… This command opens a dialogue window where the buffers and the ninhydrin batch numbers can be entered. This table can be printed on a program header if required.

Control\Autosampler… Similar function to the Sample Load button.

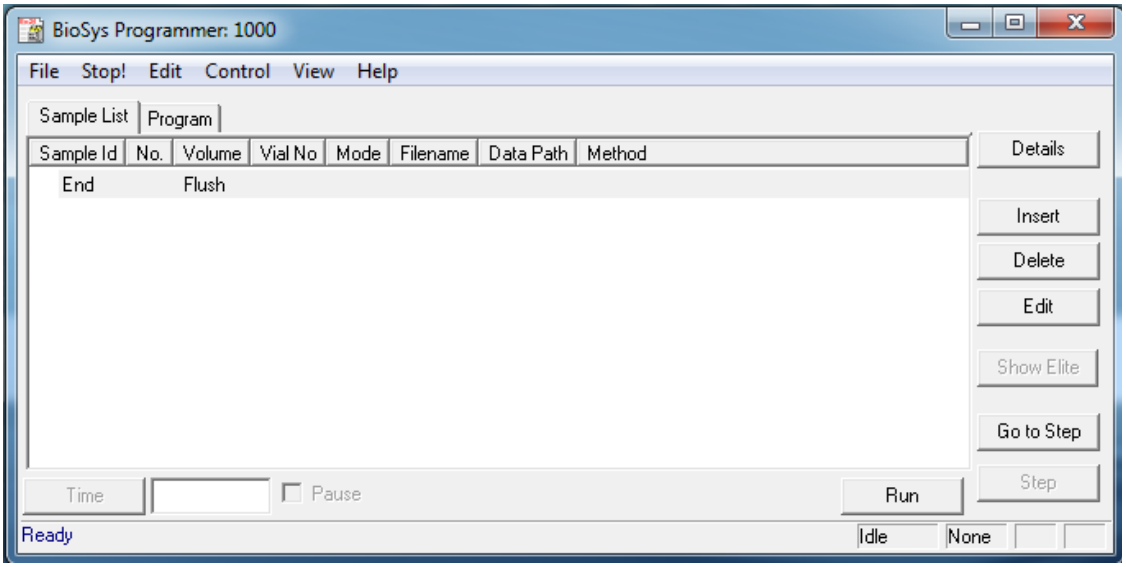
Control/Reagent Management… This command opens a dialogue window where the volume of the buffers and reagent can be entered. These volumes will be used by the system to calculate the usage of buffers and reagents for a Sample List.

View/Fluidics Diagram This opens the Fluidics Diagram window, where the instrument can be manually operated.

View/Program Control… This opens the BioSys Programmer where the Sample List is created and run.
View/Program Editor… This opens the BioSys Program Editor where programs can be edited or created.

4.3.2 The BioSys Programmer Window

This window can be opened from the Manual Operation window View menu or via the Start\All Programs\Biochrom\BioSys path.



The menus give access to files, save commands, instrument control functions and the other main application areas of the software

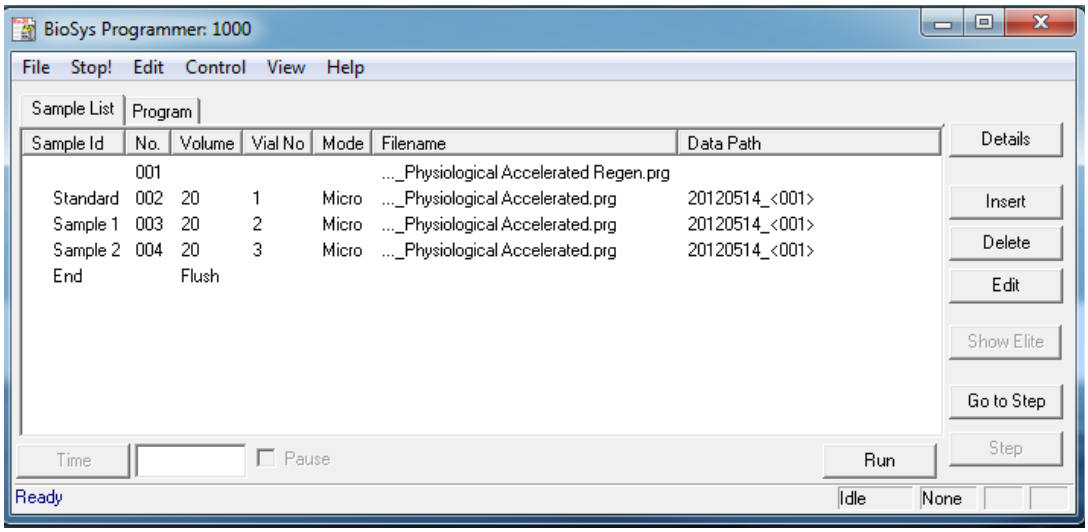
The **Details** button opens a window where information about the Sample List can be entered, this can be useful later when viewing older Sample Lists on the Program Editor.

The Sample List is built up by addition of programs. These are called up from the appropriate directories using the **Insert** then **Program Filename** buttons. The required program can now be loaded along with the regeneration program if required.

Subsequently, the buttons **Insert**, **Delete** and **Edit** on the right hand side are used to build up a list of programs to analyse each sample loaded.

The **Elite** button in the Sample Details window opens another dialogue window where a data collection method can be set for each sample in the list. The Data directory and the data filenames can also be set here.

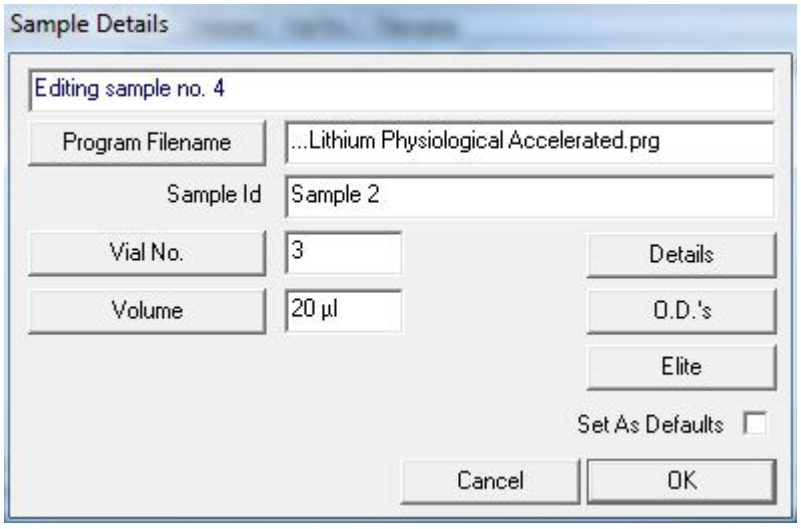
A completed Sample List would look like this:



The **Go to Step** button can be used to go to a selected sample later in the list, it can also be used to run the Sample List from a selected sample. The **Step** button moves the sample list to the next available step.

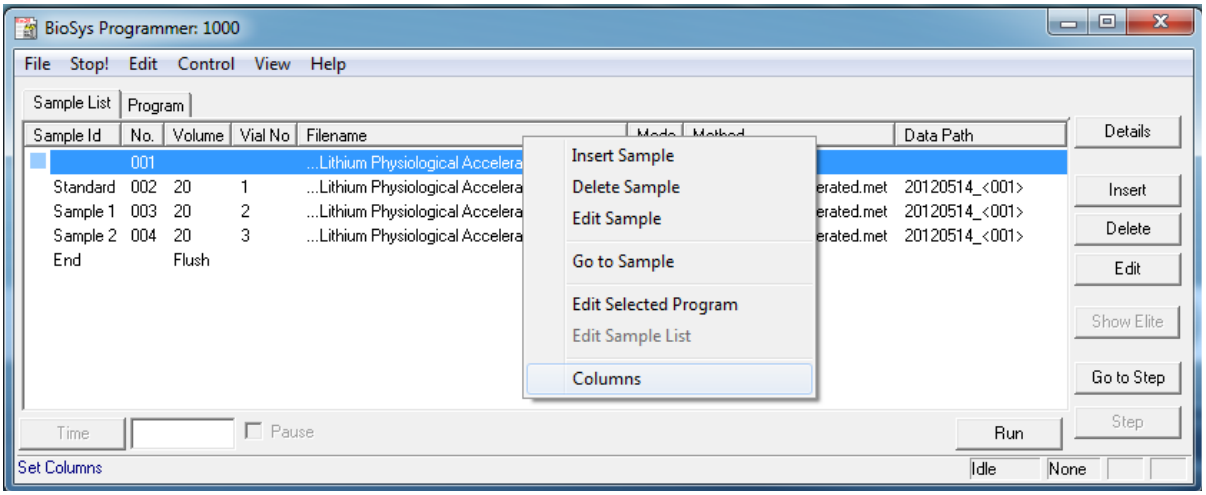
The **Edit** button can be used to call up the Sample Details dialogue box and then change the required parameters of a selected sample.

An alternative method is to select a sample line and then right-click and select **Edit Sample** to modify it as shown below:

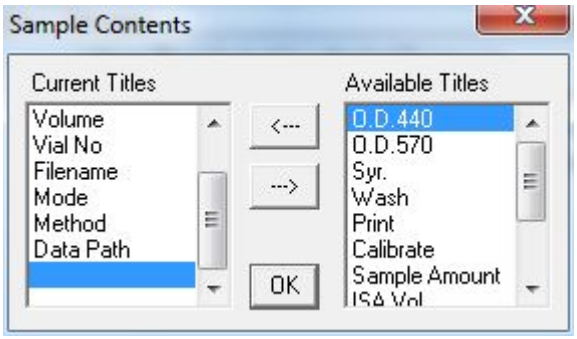


Column display:

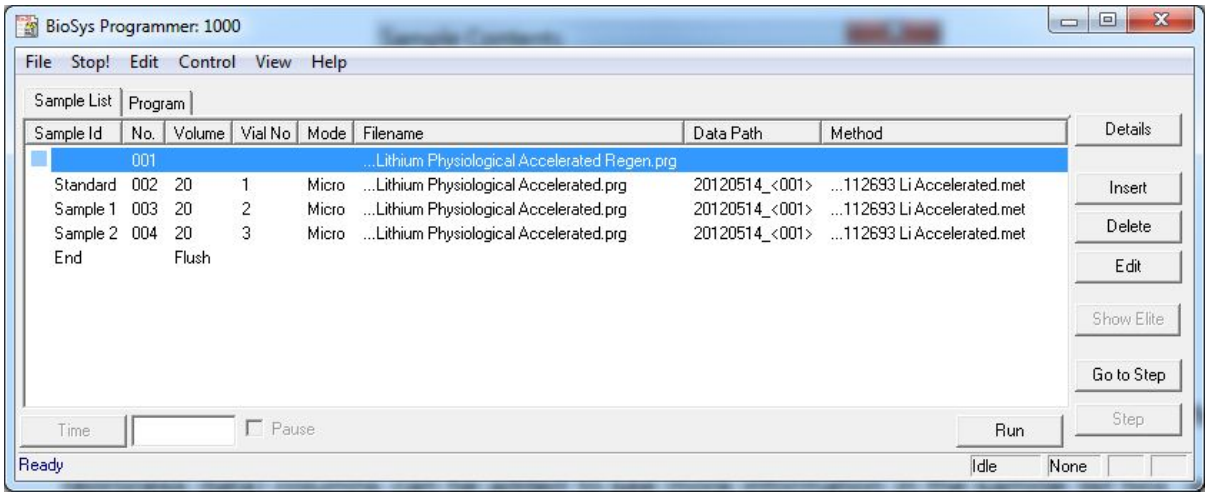
Right-click on the Sample List window and select **Columns** from the menu.



The **Columns** command is used to customise the sample list layout. Columns can be added by highlighting them in the **Available Titles** box and using the arrow to move them to the **Current Titles** box. The reverse can be done to remove columns.



For example, the **Mode** (autosampler injection mode), **Filename** (BioSys program to effect separation), **Data Path** (data filenames) and **Method** (OpenLAB method to collect and reprocess data) columns can be added to see more information in the sample list box, as shown below.

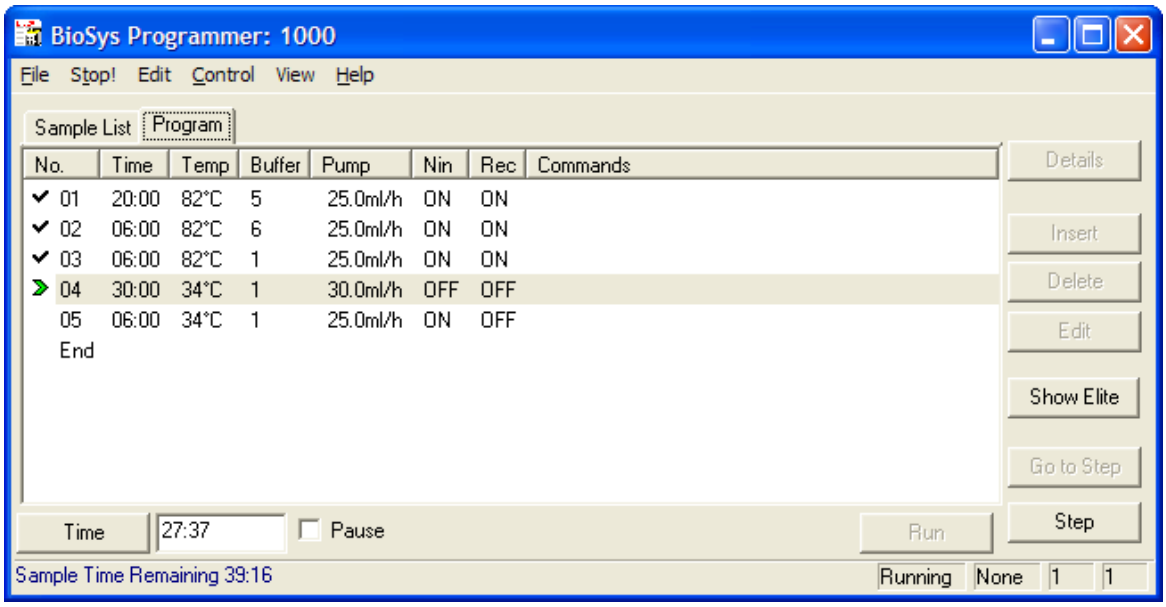


4.3.2.1 Running the Sample List

The sample list is started by clicking on the **Run** button. If OpenLAB security is enabled, a dialogue window opens to enter the name and password required to access OpenLAB.

Click on the **Program** tab to see the current program steps and details.

Completed steps are indicated by a tick mark on the left hand side of the window and the current step by a green arrow.



At the bottom of the window, the Status bar shows the current instrument status.

Click on the **Show Elite** button to open the OpenLAB Online chromatogram window.

4.3.3 The BioSys Editor Window

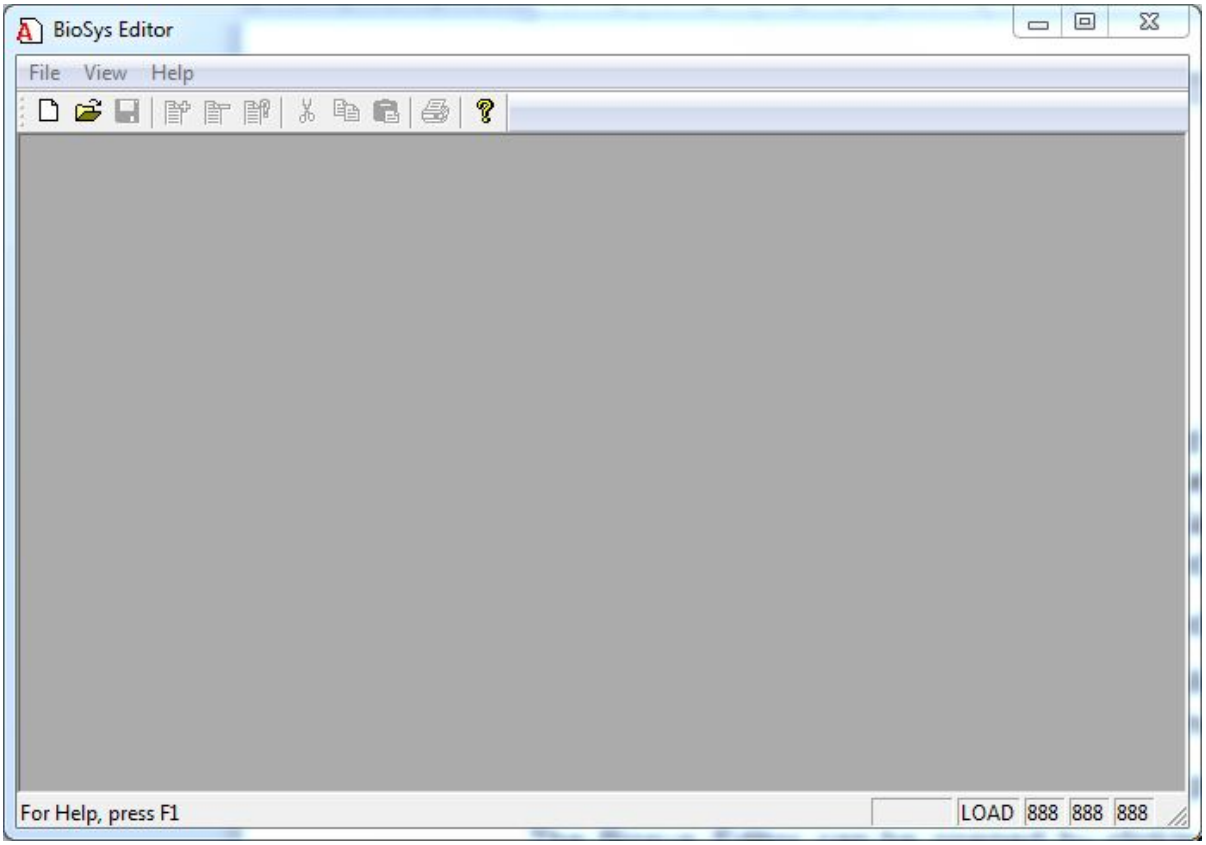
The BioSys Editor is required to create and edit the programs required by the BioSys Programmer. The Program defines a sequence of steps that perform an injection, an analysis and the column regeneration. A Sample List is a list of samples to be run, each of which consists of a program and associated sample details.

Both Programs and Sample Lists can be edited in the same editor window.

All editing is performed through the Main Menu, the Toolbar or a context menu. The menus contain all of the available commands, some of which are found on the Toolbar.

The status bar shows the total time of the program being edited.

The Biosys Editor can be opened by clicking on *Start\All programs\Biochrom\Biosys\Launch Biosys editor* or by clicking on *View\program editor* from the BioSys Manual window.



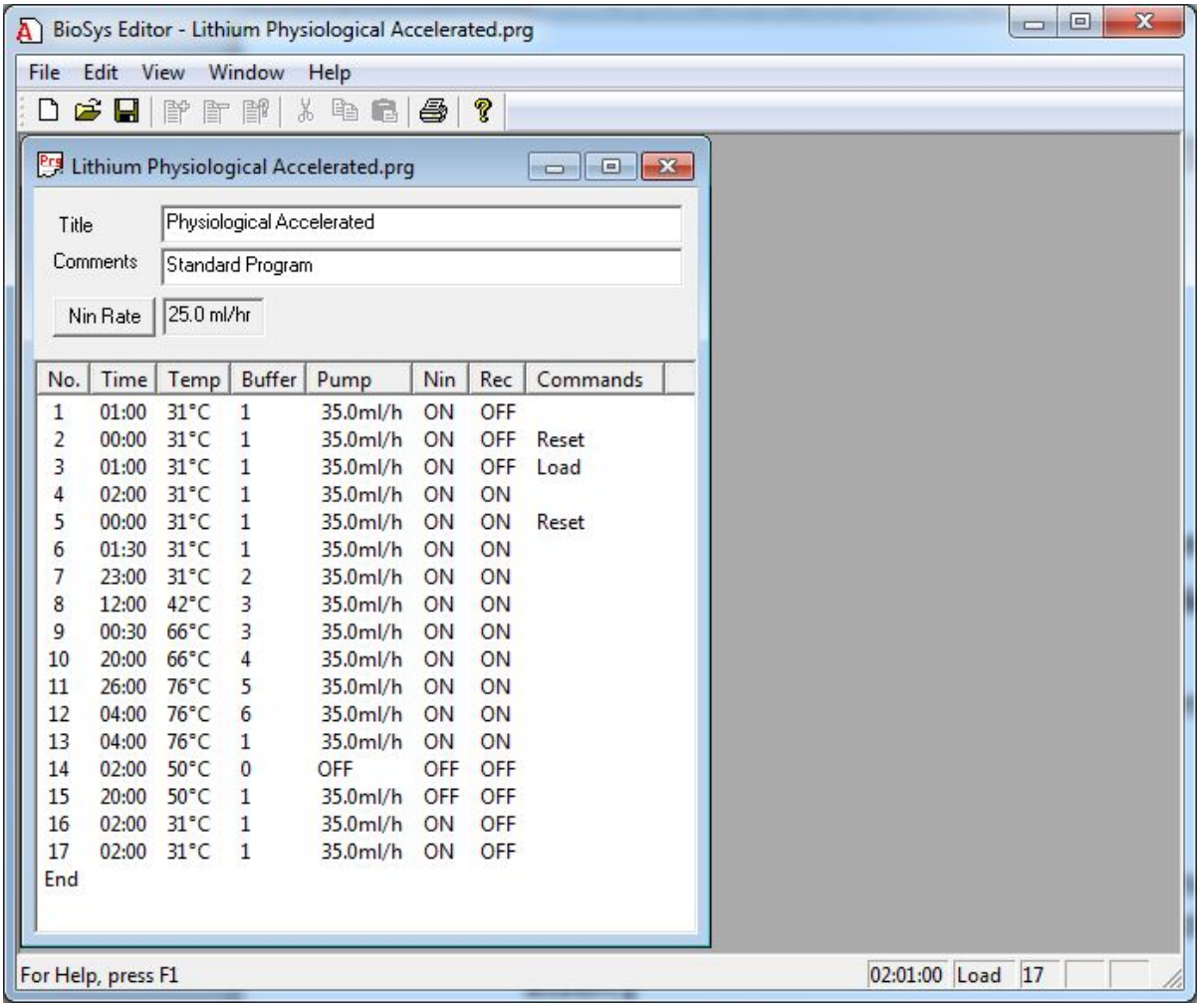
4.3.3.1 The Program Editor

The Program Editor is used to create and edit programs.

New programs are created by clicking on **New** from the Main Menu. The choice of creating a Program or a Sample List is given. Please select **Program**.

A default program can also be opened by clicking on **Open** and by selecting a default program located in the **Standards** folder.

The program title and comments can be entered at the top of the program.



The list of Program Steps is created and maintained by Inserting, Editing and Deleting steps and Cutting, Copying and Pasting groups of one or more steps. These functions are accessible by right-clicking on the program.

Insert
A new step is inserted at the position of the cursor. The content of the step is either copied from the preceding step, otherwise default values are used. The Step Editor is then displayed, so that these values can be edited.

Edit
The currently selected step, or steps, are opened in the Step Editor

Delete
The currently selected step, or steps, are deleted.

Cut
The currently selected step, or steps, are copied to the clipboard before being deleted.
Copy
The currently selected step, or steps, are copied to the clipboard.

Paste
Any steps currently stored in the clipboard are pasted into the Program List at the current cursor position.

- Program Step Editor

The Step Editor can be used to edit a single step or group of steps. Double-click on the step to be edited to open the Step Editor. Any of the parameters on the step can now be changed.

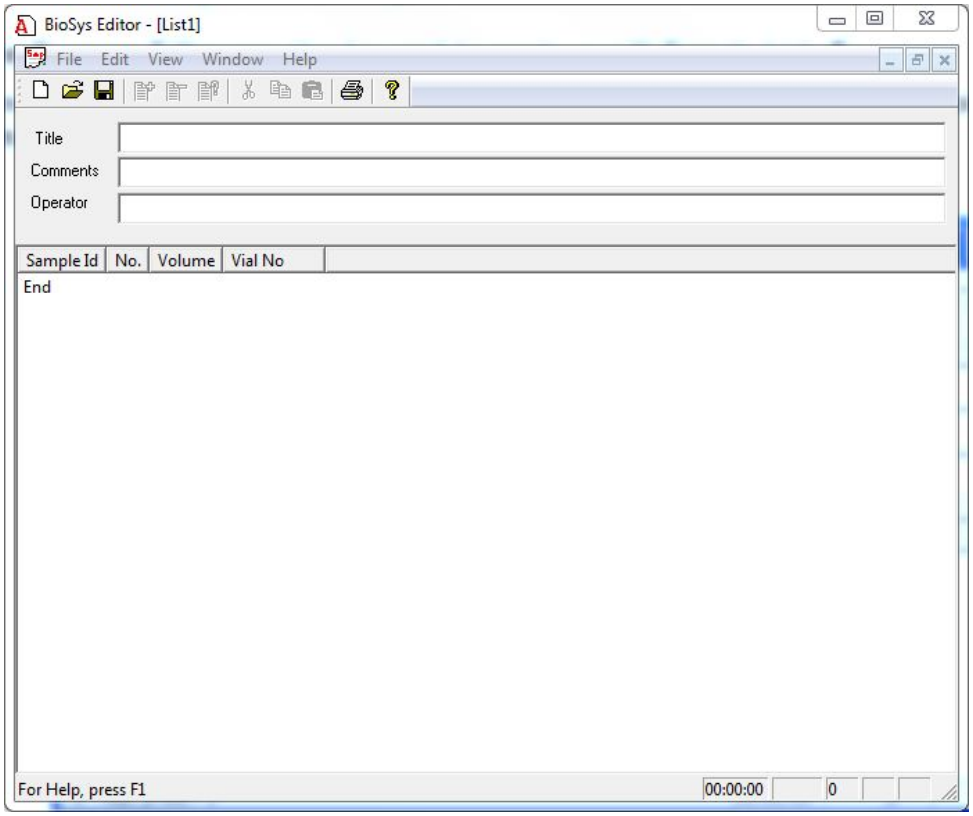
To edit a group of steps, select the first step, hold the Shift key down and click on a step below the first one selected.

Right-click on the highlighted group and select Edit Steps editor from the menu. The Step Editor displays the various options to be edited.

4.3.3.2 The Sample List Editor

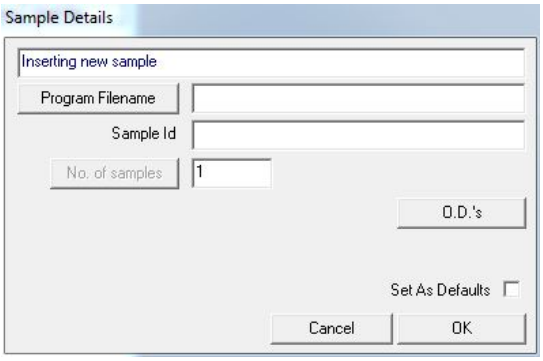
The Program Editor can also be used to create or edit Sample Lists.

New sample lists are created by clicking on **New** from the Main Menu. The choice of creating a Program or a Sample List is given. Please select **List**.



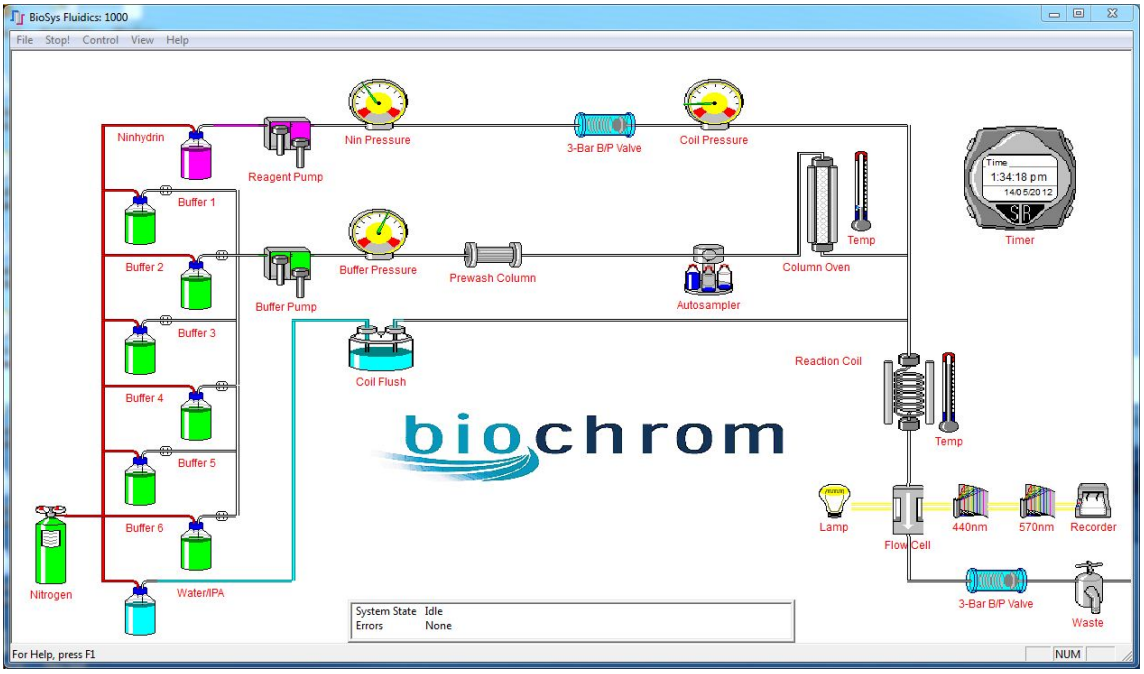
Click on the **End** line on the Sample List window, this action enables the **Insert** command in the Edit menu and the Insert, Remove and the Edit buttons on the button ribbon.

Insert a new sample by right clicking on the window and select **insert step**.



Then select the relevant program and OpenLAB methods required and save the Sample List.

4.3.4 The BioSys Fluidics Window



This application enables the operator to view all system functions during operation in a convenient layout and gives a quick overview of system status.

Before start up the fluidics screen shows the main system components and the way in which they are connected in the analyser. Analyser functions can also be controlled in a similar way to the Manual Operation window.

Any component that can be controlled or read will display its current status. Right-clicking on the system components will bring up a context sensitive menu, which will allow any operations to be performed on the components.

During analysis several components are displayed in an animated format. When a program is running the functionality of this window is disabled to prevent accidental changes to the program parameters.

4.3.4.1 System status information

Various types of information are provided by the fluidics window icons depending on the components they represent.

The icons listed below represent components that have no function other than representing a physical component in the instrument. These have no controllable features and no displayable states and are included only to show their location in the fluidics system. The cursor will remain as a pointer whilst positioned over these components. These are:

- The Pre Wash Column
- The Reaction Coil
- Ninhydrin Backpressure valves

The following components have a displayable state that is shown on the screen. The cursor will remain as a pointer whilst positioned over these components.

Nitrogen Cylinder
The Nitrogen Cylinder can be displayed in two states representing the current pressure level form the nitrogen supply system:

Green = OK
Red = Pressure Low

Pressure Gauges
The 3 Pressure Gauges will show the actual pressure for the **Ninhydrin**, **Buffer** and the **Reaction Coil**. When the pointer is placed on the icon the pressure in bar is shown in a small message box.

Coil Flush
The Coil Flush icon is animated when a Coil Flush command has been triggered, such as when the instrument has been shut down. This is a representation, and is not directly linked to the actual component.

The following components are active. They either have a menu, or have a function that can be triggered by double clicking on them. When the mouse pointer hovers over any of these, the cursor will appear as a hand.

The Bottle control
This represents the 6 Buffer bottles, the Ninhydrin bottle and the Coil Flush water bottle. A bottle is represented with a liquid level indicating the actual reagent volume. The valve on the right hand side of the buffer bottles shows whether the bottle is currently in use and the corresponding fluidic line is coloured green. When the mouse pointer is positioned over the bottle during a run, the actual current volume remaining is displayed. These readings are taken from the Reagent Management system. The Coil Flush water bottle is not part of the Reagent Management system, the volume shown is not actual. The water bottle volume should be checked before running the instrument.

The Pump control
These represent the Buffer pump and Ninhydrin pump. These will be animated when any pump is active. The speed of animation will reflect the pump rate.

The Autosampler control
This represents the Autosampler. This has options to start a Load sequence after setting the Load parameters. The autosampler icon will be animated when an injection is taking place.

The Column
This is associated with a temperature gauge that displays the current temperature. The heating bars of the column will be shown as red when heating, blue when cooling and grey at temperature.

The Reaction Coil
This is associated with a temperature gauge that displays the current temperature. The heating bars of the coil will be shown as red when heating, blue when cooling and grey at temperature.
The Reaction Coil also has an option to Enable/Disable the heating element.

Monitoring Devices
The Detection system is a set of components that contain the Lamp, Flow Cell, Detectors and Chart Recorder. The Lamp and Recorder can be switched On/Off, and the Detectors can have their O.D. settings and baseline positions set.

The Lamp
Can be switched On or Off.

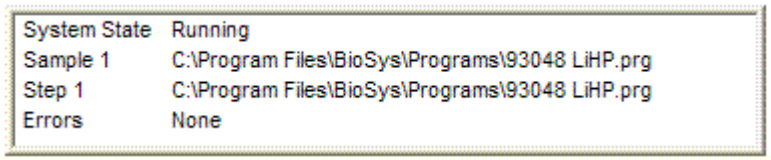
Flow Cell
This symbol has no active function apart from denoting its position in the analyser system.

The Recorder
Can be switched On or Off

The Detectors
Will define the O.D settings and baseline position for each channel, 570 and 440.

The Timer Control
This is used to display one of four timers: the system time, the step time, the elapsed sample time or the run elapsed time.

The Status Pane



The Status Pane shows the current system status. It will indicate whether the system is running, whether any outstanding errors need to be cleared and if the system is running a Sample List, the sample and program details will be displayed.

4.3.4.2 Analyser operation from the menus

The BioSys Fluidics window also contains a menu structure, which allow access to other features and applications. The various options available under each menu are given below.

File/Setup
Opens the BioSys Setup application

File/Shutdown
This will shut the instrument down and close all BioSys applications.

File/Exit
This will close the BioSys Manual program, leaving the instrument running.

Stop!
This will stop the instrument immediately. This is only available during manual operation.

Control/Fluidics/Buffer Number…
This will set the required Buffer and start the buffer pump.

Control/Fluidics/Buffer Assignment
The option will open the Assign Buffers Dialog box to define the buffer and ninhydrin details.

Control/Fluidics/Buffer Pump
This will switch On/Off the Buffer pump.

Control/Fluidics/Ninhydrin Pump
This will switch On/Off the Ninhydrin pump.

Control/Fluidics/Buffer Pump Rate…
This will set the Buffer pump rate.

Control/Fluidics/Ninhydrin Pump Rate…
This will set the Ninhydrin pump rate.

Control/Fluidics/Calibrate Pumps…
This will open the Flow Rates dialog to calibrate the pumps.

Control/Fluidics/Coil Heater
This will enable the Coil Heater.

Control/Fluidics/Coil Temperature
This will set the required Coil Temperature.

Control/Fluidics/Column Temperature…
This will set the required Column Temperature.

Control/Autosampler/Load
This performs an Autosampler Load operation.

Control/Autosampler/Parameters
The option will open the Autosampler Dialog to set the Autosampler parameters such as volumes and wash functions.

Control/Optical/Lamp
This switches the photometer lamp on or off

Control/Optical/Recorder
This switches the Recorder on or off

Control/Optical/Baseline/440… or 570…
This sets the selected channels baseline position

Control/Optical/Optical Density/440… or 570…
This sets the selected channels O.D setting for the recorder output

Control/System/Override
This toggles the Override parameter.

Control/System/Hold
This toggles the Hold state.
Control/System/Timer…
This sets the Step Time.

Control/Reagent Management…
This will open the Reagent Management Dialog box to update the bottle volumes.

View/Manual Control…
This opens the BioSys Manual Control application.

View/Program Control…
This opens the BioSys Programmer application.

View/Program Editor…
This opens the BioSys Editor.

View/Status Bar
This will Display/Hide the Status Bar.

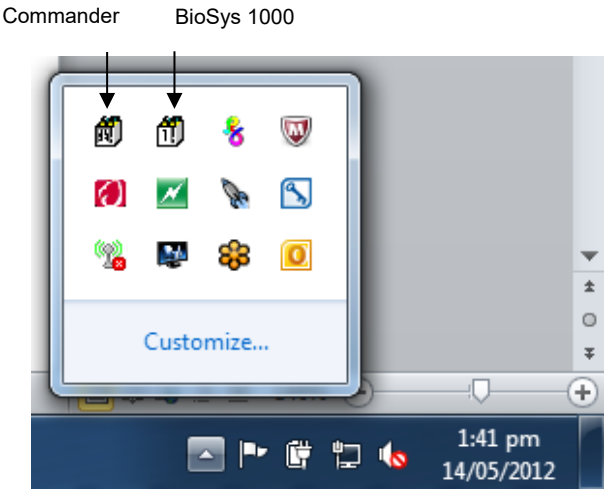
Help/Help Topics
This opens the Help Index, which provides On line help pages.

Help/About BioSys Fluidics
This displays the About box, showing software reference details.

During operation parameters are continually updated and the pumps are animated.

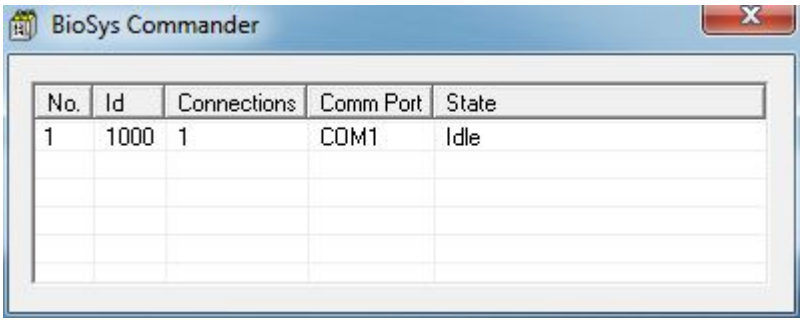
4.3.5 The BioSys Utilities

These are accessed by clicking on the arrow in the task bar and by right clicking on any of the two icons on the task bar.



4.3.5.1 The BioSys Commander

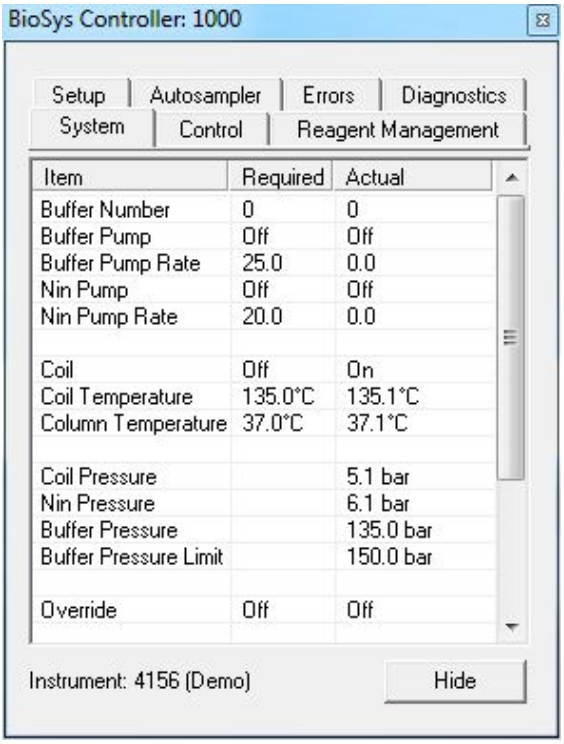
The BioSys Commander has a simple menu, selected by right-clicking on the icon in the task bar showing **Display Commander**, **Display Traces** and **Help**.



The BioSys Commander displays a dialog box that shows the number of connections made to each instrument attached to the system, if more than one analyser is controlled by the same computer. For each Instrument connected the Dialog box shows the **Instrument Number**, the **Instrument Id**, how many applications are **Connected** to the instrument, the instrument **Comm Port** and the Instrument **Status**.

4.3.5.2 The BioSys 1000 Controller

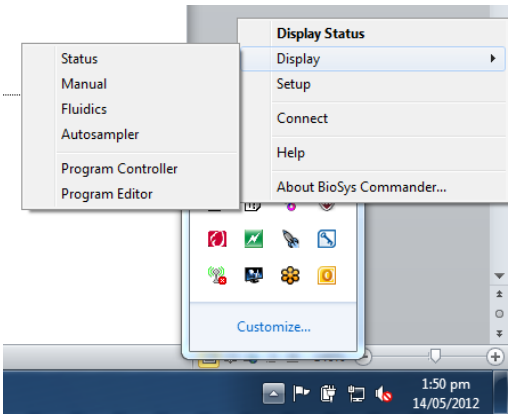
The current status of the instrument and the autosampler can be displayed from the BioSys 1000 Controller. Simply right click on the BioSys 1000 icon in the task bar and select **Display status**



The window shows the entire instrument status. It is divided into a number of tabs: Setup, Autosampler, Errors, Diagnostics, System, Control and Reagent Management.

Each tabs shows the respective status/configuration for different areas of the system.

The BioSys 1000 Controller menu offers other choices to open different parts of BioSys, such as the Manual Operation window, the Fluidics window, check the autosampler status, etc.



4.4 Automatic Operation

4.4.1 Operating the BioSys software

To make a full start up of the system and its functions it is necessary to create all programs and lists with appropriate set up details.

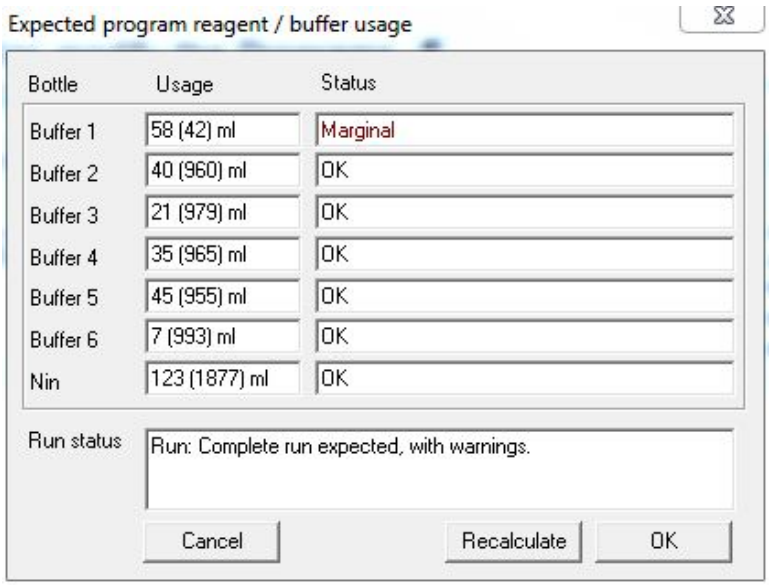
This process is done in a number of steps, as follows:

- Create and/or modify the Programs.
- Create and/or modify a Sample List to be run.
- Setup the instrument using the BioSys Manual Operation screen.
- Run the Sample List.
- Monitor and interact with the system during a run.

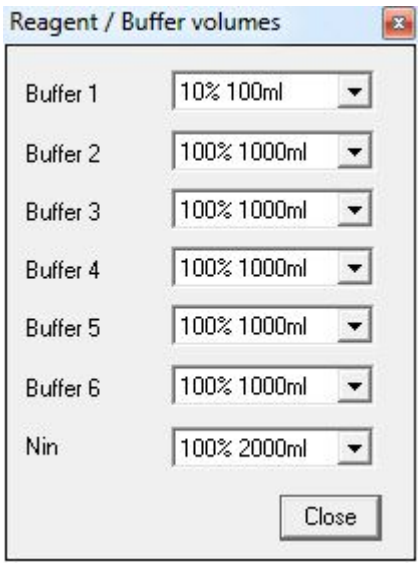
4.4.2 Additional checks before the start-up procedure

When the Reagent Management System is enabled, the reagent levels check is made automatically before a run is started. If the program determines that the run might not complete with the current reagent levels then the window below will be automatically displayed.

See the Reagent Management tab on Setup, Section 4.6.6, to enable or disable the Reagent Management System.

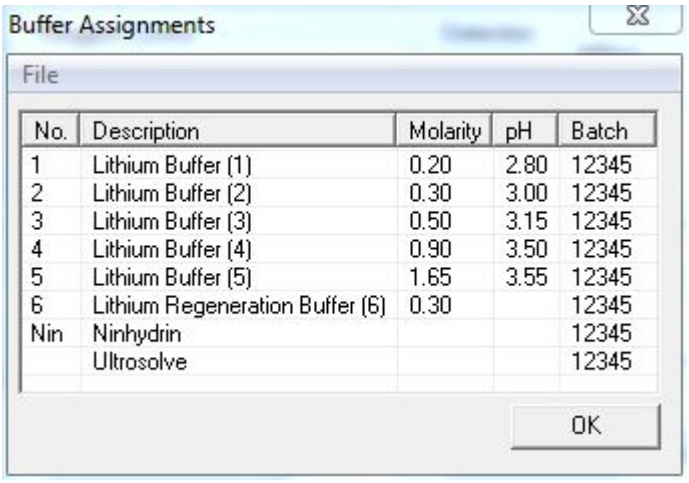


Top up bottles if required and click on the **Recalculate** button to update the volumes.



Click **Close** after the bottle volumes have been updated.

Check that the buffer assignments are correct. This can be accessed by clicking on *Control\Assign buffers* from the BioSys Manual window.



The buffer configuration can be saved by clicking on *file\save* or a previous buffer configuration can be loaded by selecting *file\load.* The buffer configuration file has **.buf** extention.

4.4.3 Autosampler Setup

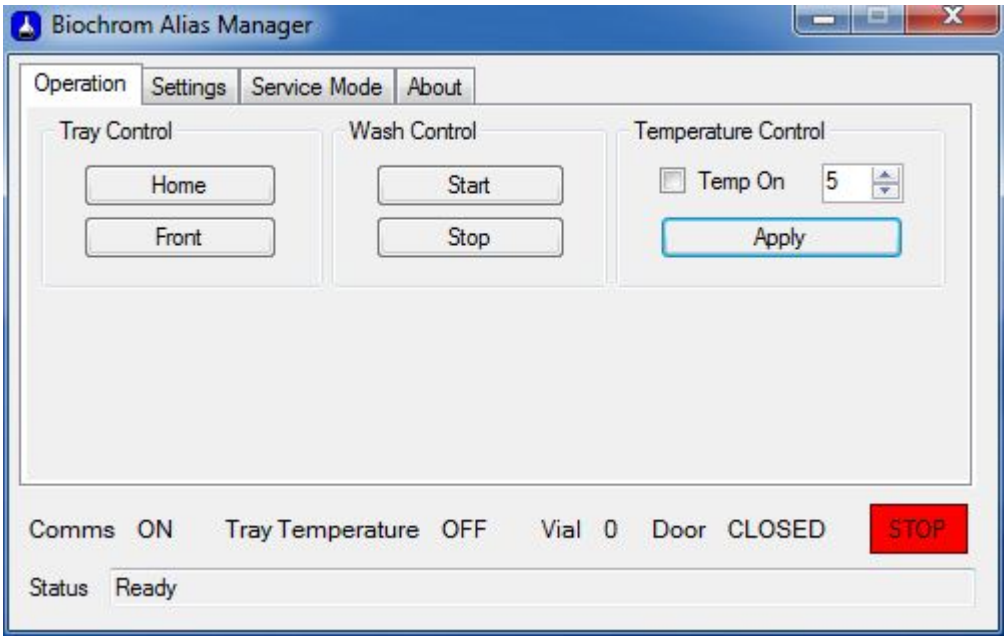
The parameters listed below are used to set up the Autosampler to run in conjunction with the analyser. Please refer to the Autosampler manual for further information on advanced functions.

Make sure the serial cable is connected between the RS232 port on the back of the Autosampler and a spare serial port on the computer.

Switch on the Autosampler using the ON/OFF rocker switch at the back. A green light should lit on the top left hand side of the front panel.

Start the Bio 30+ Autosampler software by double clicking on the icon 

on the desktop. The Bio 30+ Autosampler Interface starts and the following window appears:



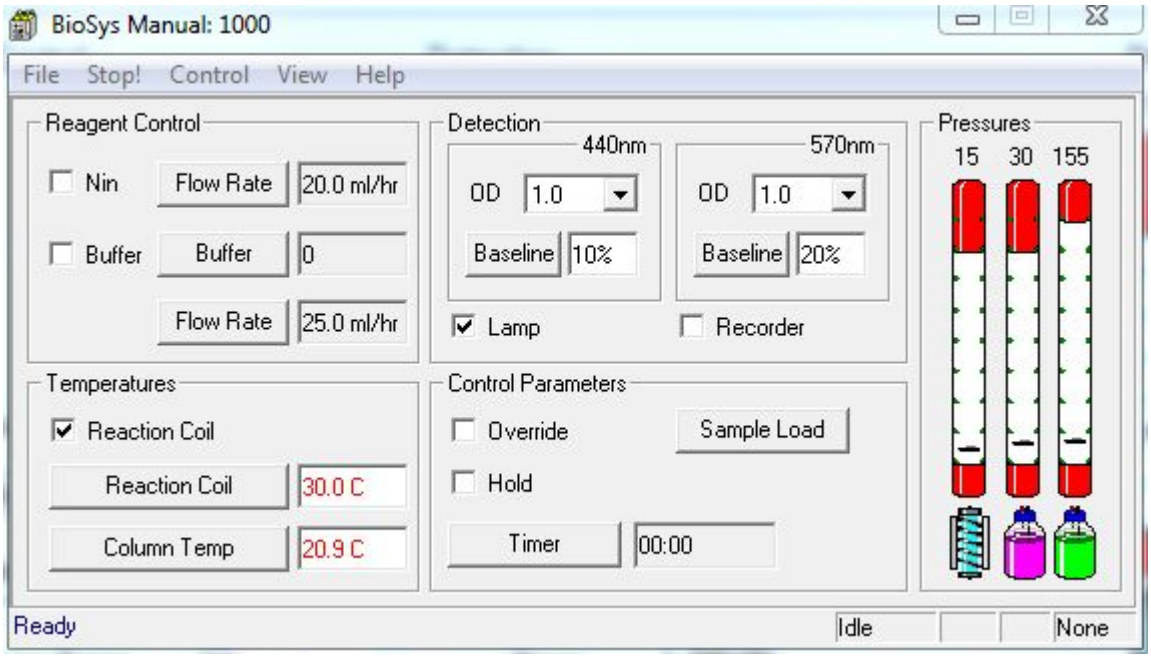
- Choose the required temperature either by typing in the number directly into the box (min 4 max 25) or by clicking up or down on the arrows.
- Tick the box next to the temperature to enable the cooling and then click on the **Apply** button.
- The cooling will now start in the autosampler. You can monitor the actual temperature in the status bar.

The vial number, sample volume, injection mode, and wash cycle are controlled from the BioSys Sample List.

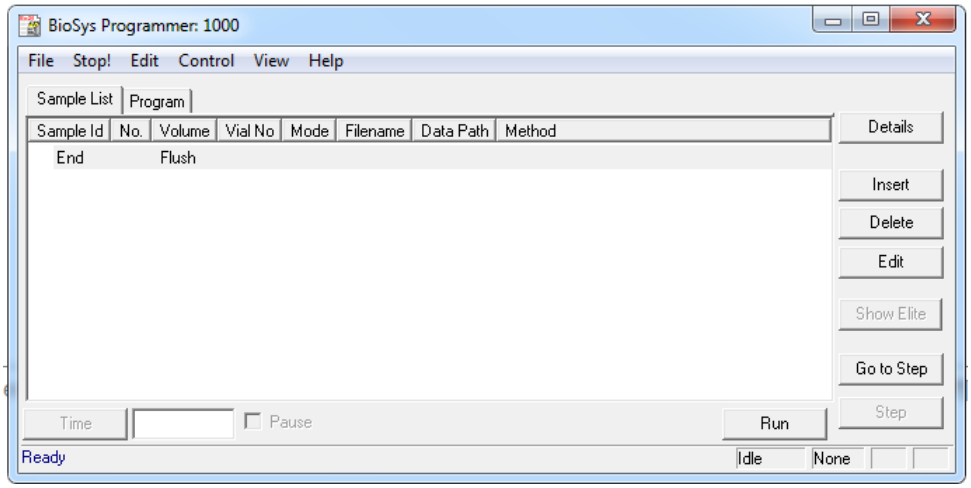
4.4.4 Start up Preparation

On the fluidics cabinet, check that the On/Off switch on the left hand side is in the **ON** position. Switch the computer on and open the Bio 30+ Autosampler Software by double clicking on the shortcut on the desktop.Then start the BioSys Manual Operation window using the usual Windows procedure: Start / All Programs / BioSys / BioSys Manual, or from a shortcut on the desktop.

Prepare the instrument using the Manual Operation window.

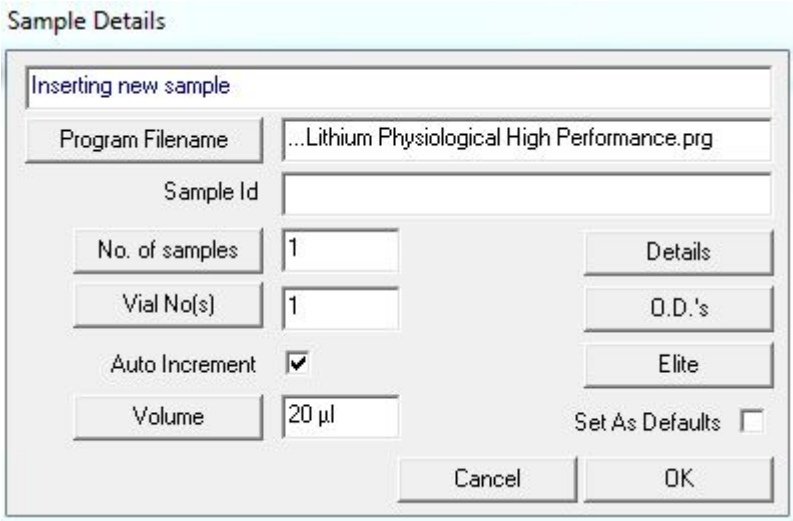


- Click on the **Coil** checkbox to turn the reaction coil on.
- Click on the **Column Temp** button and set the column temperature to 50°C.
- While the reaction coil warms up, you may check the buffers and ninhydrin levels and set up the Reagent Management system if enabled.
- When the reaction coil temperature reaches 101°C, click on the **Buffer** button and select buffer 1. When the pump starts the sound of gas being released can be heard. It comes from the Coil Flush device, allowing the device to fill up with the UltraClean Plus liquid.
- Start the ninhydrin pump by clicking on the **Nin** checkbox.
- The actual coil and column temperatures and pressures can be easily monitored on this window.
- Create the Sample List. To set up the instrument for running samples click on the **View** menu then **Program Control**.



- Click **Insert** then **Program Filename** on the **Sample Details** window to select the first program. This is usually the regeneration program, which runs first to prepare the analytical column. Click **Open** to accept the selection. Click **OK** on the Sample Details window. This program becomes the first step of the sample list.

- To add the analytical program/s click **Insert** and select the program required for the analyses by clicking on the **Program Filename** button.



- Set the following:

    - **Sample ID**: This entry will be applied to all samples but each sample ID can be individually edited when the Sample List is completed.

Note: Only use alpha numeric characters, no signs such as * / or comma are accepted by the software.

    - **No. of samples:** The total number of injections (standards plus samples).

    - **Vial No(s)**: The ID number of the first vial in the sequence.

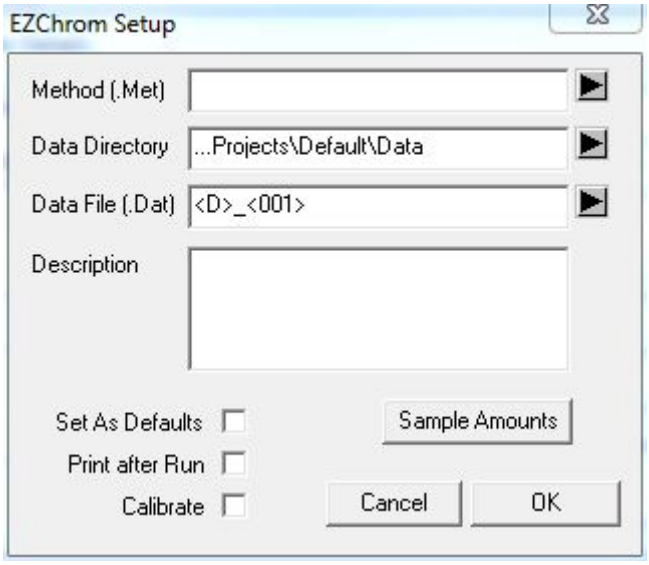
- **Auto increment**: If the samples are going to be in different vials, leave the checkbox ticked on. If more than one injection is required from the same vial, e.g. standard, untick **Auto Increment**.

- **Injection volume**: This entry will be applied to all samples, but each injection volume can be individually edited when the Sample List is completed.

- **Details**: Click on this button to select the injection mode and to enable the needle wash.

- **Set As Defaults:** When checked, this parameter allows the injection volume and the injection mode to become default parameters.

- Click on the **Elite** button to select the Method for data collection.



- **Method (.Met)**: Click on the ▶ arrow to select the method required.

- **Data Directory**: This is the directory where the data will be stored. Click on the▶ arrow to select a different data directory if required.

- **Data File (.Dat):** This is the name given to each run when it is saved at the end of data collection.
  The default filename is <D>_<001>, where <D> is the date (YYYYMMDD) and <001> is a counter.

  For example, running 5 runs on August 19th 2013 will create 5 filenames in the data directory of Elite:

  20130819_001.dat
  20130819_002.dat
  20130819_003.dat
  20130819_004.dat
  20130819_005.dat

- **Print after Run**: Click on the checkbox to print the Custom Report at the end of each run if required.

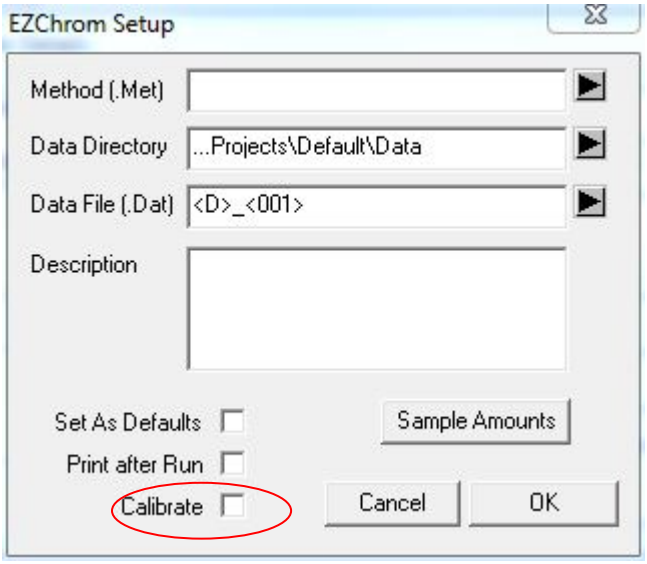
- Click OK when the sample list is completed, and OK on the Sample Details window.

The individual sample IDs can now be edited:

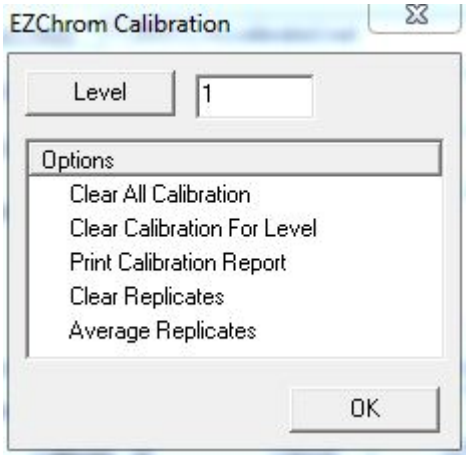
- Double-click on the sample to be edited, the Sample Details windows opens with the details for that sample.
- Highlight the Sample ID and enter the correct one. Click OK when finished.
- Do the same for all samples.

The following parameters can be edited after the sample list is complete:

- **Calibrate:** Double-click on the standard, then click on the **Elite** button. Tick the **Calibrate** checkbox to calibrate the standard if required. This will update the calibration table in the OpenLAB method.

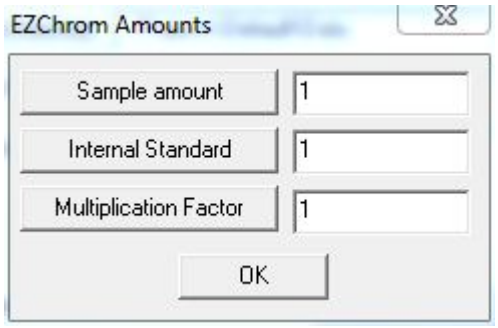


Select the calibration level and the options required.



Click OK to close the window.

- **Sample Amounts**: This button opens a window where the Sample Amount, the Internal Standard amount and the Multiplication Factor can be set.



  Click OK to close the window.

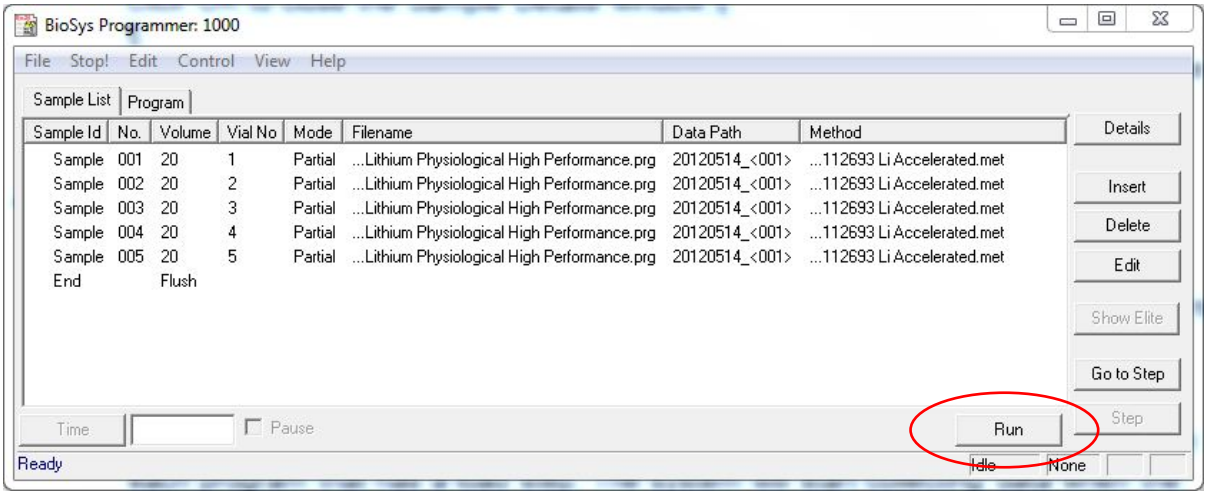
- **Set As Defaults:** When checked, this parameter allows the method, calibration, amounts, print after run and the filename to become default parameters.
  Click OK to close the EZChrom Setup window.

  Click OK to close the Sample Details window.

The Sample List can be printed and can be used as a guide for positioning the vials on the autosampler tray.

4.4.5 Starting The Sample List

- Click on the **Run** button to start the sample list. The regeneration program runs first, followed by the full analytical programs.



- When the sample list is running, OpenLAB is automatically activated at the start of each program that has a load step. The system will start collecting data when the injection is completed.

- Click on the **Program** tab to see the current program list. The steps completed so far are marked with a tick mark and the current step is marked with a green arrow. The same applies to the sample list.

- Click on the **Show Elite** button on the Sample List window to see the data being collected. The OpenLAB Online window opens up locked, showing a grey screen, click on the **Window** menu and untick ✓**Lock**. Click on the **View** menu and select **Tile Data**, the chromatogram will be displayed.

Note: The OpenLAB sequence is created by the BioSys Sample List, every new run adds one line to the sequence. Each run will be saved with the data filename chosen when the Sample List was created. The Sequence will be saved as a csv file in the Sequence directory of OpenLAB with the filename _Current Sequence.csv.

- At the end of the sample list the OpenLAB Online window is closed.

Post processing can be carried out using the sequence and other functions in OpenLAB. Please see the OpenLAB user manual for more details.

Note: do not run the instrument unattended with the *override* function selected

4.4.6 Access During The Run

A number of functions can be accessed from the Sample List menus during the run, these functions show information that may be useful.

The BioSys control program continuously monitors the instrument, if there is any fault during the run the instrument shuts down and a message is displayed on a floating window.

- **Run Times**

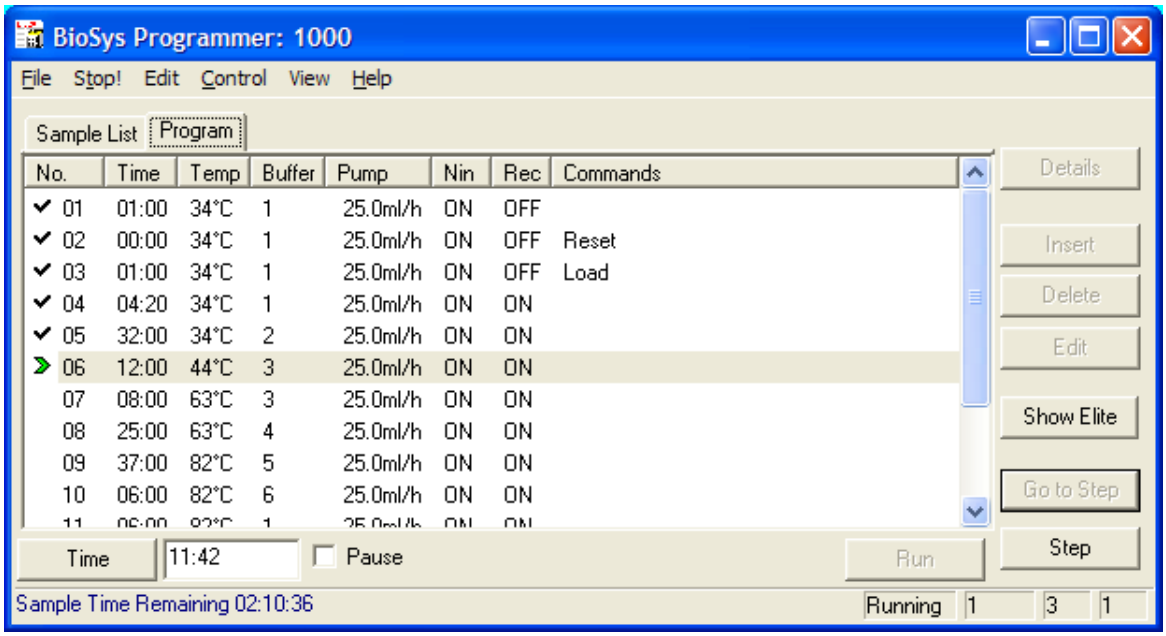
  Click on **View** then **Run Times**, there are four selections:

  - **Elapsed Run Time:** is the elapsed time since the beginning of the sample list.
  - **Elapsed Sample Time:** is the elapsed time of the current sample.
  - **Remaining Run Time:** is the remaining time for the completion of the sample list.
  - **Remaining Sample Time:** is the remaining time for the completion of the current sample.

Any of these times selected will be displayed at the bottom of the window, on the status bar.

- **Editing the current program**

  Any step below the current step can be edited if required. Double-click on the program line to open the Step Editor to change any parameter on that step. These changes are not saved. For these changes to become permanent, edit the program and save it on the BioSys Editor.



  - **Stepping forward**

    Clicking on the **Step** button advances the Sample List or the program to the next step.

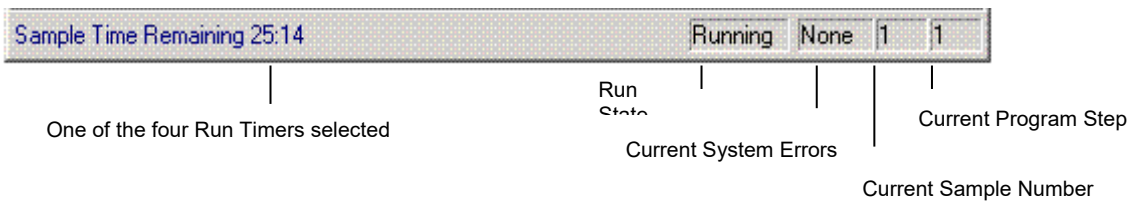
    The Sample List or the program can be advanced a number of steps by highlighting a step below the current step and clicking on the **Go to Step** button.

- **The Fluidics window**

  The fluidics window can be opened at any time to see a graphical view of the instrument operation. The functionality of this window is disabled during the sample list run. Click on the File menu then Exit to close this window.

- **The Status bar**

  At the bottom of the Sample List window is the status bar, which shows some useful information:



- **The Manual Operation Window**

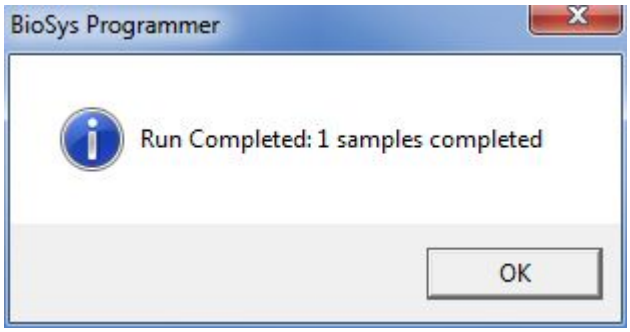
This window displays the current operating parameters such as buffer selected, ninhydrin On or Off, pressures and temperatures.

- **Stopping the Sample List**

  The sample list can be aborted at any time by clicking **Stop!** on the menu bar on the Program Control window. This action stops the complete operation of the instrument. The Coil Flush device cleans the reaction coil.

- **End of Sample List**

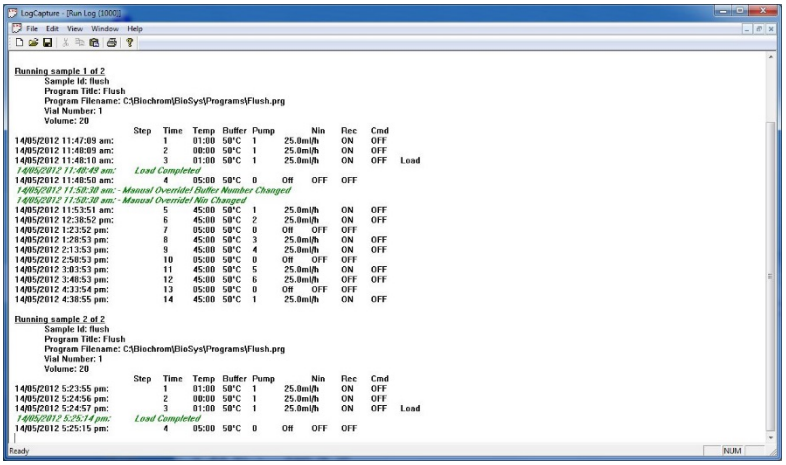
  At the end of the Sample List a message is displayed. The instrument performs a shutdown procedure. The ninhydrin pump stops, the coil is set to off, the column temperature is set to 37°C and the buffer pump pumps buffer 1 for approximately 3 minutes. When the buffer pump stops, the coil flush device washes the reaction coil with approx. 15 ml of distilled water.



4.4.7 Run and Sample logs

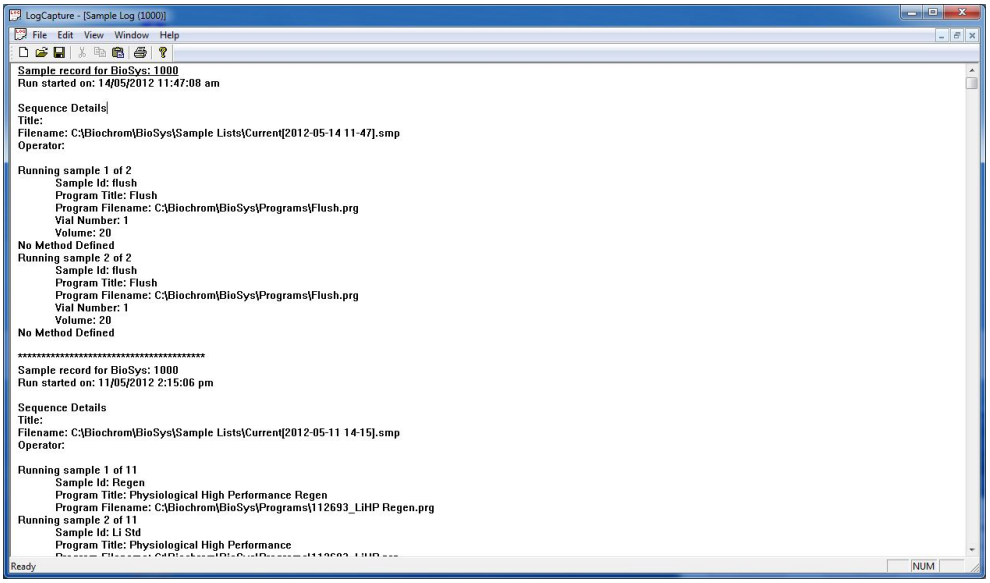
During operation and after completion of a series of analyses the Run and Sample logs accumulate all operating details for archiving.

The Run log is created every time a new Sample List is started. It is available on the *View/Show Run Log* menu. This menu item is only available during the run and after the end of the Sample List.



The Run log lists all the events during the run, showing the date and time of every step in the current program and all parameters in operation. Changes in the Sample List by the user and any error conditions will also be listed.

The Sample log is created from the first Sample List ever ran and is continuously updated with each new Sample List. The Sample log may get quite big with time.



These logs can be filed to provide a complete record of samples analysed with their related control programs.

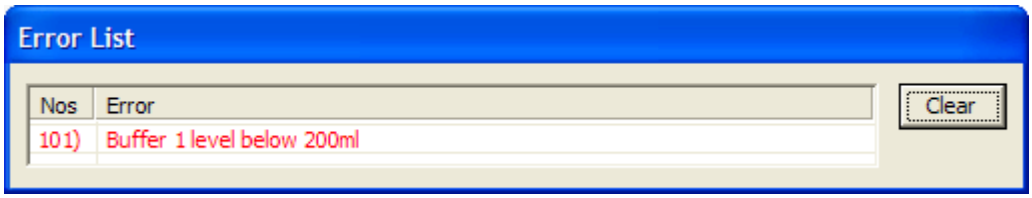
4.5 Reagent Management System

This part of the control software can be used to calculate reagents usage for a series of analyses. Under the **Setup** button click on the Reagent Management tab and click the check box to activate the system. The minimum warning level can also be specified (default 300ml).

The calculations are based on the flow rates and the number of samples in the sample list. Therefore, if the reagent management system is enabled, the sample list must be accurately set to the total number of analyses.

ERRORs 100 to 106 are displayed when any bottle is calculated to have reached the warning level set in the Reagent Management Setup, where Error 100 is Ninhydrin, and 101 is Buffer 1 etc. This error message is only a warning and will not shutdown the instrument.

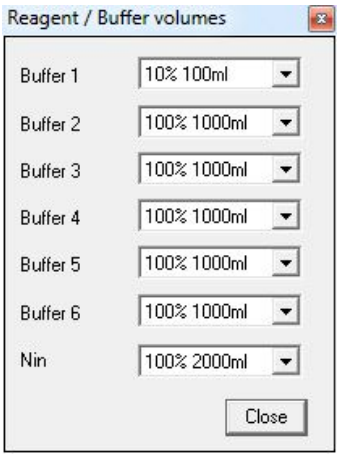
Note: once an error has been triggered, it will not be triggered again until the software is restarted or the relevant reagent level is reset, i.e. after the bottle has been refilled.



4.5.1 Using the Reagent Management System

The **Actual** buffer and ninhydrin flow rate boxes shown in the main dialogue box and manual box shows the current flow rates. These are used to calculate the buffer usage and will be the same as the default setting unless the user has manually altered them via the manual dialogue box.

From the fluidics window either right click over each buffer bottle or click the Control, Reagent Management tabs on the top menu bar to get the dialog box below.

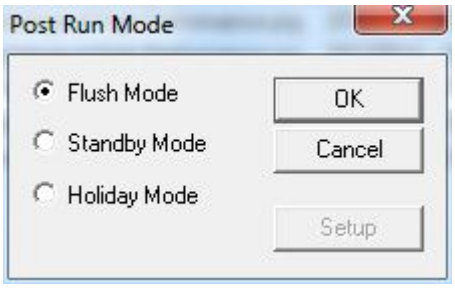


Enter the ninhydrin and buffer bottle levels in percentage. The bottle levels are now saved.

NOTE: When fluorimetric reagent is used enter 50% (1L) on the OPA box, as this reagent is supplied in one litre volume.

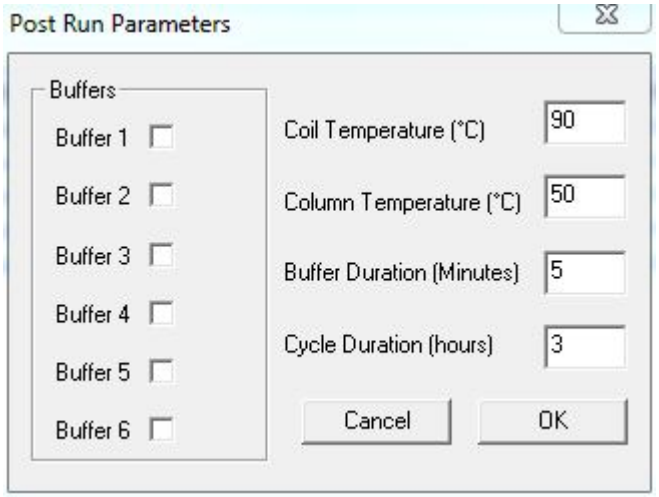
4.6 Standby and Holiday Programs

Within the programmer control window, post run modes can be selected by clicking on *Edit\post run modes*. The mode will also be shown at the end of the sample list. **Flush** is the default mode, which will cause the instrument to go through the automatic shutdown routine.



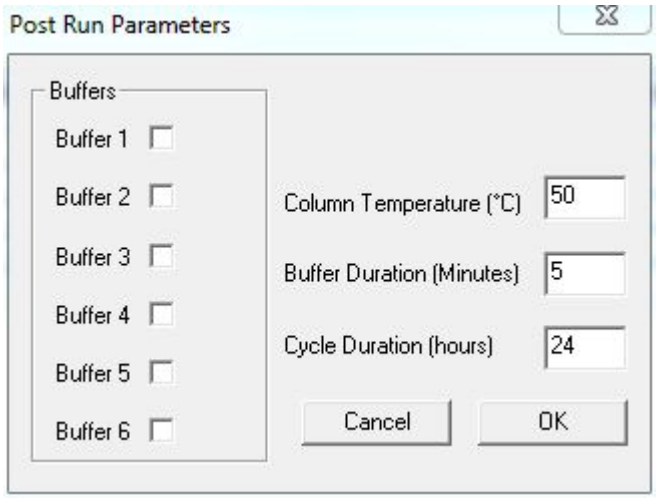
- **Flush Mode** – the instrument automatically shuts down, switching the coil and the ninhydrin pump off, setting the column temperature to 37°C, then pumping buffer 1 for three minutes before switching off. The coil flush device operates automatically.

- **Standby Mode** – this allows the instrument to be left in a quick start mode. The column and coil temperatures will be set and selected buffers will be pumped for a defined duration (in minutes) over a chosen cycle duration (in hours). Clicking on **Setup** will open up the Post Run Parameters Dialogue box for this mode (see below) and enable the various parameters to be set.



- **Holiday Mode** – this allows the buffer pump and valves to be operated over a sustained shut down such as over a holiday period. The coil is switched off (and it is recommended that the ninhydrin reagent is removed and the line flushed with water) but the column temperature is set and the selected buffers pumped for a defined duration over a chosen cycle duration (which is usually longer than that under Standby

Mode). Clicking on **Setup** will open up the Post Run Parameters Dialogue Box for this mode and enable the various parameters to be set.



5 OPERATING INSTRUCTIONS

CONTENTS

5 OPERATING INSTRUCTIONS

5.1 Equipment And Sample Preparation

The following sections detail the procedure to be adopted to prepare the Bio 30+ for operation from the dry state. Also included in this section are the detailed instructions for the preparation of samples.

5.1.1 Installation of the Buffers

Due to the high sensitivity of the Bio 30+, the buffer solutions supplied with the instrument are high quality, filtered buffers; these being used during installation and initial operation. Biochrom buffers must be used to comply with the warranty conditions.

The buffer reservoir bottles are designed to contain one litre of buffer solution. For ease of use, each buffer is colour coded on the label of the 2L plastic container, the buffer line and the 1L glass bottle as shown below:

Bottle line number/name	Bottle Colour code	Additional marking
1	RED	Red line tag
2	ORANGE	Orange line tag
3	GREEN	Green line tag
4	BLUE	Blue line tag
5	PURPLE	Purple line tag
6	GREY	Grey line tag
Coil flush solution	-	Yellow line tag
Piston wash	-	White line tag
Reagent	-	Black line tag

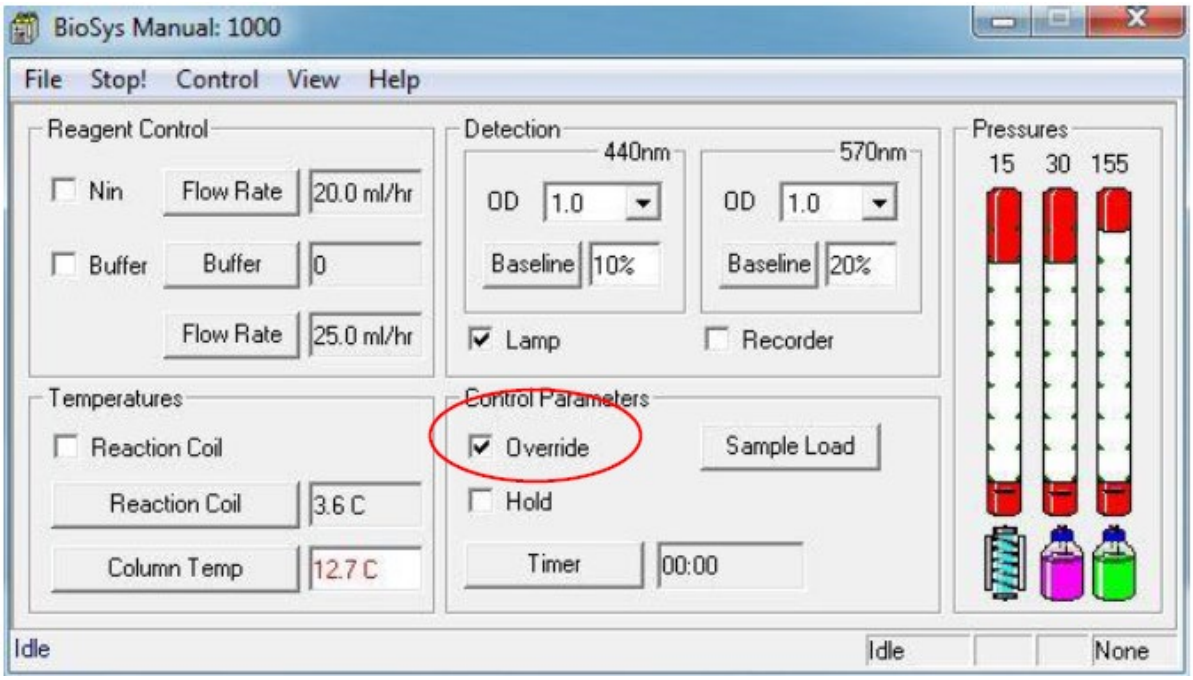
The table below shows the correct positions for each of the buffer systems.

System	B1	B2	B3	B4	B5	B6
Lithium Physiological System	Lithium Buffer 1, pH2.80	Lithium Buffer 2, pH3.00	Lithium Buffer 3, pH3.15	Lithium Buffer 4, pH3.50	Lithium Buffer 5, pH3.55	Lithium Regeneration Buffer 6

Don’t refill incorrect buffer into the bottles, this may lead to the incorrect analysis. Follow the colour coding provided in the bottle.

5.1.1.1 Initial buffer line bleeding

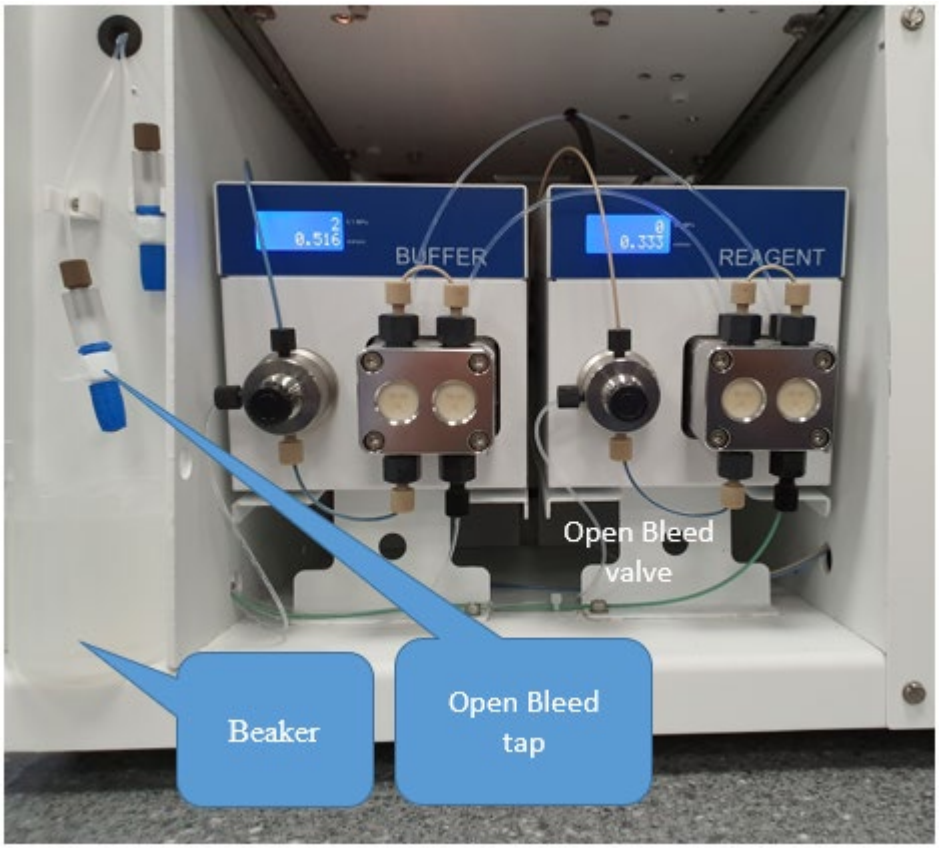
1. Go to the **BioSys Manual control** window and activate the Override system:



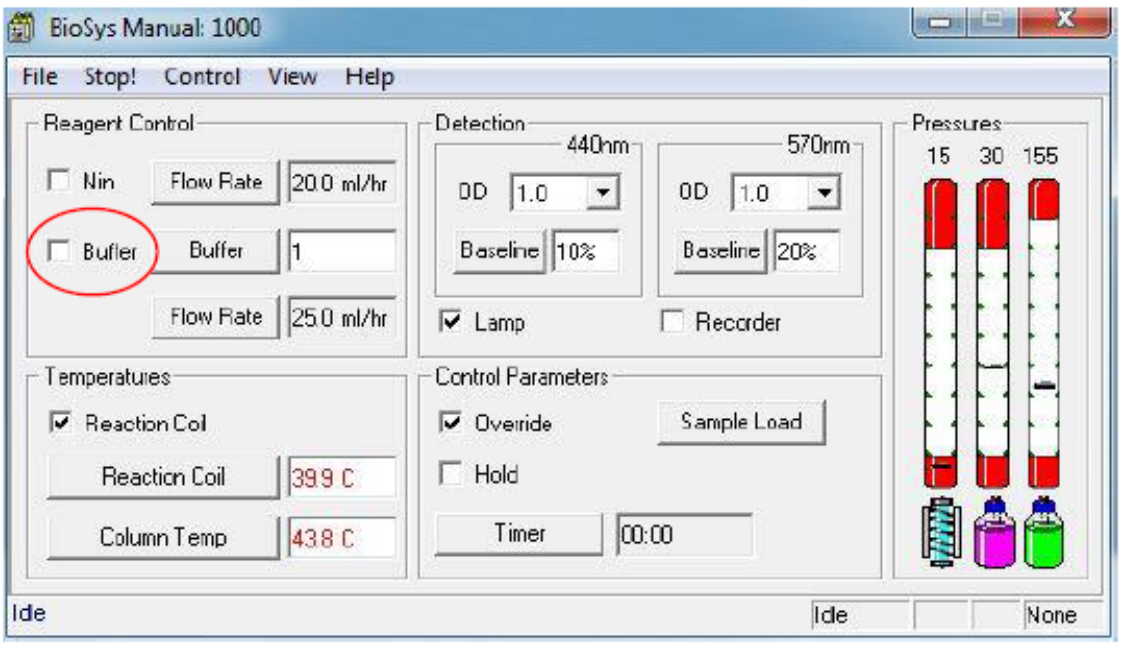
2. Install the buffers required by the analytical system and label the bottles with the buffer batch number. The buffers and reagent lot numbers can be entered on the Assign box, to do this click on the Control menu and select Assign Buffers…, you can then fill in the appropriate information.

3. Turn on the nitrogen supply to the reservoirs and set the pressure to **0.2 bar/3 psi.**

4. Place a beaker under the buffer pump filter bleed tap and open it.



5. From the Manual Control window select **buffer** and press **1** followed by **Enter** then **untick** the buffer pump check box in order to switch the pump off and leave the buffer solenoid valve #1 opened.



6. Air should come out from the buffer line, followed by the buffer. If the buffer does not appear at the tap check that the nitrogen pressure to the bottles is set to 3psi. If the nitrogen pressure is correct, fit the syringe adaptor to the bleed tap and connect the 20ml syringe. Gently operate the syringe plunger to draw the buffer through the feed pipe until the syringe is filled with liquid.

7. Select **buffer 0** to close the buffer solenoid valve.

8. Select each of the remaining buffers and repeat steps (5) to (7). This will prime all of the buffers and the regeneration solution.

9. Close the bleed tap when all of the buffers have been primed.

5.1.1.2 Buffer change during routine operations

When changing a buffer reservoir during normal operation, adopt the following procedure:

1. Ensure that the buffer to be changed is not being pumped.

2. Remove the buffer reservoir bottle cap and lift the bottle out of the buffer compartment. Clean and refill the buffer reservoir. Once clean and dry it is best to rinse the bottle with a small amount of the new buffer.

Note: Never mix new and old buffer lot numbers.

3. Replace the buffer bottle onto the buffer compartment in the Bio 30+ and refit the buffer tube and cap.

4. If a small amount of air enters the system during this procedure, it will be removed at the buffer pump inlet filter.

5. If an air lock does form in the buffer feed line then bleed this line, as described in section 5.1.1.1.

5.1.2 Controlling the Nitrogen Pressure into the Buffer Bottles

The nitrogen pressure in the buffer and ninhydrin bottles is factory set to the level required for the routine use of the Bio 30+. The nitrogen pressure regulator is mounted on the top left hand side on the front of the instrument, the pressure gauge above the adjuster indicates the pressure of the nitrogen feed. To increase the nitrogen pressure, pull the knob and turn the knob clockwise slightly. Monitor the pressure on the gauge above the knob.

When the nitrogen pressure is being reduced, a considerable delay may occur between the knob adjustment and the visible indication on the pressure gauge.

A pressure relief valve in the nitrogen low pressure manifold operates if the nitrogen pressure rises to 6psi, thus preventing any possibility of damage due to overpressure.

Note: Always adjust the operating pressure to 3psi for safe operations and reproducible results.

5.1.3 Bleeding the Buffer Pump

Air may get to the buffer pump when a buffer runs out during the analysis. Any air present in the check valves will prevent the pump from operating, therefore, if the pump fails to operate perform the following procedure to remove all air from the buffer pump system:

1. Open the Bio 30+ front door.

2. Go to the **Biosys Manual** control window and activate the Override system as described in section 5.1.1.1, set the column to 50°C and turn on the reaction coil.

3. Open the pump bleed valve located on the integrated pressure transducer on the front of the pump by turning the knob anticlockwise (see fig 5.1).

4. Start pumping buffer 1 and increase the flowrate to 50ml/h.

5. After 1 minute (when the pump has stopped) close the buffer bleed valve located on the front of the pump by turning the knob clockwise.

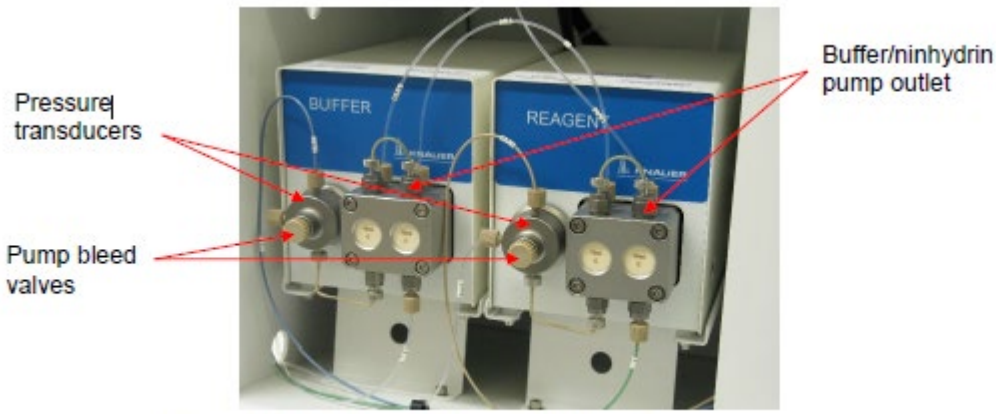


Figure 5.1 Buffer/Reagent Pumps with integrated pressure transducers

If this procedure did not remove all of the air from the pump then proceed as explained in the following:

1. Enter **Buffer 0** to stop the pump and close the solenoid valve.
2. Remove the buffer outlet tubing by unscrewing the fingertight connector from the top of the outlet check valve (See figure 5.1).
3. Select **buffer 1** and activate the override function.
4. Attach the pump bleeding syringe to the outlet.
5. Slowly draw the plunger out of the syringe until all the air is removed from the system.
6. Enter **Buffer 0** to stop the pump and close the solenoid valve.
7. Reconnect the tubing to the outlet check valve.
8. Deactivate the Override function.

5.1.4 Bleeding the Ninhydrin Pump

Air may get to the ninhydrin pump when the reagent runs out during the analysis, or when the reagent is replaced and a new filter cartridge is fitted. Any air present in the check valves will prevent the pump from operating, therefore, if air is present in the pump, perform the following procedure to remove this air:

NOTE: check section 5.1.9 for instructions on how to prime the ninhydrin tubing and pump when replacing the ninhydrin bottle.

1. Open the Bio 30+ front door.
2. Open the ninhydrin pump bleed valve located on the front of the ninhydrin pump by turning the knob anticlockwise (see fig 5.1).
3. Leave the knob open for 1 minute. Any air bubbles in the line should go through the waste line.
4. Close the ninhydrin pump bleed valve by turning the knob clockwise.

If this procedure did not remove all of the air from the pump then proceed as explained in the following:

1. Remove the ninhydrin pump outlet from the top of the check valve. Attach the pump bleed syringe.

Note: do not use the same syringe as for the bleeding of the buffer pump to avoid cross contamination.

2. Slowly draw the plunger out of the syringe until all the air is removed from the system.

3. Reconnect the ninhydrin outlet pipe.

4. Deactivate the Override function.

Note: The ninhydrin pump will not start if the coil temperature is below 101°C.

5.1.5 Pressure Transducers

The pressure transducers are maintenance free as they are integrated to the pumps.

5.1.6 Setting the Buffer and Ninhydrin Flow Rates

Flow rates for a particular analysis are chosen to provide adequate separation of the amino acids present in the sample. Ideally this separation should be achieved in the minimum possible time and at an operating pressure of less than 145 bar. If temperature changes are made during the analysis then the maximum flow rate is limited by the pressure at the lowest operating temperature.

The buffer and ninhydrin flow rates should be maintained at the values recommended for the program in use. During a series of analysis the flow rate must remain the same to obtain reproducible retention times.

NOTE: The maximum operating pressures of the system are as displayed on the Pressures Display box. The levels are:

Buffer	**150 bar**
Ninhydrin	**30 bar**
Reaction coil	**15 bar**

The buffer and ninhydrin flow rates should be maintained at the values recommended for the program in use. During a series of analysis the flow rate must remain the same to obtain reproducible retention times.

The default buffer and ninhydrin flow rates are set in the Setup window. These flow rates are used when operating the system manually.

To set flow rates for specific programs simply enter the value in the program editor, these flow rates will overwrite the default flow rates if they are different.

To manually change the current flow rates, go to the **Manual Control** window. Press the **Nin Flow Rate** or **Buffer Flow Rate** buttons to change the current flow rates.

The default flow rates can be set as follows:

Click **File** then **Setup…** followed by the **Default Pump Rates** tab.New default flow rates can now be entered for the buffer and reagent pump. The standard flow rates are shown below:

System	Buffer Flow Rate	Ninhydrin Flow Rate
Physiological accelerated	35 ml/h	20 ml/h
Physiological High Performance	25 ml/h	20 ml/h
Physiological High Resolution	20 ml/h	20 ml/h

5.1.7 Bio 30+ Flow Rate Calibration

The buffer and ninhydrin flow rates should remain quite stable during normal operation. If it is necessary to check them, just follow the steps below.

It is recommended that the supplied 2.0 ml burette is used for this operation. The volume to measure for the calculation is 0.5 ml.

First measure the buffer flow rate, then measure the combined buffer and ninhydrin flow rates.

The buffer or ninhydrin pump speed is automatically adjusted by a coefficient given by the program.

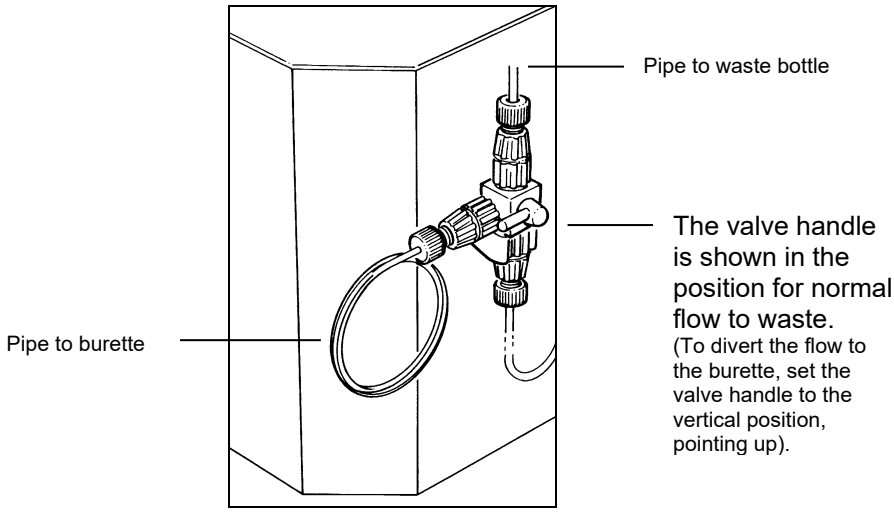
5.1.7.1 Calibration Procedure:

Please follow the following steps when checking the buffer and ninhydrin flow rates:

1. Set the coil to on and the column to 50°C.

2. When the column temperature has settled down, start pumping buffer 1.

3. Place the burette on a stand. Divert the waste liquid to the burette by switching the 3-way valve mounted on the reaction coil.



4. Click on the **Control** button then **Flow Rates…**

5. Click on the **Measure Flow Rate** button in the buffer pump section, the flow rate counter appears.

6. Close the burette valve and when the liquid reaches the zero mark click on the Start/Stop button to start the counter. Stop the counter when the liquid reaches the mark corresponding to 0.5 ml. The display on the counter reads the actual flowrate in ml/hr. Measure a couple of times to confirm that the flowrate is stable.

7. Close the flowrate counter. At this point the program asks whether the Calibration Coefficient needs to be updated using the new flow rate. When the Yes button is clicked, the software adjusts the pump flowrate. Now click on the Verify Flow Rate button and repeat the measurement to confirm that the flow rate is now OK.

8. To adjust the ninhydrin pump flowrate, click on the **Measure Flow Rate** button in the ninhydrin pump section, the flow rate appears. If the ninhydrin pump is off and the coil temperature is >101°C, the pump will start on clicking the button.

9. Allow the ninhydrin flow rate to stabilise and measure the flow rate as explained in step 6 above.

Note: At this point the combined flow rate is measured but the program calculates the actual ninhydrin flow rate.

10. Same as steps 7 and 8 above.

5.1.8 Preparing the Ultra Ninhydrin Reagent

IMPORTANT: Always wear gloves and safety glasses when handling the Reagent.

A ninhydrin preparation kit is supplied to ensure that the ninhydrin used is of the required quality. This kit comprises a polythene bottle containing Ultrosolve Plus (a solvent/buffer mixture), a smaller bottle of Ultra Ninhydrin Solution. To prepare the ninhydrin reagent proceed as follows:

1. Pour approximately 1700ml of the Ultrasolve Plus 1.75L solution into a clean empty Analyser reagent bottle.
2. Add a clean magnetic stirrer.
3. Bubble the solution with Nitrogen for approximately 10 minutes under constant stirring.
4. Shake the Ultra Ninhydrin solution bottle.
5. Pour the content of the Ultra Ninhydrin solution into the Analyser reagent bottle containing the Ultrosolve Plus previously prepared at step 3.
6. Use the remaining 50ml of the Ultrosolve Plus 1.75L solution to wash the empty Ultra Ninhydrin solution bottle of any remaining residue.
7. Add this to the Analyser reagent bottle.
8. Continue bubbling and leave the reagent mixing for a further 10 minutes.

IMPORTANT: do not bubble the solution for more than 10min as this might evaporate the solvents in solution.

9. When ready a clear red/orange coloured solution should be present without any deposit visible.

5.1.9 Replacing the Ninhydrin Reagent Bottle

To replace Ultra ninhydrin reagent in the Bio 30+ proceed as follows:

IMPORTANT: The Ultra ninhydrin reagent must always be prepared in the supplied 2L plastic coated bottle, using the procedure described in section 5.1.8 to avoid any reagent degradation.

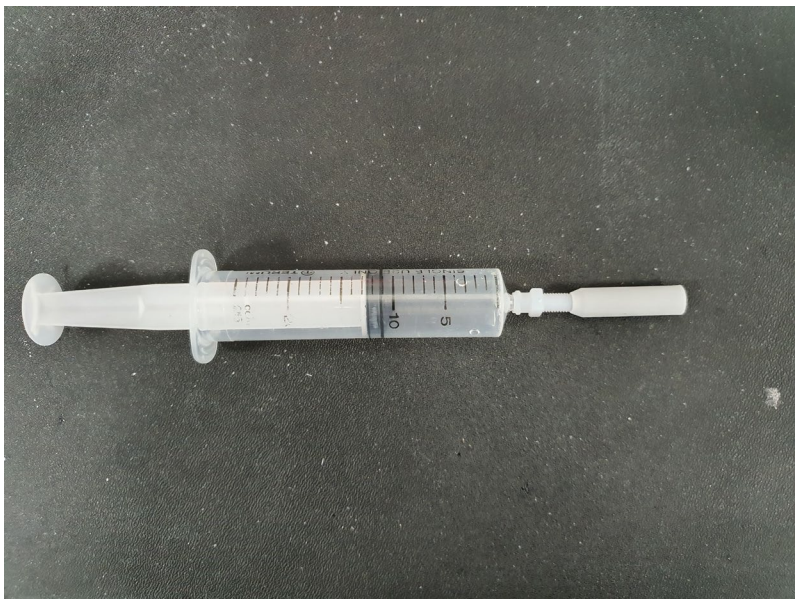
1. Select Override, column temperature to 50°C and the reaction coil to ON.

2. Install the ninhydrin bottle on the instrument, place the ninhydrin feed line into the bottle and.tighten the cap.

5.1.10 Ninhydrin filter cartridge replacement

It is important to remove as much air as possible from the new microfiber filter element to prevent air getting into the Reagent pump where it may cause pressure fluctuations

1. Flush the new filter element with IPA using a syringe as shown below



2. Replace the ninhydrin filter cartridge and place the ninhydrin bottle in position.

3. Open the ninhydrin air bleed tap on the side of the instrument and place a beaker underneath, the nitrogen pressure in the bottle will push ninhydrin solution through.

4. Leave the tap open for 10 min in order to flush any air coming out from the new filter.

5. Close the tap and start pumping buffer 1. If the coil temperature is over 101°C, start the ninhydrin pump **with the ninhydrin bleed valve opened on the pump**.

6. Pump to drain for a few seconds then close the ninhydrin bleed valve. The ninhydrin pressure should increase.

If the ninhydrin pressure fails to increase, the pump may have to be primed by hand:

1. Stop the ninhydrin pump.
2. Place the ninhydrin bottle on the bench and loosen the bottle cap to release the nitrogen pressure.
3. The reaction coil should be switched on and allowed to reach its operating temperature before liquids are pumped through the system. From ambient temperature, the coil will require up to 30 minutes to reach the standard operating temperature. The coil operating temperature can be adjusted to between 40°C and 145°C.

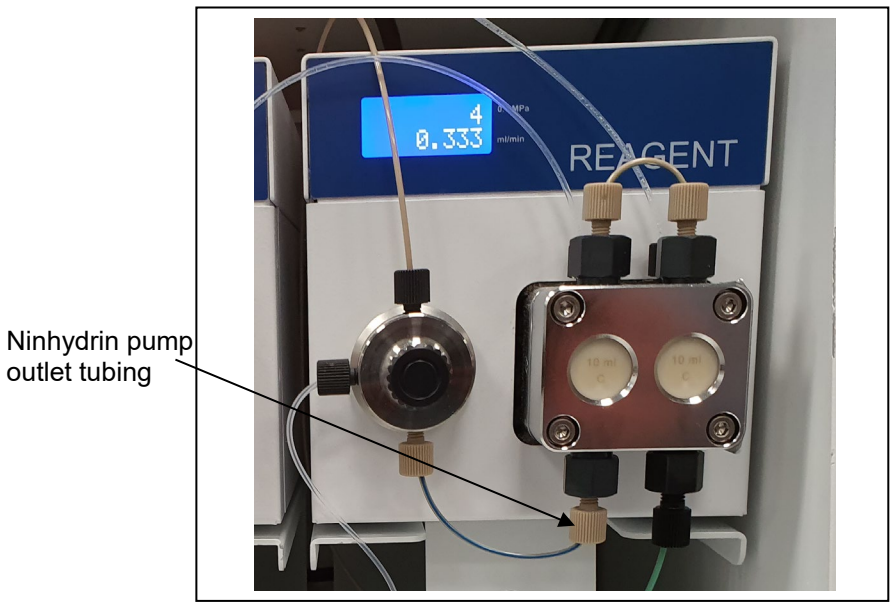


Figure 5.2: Ninhydrin pump

4. Slowly draw the plunger out of the syringe until the air is removed from the system.
5. Reconnect the ninhydrin outlet tubing to the bottom of the check valve assembly. Tighten the bottle cap and place the bottle in position.
6. Pump ninhydrin to drain for a few seconds then close the bleed valve.
7. The ninhydrin pressure gauge on the Pressures Display box should now read approximately 10 bar and the pressure in the reaction coil should have increased by about 1 to 2 bar as the ninhydrin is added to the buffer flow.
8. Remove the Override function.

The system is now primed with ninhydrin and ready for normal operation.

NOTE: The filter fitted to the ninhydrin line in the ninhydrin reservoir should be replaced at least every month. A blocked filter can produce pump starvation and low pressure errors.

5.1.11 Reaction Coil

The reaction coil should be switched on and allowed to reach its operating temperature before liquids are pumped through the system. From ambient temperature, the coil will require up to 30 minutes to reach the standard operating temperature. The coil operating temperature can be adjusted to between 40°C and 145°C.

Normal operating temperature for Ultranin is 135°C and for EZNin 138°C

5.1.12 Photometer

The only preparation required to use the photometer, prior to operation, is to ensure that the lamp is on. In the event of a lamp failure, replace the bulb as described in section 6.2.11.

5.1.13 Recorder (optional)

The output from the photometer is 100mV, therefore, set the recorder input range selector switches to the 100mV position. The recorder is a completely self-contained unit and is separately fused. For detailed description of the recorder see the recorder Instruction Manual.

5.1.14 Sample Preparation for Physiological Fluid Analysis

De-proteinised blood plasma sample preparation instructions are provided in the Instructions for Use for Reagents and Kits.

In the preparation of all samples, for the best results the following precautions must be observed.

1. The sample must be completely free of proteins and large peptides.
2. Where possible maintain the molar concentration of salts in the sample below the level where they interfere with the application of sample to the analytical column.
3. The pH of the sample should be controlled so that chromatography of the acidic amino acids is not affected.
4. When heat labile amino acids are being measured the sample preparation must be performed at +4°C.
5. It is recommended that all samples are filtered using a 0.2µm membrane filter before loading into the vial.
6. Centrifugation must always be performed at the highest speed, greater than 10^4g.

5.1.15 Standard Amino Acid Solutions: Preparation

5.1.15.1 Biochrom Physiological Standard (80-6002-80) –

To prepare a Physiological standard, mix the solutions in the following proportions:

Physiological Standard	1 Volume
Norleucine Internal Standard	1 Volume
pH 2.2 Lithium Loading buffer	3 Volumes

The concentration of norleucine internal standard solution should be prepared at 2,500umol/L. This produces a final working concentration of 500umol/L of most constituents except cystine and homocystine, which are 250umol/L and Urea which is 5000µmol/L.

If required, prepare fresh glutamine solution at a concentration of 2,500umol/L and add one volume of this solution and two volumes of Loading buffer instead of three volumes of Loading buffer.

5.1.16 Autosampler Operation

The autosampler settings are controlled by the Biochrom Alias Manager software while the autosampler operations are controlled by the BioSys control program. During the autosampler operation, the needle is inserted into the sample vial and a programmed volume is loaded into the sample loop. The injection valve then operates to pump buffer through the loop which carries the sample onto the analytical column.

At the end of a series of analyses the Bio 30+ will pump buffer 1 for five minutes and then automatically switch-off.

When loading the autosampler vials the length of time required for each analysis must be considered, as storing the sample at 4°C for more than 72 hours will result in the partial loss of the labile components (e.g. glutamine).

See Section 4.2.2 for further information about the settings.

5.1.17 Bio 30+ Safety Systems

A safety system within the Bio 30+ monitors the state of various parameters, providing an automatic shut-down facility in the event of a fault condition occurring. The conditions which cause automatic shut-down are as follows:

High coil temperature	Low buffer pressure
High column temperature	Low coil pressure
High ninhydrin pressure	Low ninhydrin pressure
High coil pressure	Low nitrogen pressure
High buffer pressure	Photometer lamp failure

5.2 Analytical Procedures

5.2.1 Initial Checks

Before commencing an analysis or a series of analyses check the following:

1. The reaction coil temperature is correctly set, the coil is switched on and the temperature is stabilised. Normally 30 minutes are required for the temperature to stabilise.
2. The analytical column temperature is set correctly and stable.
3. The buffer reservoirs contain sufficient buffer for a series of analyses.
4. The ninhydrin reservoir contains sufficient ninhydrin for a series of analyses.
5. The Coil Flush bottle contains enough liquid.
6. The nitrogen cylinder contains sufficient nitrogen for the analyses and the regulator is set to deliver a pressure of 5 bar.
7. The integration system is set up.
8. The chart recorder, if present, has sufficient supply of paper and that the pens are working correctly.
9. The printer has sufficient supply of paper.
10. The drain reservoir is empty or has enough capacity.
11. The piston flush reservoir contains sufficient distilled water.

5.2.2 Setting the Chart Recorder (optional)

1. Chart Speed. Rotary switch to select the chart speed and a two-position key to select mm/min or mm/ sec. Set these to a suitable value (e.g. 2mm/min for Lithium system).

2. Input Range. Rotary switch to select the input ranging for each channel in mV. Set these to 100mV for both channels.

3. Pen Up/Down. Two-position key to activate the electrical pen lift. Set this to place the pens in contact with the paper.

4. See Section 9, Appendix C for the recorder setting up.

5. In addition to these controls on the chart recorder unit, the following programming functions on Biochrom 30 also affect the chart recorder.

6. Linear/Logarithmic Amplifier Response. This programming function controls the form of the display on the chart recorder so that the chart peak heights are either linearly or logarithmically proportional to the amino acid concentrations.

7. Optical Density Range (OD). This determines the sensitivity of the Biochrom 30 which should be commensurate with the expected amino acid concentration. The following is intended as an approximate guide to the OD setting for various amino acid concentrations.

Amino Acid Concentration		Optical Density Setting
Less than	1 nanomole	0.1 OD
	2 nanomoles	0.2 OD
	5 nanomoles	0.5 OD
	10 nanomoles	1.0 OD
Greater than	10 nanomoles	2.0 OD

8. Baseline Offset. This determines the baseline position on the chart recorder. In the linear mode of operation the baseline is usually set to the right of the recorder chart whereas, in the logarithmic mode it is usually set to the left of the chart.

Evaluation and Record Keeping

During Bio 30+ operation, complete records of all operating parameters, reagent changes and operating pressures must be maintained. Only by maintaining these records can the Bio 30+ performance be monitored and any degradation detected. See Qualification & Performance Verification document.

Quantitative analysis of each amino acid is performed by determining the area under each peak using the data integration software (e.g. OpenLAB EZChrom). Alternatively, a suitable integrator can be connected to the integrator socket on the mains panel.. Three possible outputs for integration are available from this socket. These are 440nm, 570nm and combined signals from both channels. The start of run for the integrator and the 0V signals are also available. See Section 9, Appendix D for more information on the integrator connections.

5.3 Shut-Down Procedure

At the end of a sample list there are three options for the shutdown procedure - Flush, Standby and Holiday Modes (see Section 4). If the instrument is going to remain unused for a few days (e.g. over a weekend) and the instrument might be needed at short notice it is advisable to set up the Standby Mode at the end of the sample list so that the pumps are cycled and the coil and column are heated ready for a quick start up.

If the instrument is going to be unused for over a week then it is better to set up the Holiday Mode at the end of the sample list which enables the buffer pump to be cycled over a longer period. In this case the ninhydrin reagent is removed (and stored at 4ºC) using the following procedure:

1. Remove the ninhydrin reservoir and replace with a reservoir of Isopropanol/distilled water 50/50.

2. Start pumping buffer 1, open the ninhydrin pump bleed valve and start the ninhydrin pump. After 10 minutes colse the ninhydrin pump bleed valve and pump for another 10 minutes.

When the ninhydrin reagent is replaced on the instrument after storage, it is advisable to make sure there is no oxygen present by de-gassing it with nitrogen.

If the instrument is to remain shutdown for a period greater than one month then it is advisable to also perform the following:

1. Remove the analytical column and the prewash column and store them in their original packaging at room temperature. Link the inlet and outlet pipes using a connector.
2. Replace the buffers with IPA/water 50/50 and pump each ones through the system.
3. Switch off the photometer lamp and the reaction coil.
4. Your instrument is now safely in long storage condition.

6 MAINTENANCE

CONTENTS

6 MAINTENANCE

6.1 Preventive Maintenance Schedule

To obtain consistent and accurate results from Bio 30+, the routine maintenance procedures described in this section should be performed carefully. These procedures are divided into daily and monthly checks.

6.1.1 Daily Checks

Each day, before commencing instrument operation, check the following:

1. The volume of buffer in the buffer bottles.
2. The volume of ninhydrin in the ninhydrin bottle.
3. The volume of solution in the coil flush bottle.
4. The volume of distilled water in the piston wash bottle.
5. The level of waste in the drain bottle.
6. The quantity of paper in the chart recorder (optional)
7. The recorder pens are functioning correctly (optional)
8. The quantity of paper in the printer.
9. The quantity of nitrogen in the cylinder.
10. The level of wash solution in the autosampler wash bottle.
11. Check condition of the ninhydrin bottle filter.

Failure to perform these checks may invalidate an analysis or a whole series of analyses.

6.1.2 Monthly Checks

Each month, before commencing a series of analyses, perform the following checks:

1. Clean the flowcell and back pressure valves with solvent, e.g., isopropanol (see section 6.2.9).

2. Replace the Ninhydrin bottle filter

Note: Never add new buffer to old. Always discard remaining buffer. Thoroughly clean and rinse buffer reservoir and refill with fresh buffer.

After performing these checks, perform the daily checks before commencing instrument operation.

6.2 Maintenance Procedures

This section describes in detail the maintenance procedures to be performed on the Bio 30+ to ensure that the best possible results are obtained. These maintenance operations are referred to in section 6.1 Preventive Maintenance Schedule and section 7 Fault Diagnostics.

6.2.1 Analytical Column

The columns produced for amino acid analysis are intended to give excellent lifetimes. The column matrix has proved to be stable for several years based on continuous operation and the column materials are stable when used with the buffers provided. Periodic cleaning and repacking is necessary to maintain optimum performance and is carried out at Biochrom for a reasonable charge (contact your local sales representative for details).

The required frequency of these procedures depends on the treatment of and the types of samples analysed. The sample hydrolysis and deproteinisation methods are described in the reagent Instructions for Use.

During normal operation the analytical column will not require any maintenance unless the buffer pressure rises above the safe maximum limit, or the resin in the column becomes contaminated. To reduce the possibility of either of these problems arising:

- Ensure that the column has attained the analytical temperature before pumping buffer.
- Do not pump buffer through the column at a flow rate greater than 40ml/hr as this may cause the resin bed to compress, creating headspace.
- Do not load dirty or improperly prepared samples onto the analytical column. Always use the recommended sample preparation methods.
- Do not use buffers which are contaminated with micro-organisms.

Before removing the analytical column, prepare the Bio 30+ as follows:

1. Switch off the reaction coil and the ninhydrin pump and pump buffer 1 through the system for 10 minutes to cool down and remove any ninhydrin from the coil.

2. Allow the column temperature to settle at the low temperature.

3. Switch off the buffer pump and allow the buffer pressure to fall to zero.

6.2.1.1 Column Headspace

If the peak resolution decreases in the first third of the chromatogram over several successive standard injections then this indicates that a headspace is being formed. If this happens, your column needs to be repacked by Biochrom. Before removing the column the instrument should run buffer 5 at 50°C for 30 mins.

Note: Some buffer will come out of the outlet pipe when disconnected from the column. This buffer is expelled by the pressure in the coil flush device. The pipe can be blocked with the PEEK coupling and fingertight blind fitting provided.

6.2.1.2 Analytical Column maintenance

During Bio 30+ installation, the analytical column will be supplied packed with the correct resin for the required analytical system. Overtime, the back pressure produced by the column may gradually increase until the buffer pressure reading rises above the recommended limits and/or the resolution drops below the specification. When this occurs, the analytical column resin must be cleaned and repacked. This procedure should only be carried out by trained Biochrom staff. Any attempt to open and repack the column is at the customer's own risk as it may invalidate the warranty.

6.2.2 Prewash Column

When the back pressure of the analytical column rises above the normal operating pressure check for blockage in the inlet and outlet frits of the prewash column, using the following procedures:

1. Turn off the ninhydrin pump.
2. Disconnect the outlet fitting from the pre-wash column.
3. Select override.
4. Pump buffer 1 and observe the buffer pressure.
5. If the pressure builds up then the column will need to be changed.
6. Stop the pump and reconnect the pre wash column outlet fitting.
7. Deactivate override.

NOTE: When the ammonia plateau interferes with the arginine peak, the prewash resin should be replaced or cleaned (section 6.2.3.1).

6.2.2.1 Prewash Column Maintenance

During Bio 30+ installation the prewash column will be supplied packed with the correct resin for the required analytical system. The prewash column contains a cation-exchange resin which removes ammonia from the buffers used in the Bio 30+. At the end of each sample analysis the prewash resin is regenerated by the regeneration solution, normally stored in the buffer 6 position. Only a limited capacity for ammonia retention exists within the prewash column, therefore excessive equilibration times and high laboratory ammonia levels must be avoided. If excessive ammonia interference occurs on the baseline with a rise after Arginine peak, the prewash column needs to be cleaned and repacked. This procedure should only be carried out by trained Biochrom staff. Any attempt to open and repack the column is at the customer's own risk as it may invalidate the warranty.

6.2.3 Photometer and Reaction Coil

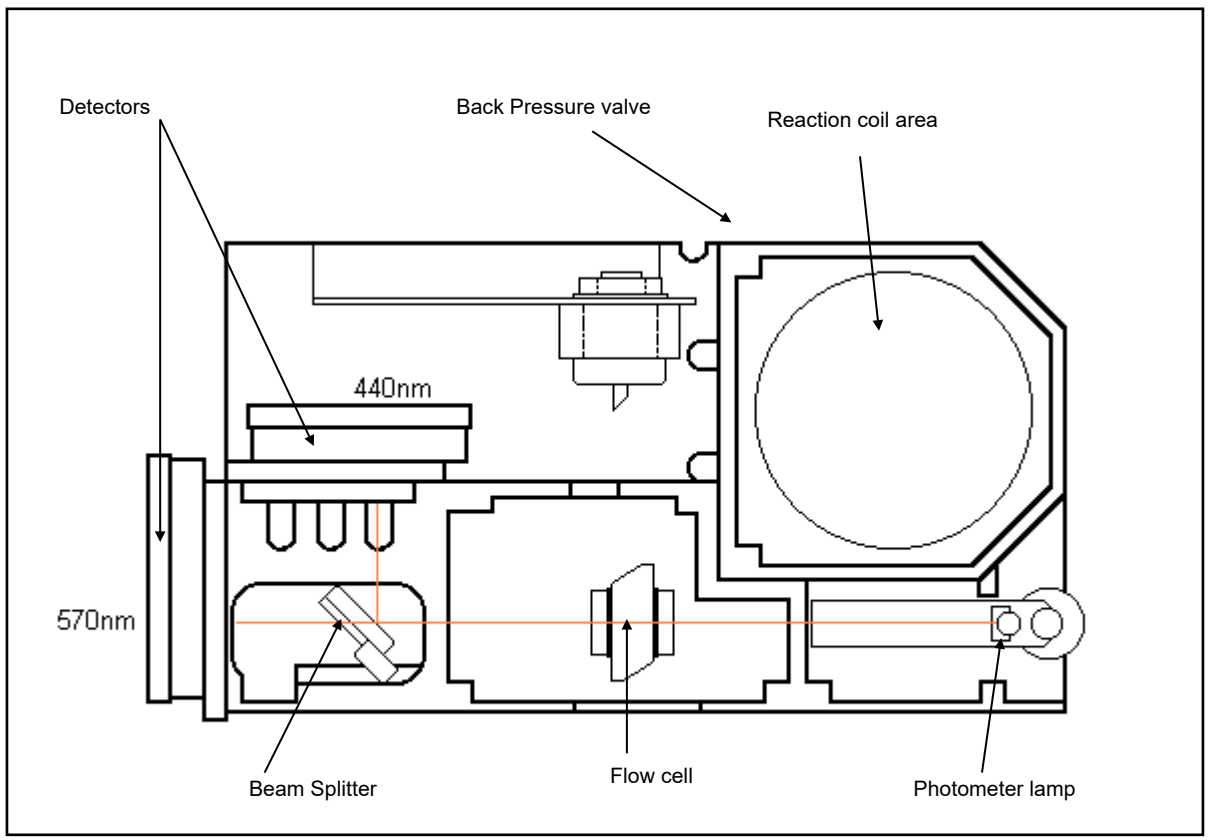


Fig 6.2 Photometer Reaction Coil Unit

During normal operation the photometer unit and reaction coil do not require any routine maintenance, the photometer and reaction coil are cleaned by the coil flush every time the instrument is stopped. Any problems which occur in either of these components are normally due to the Bio 30+ being switched off for prolonged periods with ninhydrin and buffer in the reaction coil. The ninhydrin may crystallise as the reaction coil cools. To prevent this fault occurring, the Coil Flush bottle in the buffer storage area must always contain Coil Flush wash solution to allow the coil flushing system to operate correctly on shut-down.

Additionally all the Biochrom elution programs contain a 2min step at the end of each run that triggers the coil flush device. This ensures a continuous and preventive cleaning of the reaction coil and the flow cell.

If the Bio 30+ is to be shut down for a prolonged period then the ninhydrin line must be filled with IPA/water 50/50. To do this proceed as follows:

1. Switch the reaction coil temperature on.

2. Remove the ninhydrin reservoir, flush the bottle headspace with nitrogen and cap it. Place the end of the ninhydrin feed line into a bottle containing a mixture of IPA/water 50/50.

3. When the reaction coil temperature is bove 100°C select buffer 1 then switch on the ninhydrin pump and operate for approximately one hour, then switch off the ninhydrin pump.

4. Select buffer 0 to stop the buffer pump and close the solenoid valve. The ninhydrin system is then filled with IPA/water solution.

The first indication that a blockage has occurred in the reaction coil or the flowcell is a continuous increase in coil pressure which is registered on the coil pressure gauge. If this rise in pressure occurs the exact location of the blockage can be determined as follows:

1. Pull the photometer/reaction coil unit tray out (see Fig 6.2).

2. Select buffer 1 and observe the rise in coil pressure.

3. Whilst observing the coil pressure, disconnect the outlet tubing at the inlet of the back pressure valve. If the pressure drops then the blockage is in the back pressure valve and this needs to be replaced.

4. If the coil pressure does not drop, then reconnect the outlet tubing to the back pressure valve. Remove the flowcell cover and while monitoring the coil pressure, disconnect the flowcell inlet tubing. If the pressure drops then the blockage is in the flowcell. The procedure for removing and cleaning the flowcell is described in section 6.2.4.

5. If the coil pressure is high after disconnecting the flowcell then disconnect the coil inlet tubing. If the coil pressure drops then the reaction coil is blocked. The procedure for cleaning the coil is described in section 6.2.6. If the pressure does not drop then check the transducer feed pipe and the transducer unit.

6.2.4 Flowcell Cleaning.

To ensure that the baseline noise is kept to a minimum, the flowcell must be cleaned periodically using the method described below.

1. Switch off the ninhydrin pump, select buffer 1 then pump for 5 minutes. Select buffer 0 to stop the pump and close the solenoid valve.

2. Remove the flowcell cover and retaining nuts, then gently lift the flowcell off the studs.

3. Disconnect the inlet and outlet lines from the flowcell. Place the inlet in a small beaker.

4. Screw a syringe adapter into the outlet port of the flowcell and connect a 20ml syringe filled with isopropanol to the adapter. Gently flush isopropanol through the flowcell.

5. Fit the syringe adapter into the inlet port to flush the flowcell in the reverse direction.

Fig 6.3 Flowcell Assembly

6. Reconnect the flowcell to the fluidics and switch the coil on. Pump buffer 1 for a few minutes and check that there are no leaks at the fittings. Pump ninhydrin to check the baseline.

Note: buffer 1 and ninhydrin baseline should be run with the reaction coil at temperature, 135°C, the column at 50°C, with the buffer and ninhydrin flow rates required for the system in use and with the front door closed. When OpenLAB EZChrom system is used, the baseline can be monitored using the Preview function and the noise level can be checked with the Threshold function.

The noise should be measured on a flat portion of baseline after it has settled down. The threshold value should be less than 30000 units when measured with OpenLAB EZChrom software threshold tool.

If the above method does not improve the baseline, the flowcell may have to be replaced.

6.2.5 Reaction Coil Cleaning

The coil may be cleaned by pumping lithium hydroxide solution from buffer 6 bottle through the coil. Perform this procedure as follows:

1. Read the reaction coil pressure on the Pressure Display box.
2. Select buffer 6. If necessary reduce the flow rate to maintain the coil pressure below 12 bar as any greater pressure will cause the Bio 30+ to shut down. Ensure that the ninhydrin pump is switched off.
3. If the blockage prevents any flow through the coil then reverse the direction of flow through the coil and repeat step (b).
4. Several reversals of flow may be necessary to dislodge the blockage.
5. If no flow can be established in either direction then an engineer must be called

6.2.6 Photometer Lamp Maintenance

A faulty photometer lamp may generate either electrical noise on the baseline or no output if the lamp has failed completely. To replace and realign the lamp proceed as follows:

1. Switch off the photometer lamp by right clicking over the lamp icon or using the manual window and allow to cool.
2. Remove the lamp cover and flowcell cover.
3. Remove the faulty lamp from the lamp holder.
4. Fit the replacement lamp in the lamp holder taking care not to touch the glass envelope with the fingers. Remove any finger marks from the envelope using a clean cloth soaked with isopropanol.
5. Refit the lamp cover and switch on the photometer lamp.

WARNING: The light from the photometer lamp is very bright. Protecting glasses must be worn if the lamp cover is removed with the lamp on to avoid eye injury.

1. From the Phoenix “Instrument settings” screen, set the column to 50°C and the reaction coil ON. Pump buffer 1 and the ninhydrin for 5 minutes to stabilise the system. Place a white card on the flowcell side opposite the lamp to observe the beam of light passing through the flowcell. Adjust the lamp position using the two lamp position adjusters until a round patch of light can be seen on the white card. The metal adjuster provides vertical movement of the lamp, the red adjuster horizontal movement.
2. The lamp position can be fine tuned when a recorder is connected to the instrument. Run a ninhydrin and buffer baseline at 1.0 O.D, with the NIN mode selected. Slightly adjust the lamp position to achieve maximum pen deflection towards zero on both channels.

3. Replace the flowcell cover.

6.2.7 Tubing and Connectors

When replacing a section of tubing, ensure that the replacement tubing is of the correct size and material. Three different types of connector are used in the Bio 30+: flangeless connectors, Swagelok connectors and PEEK fingertight connectors. The preparation of these connections is described below.

6.2.7.1 Flangeless Connectors

The flangeless joint is shown below. To produce this joint proceed as follows:

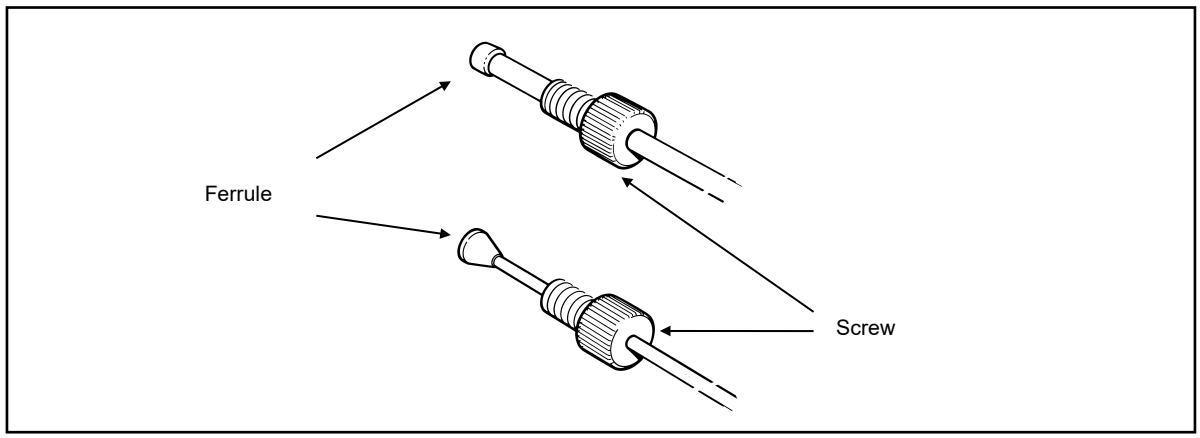


Fig 6.5 Flangeless Connectors

1. Using a sharp knife, cut the end off the tubing to produce a clean square end on the piece to be joined.

2. Slide the connector components onto the tubing in the following order: screw connector and ferrule.

3. Fit the joint into the fitting and tighten until fingertight.

6.2.7.2 PEEK Fingertight Connectors

The PEEK fingertight fitting is shown below.

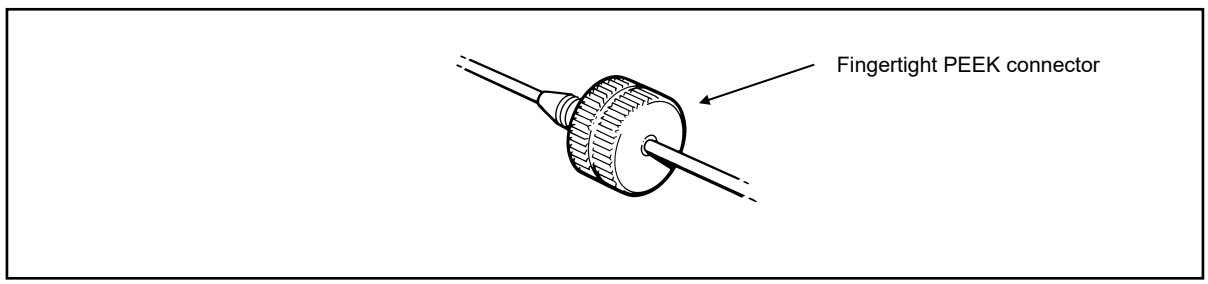


Fig 6.7 PEEK Connector

To produce this joint proceed as follows:

1. Using a sharp knife, cut the end off the tubing to produce a clean square end on the piece to be joined.

2. Slide the fitting onto the pipe.

3. Fit the joint into the fitting and tighten until fingertight.

6.2.8 Replacing the Buffer and Ninhydrin Filters

Note: Replace the buffer and ninhydrin filters when there is frequent filter related issues; not using filter may cause blockage in the fluid lines and damage the system

6.2.8.1 Ninhydrin bottle filter replacement

Caution: always wear gloves when working with ninhydrin reagent

1. Replace the PTFE filter fitted to the ninhydrin feed line using the following procedure:

2. Remove the cap from the ninhydrin reservoir.

3. Remove the filter from the end of the ninhydrin feed line and discard it.

4. Fit the new PTFE filter by holding the filter adapter and carefully inserting into the holder.

5. Refit the cap to the ninhydrin reservoir and prime the line using the ninhydrin in-line bubble trap and the ninhydrin pump bleed valve to remove any air trapped in the filter.

6.2.8.2 In-line Buffer filter replacement

Replace the PTFE filter fitted to the inlet of the buffer pump in the bubble trap using the following procedure:

1. Remove the left hand side panel on the instrument.
2. Remove the inlet and outlet connections from the filter unit and pull the filter unit out from the clip.

3. Loosen the locking screw and pull the white cover off the filter.

4. Pull the filter out from the holder.

5. Fit the new filter to the holder and reassemble the filter unit.

6. Refit the filter unit into the buffer feed line.

7. Prime the filter assembly to remove the air.

6.2.8.3 In-line Ninhydrin filter replacement

Replace the PTFE filter fitted to the inlet of the ninhydrin pump in the bubble trap using the same procedure as in 6.2.9.2.

6.2.9 Pump Maintenance

The pumps fitted to the Bio 30+ are designed to provide consistent, reliable operation under normal operating conditions.

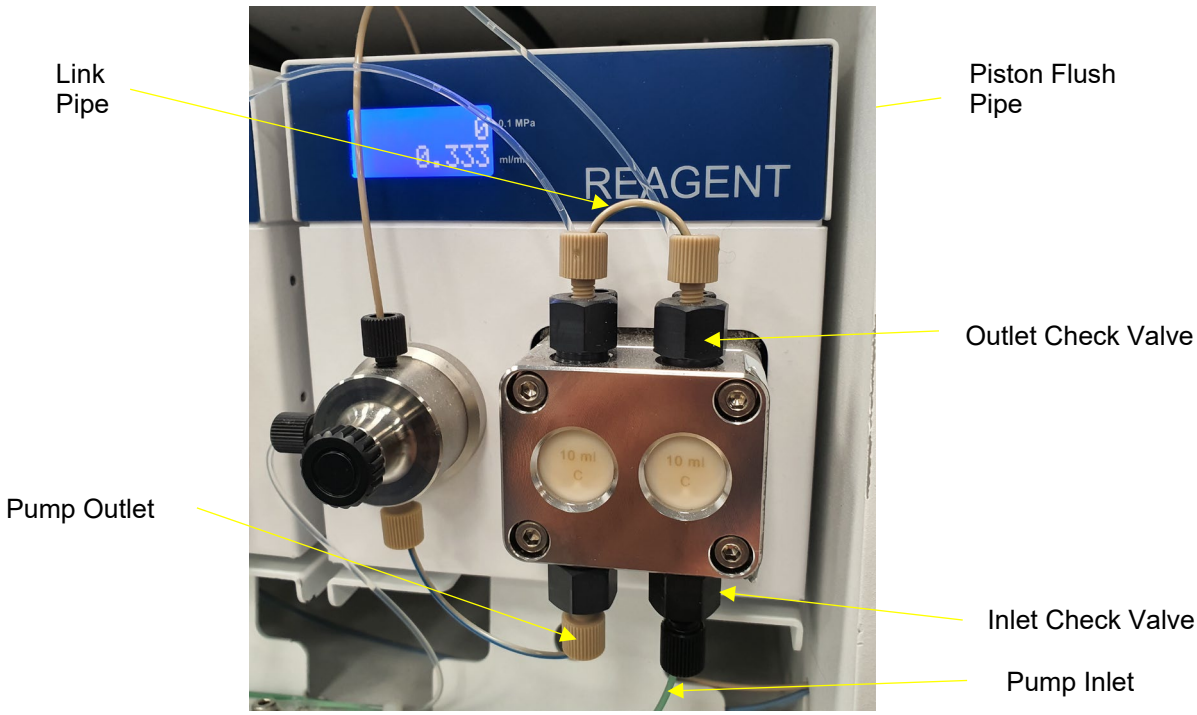


Figure 6.8 Pump head assembly

Before performing any maintenance operations on the pumps read the instructions in the relevant paragraphs of this section.

If any problems not covered in this section occur with the pump then contact your local service office.

6.2.9.1 Cleaning the Check Valves

- **Buffer Pump**

1. Flush ninhydrin from the coil by pumping only buffer 1 for 5 minutes, then select buffer 0 to stop the pump and close the buffer solenoid valve.

2. Remove the buffer pump check valve inlet and outlet connections .

3. Remove the pump inlet and outlet connections.
4. Remove the PEEK pipe connection between the two pump chambers.
5. Remove the lower inlet check valve and housing. Note the check valve direction.
6. Remove the upper outlet check valve and housing. Note the check valve direction.
7. Rinse the two check valves in isopropanol. This is best achieved by placing the valves upright on a gauze and immersing them in an ultrasonic bath for 5 minutes.
8. Rinse the check valves thoroughly in clean isopropanol.
9. Make sure the area is dry then refit the check valves and housing into the pump unit, locating the valves correctly for the inlet/outlet functions.

Caution: always wear gloves when working with ninhydrin reagent

Caution: Do not over tighten the PEEK check valve housings

- **Ninhydrin Pump**

The same procedures to clean the buffer pump valve can also be used for the ninhydrin pump check valves. Please note the Ninhydrin bottle should be placed at the same level as the pump and the cap loosened to prevent the flow of Ninhydrin.

The check valves can also be cleaned in an ultrasonic bath.

6.2.9.2 Replacing Check Valves

- **Buffer Pump**

To replace the buffer pump check valves proceed as follows:

1. Remove the check valve inlet and outlet connections.
2. Remove the PEEK pipe connection between the two pump chambers.
3. Remove the check valve housings containing the cartridges.
4. Clean the check valve area with distilled water to remove any traces of buffer.
5. Replace the faulty valve cartridges as required.
6. Refit the check valves into the housing and then into the pump unit, locating the valves correctly for the inlet/outlet functions.

- **Ninhydrin Pump**

The same procedures to clean the buffer pump valve can also be used for the ninhydrin pump check valves. Please note the Ninhydrin bottle should be placed at the same level as the pump and the cap loosened to prevent the flow of Ninhydrin.

7 TROUBLESHOOTING CHART

CONTENTS

7 TROUBLESHOOTING CHART

7.1 Introduction

This chart is intended as a quick guide to problems most likely to occur when using the Bio 30+. This section is also found in the Short Form Operation Instructions with references to relevant sections in this manual.

7.2 Error Messages

If a fault occurs during normal instrument operation (i.e. OVERRIDE not selected) then an error message will appear and the Bio 30+ may shut down. The error code number is related to the fault condition as described below.

ERRORS MUST BE CLEARED BEFORE THE INSTRUMENT IS RESTARTED

7.2.1 Analyser Errors

ERROR NUMBER OR MESSAGE	POSSIBLE CAUSE	REMEDY
ERROR 1: Reaction coil over temperature.	Control circuitry failed.	Switch off instrument. Call service engineer.
ERROR 2: Photometer lamp not lit.	a) Lamp not in position b) Lamp failed	a) Install lamp correctly. b) Replace lamp.
ERROR 3: High coil pressure.	a) Blockage of reaction coil, flowcell or back pressure valve. b) Reaction coil cold. c) Blockage in drain line pipe	a) Isolate area of blockage and clean. b) Set correct reaction coil temperature c) Disconnect and flush.
ERROR 4: High ninhydrin pressure	a) Ninhydrin flow rate too high. b) Ninhydrin back pressure valve blocked c) Blocked pipe at inlet to mixing T.	a) Set flow rate to correct value. b) Strip and clean the valve, change O ring. c) Remove and check pipe
ERROR 5: High buffer pressure.	a) Column inlet frit dirty b) Column temperature too low. c) Resin contaminated. d) Prewash column blocked. e) Buffer flow rate too high.	a) Column needs servicing. b) Check programming and check heating is functioning. c) Column needs servicing d) Column needs servicing e) Set flow rate to correct value.

	f) Autosampler loop blocked. g) Restriction in column inlet pipe.	f) Check autosampler g) Remake column inlet pipe connection.
ERROR 6: Low coil pressure.	a) Leak in fluidics. b) Coil back pressure valve faulty. c) Air in coil pressure transducer.	a) Check for leaks. Remake or tighten fittings as required. b) Examine and clean valve. c) Bleed and prime pressure transducer.
ERROR 7: Low ninhydrin pressure.	a) Ninhydrin reservoir empty. b) Flow through ninhydrin bottle filter restricted. c) Air in ninhydrin pump. d) Leak in ninhydrin fluidics. e) Dirt in check valves. f) Pump sapphire plunger broken. g) Pump motor stopped. h) Bleed valve opened	a) Refill ninhydrin reservoir. b) Clean or replace filter. c) Bleed ninhydrin pump. d) Check for leaks. Remake or tighten fittings as required. e) Clean valves. f) Replace plunger. g) Check programming. If pump faulty, call service engineer. h) Close valve.
ERROR 8: Low buffer pressure.	a) Air in pump. b) Leak in buffer fluidics prior to column. c) Bleed valve opened e) Dirt in check valves. f) Buffer solenoid not operating. g) Buffer pump sapphire plunger broken. h) Pump motor stopped.	a) Check buffer reservoir. Refill and prime as required. Bleed pump. b) Check for leaks. Remake or tighten fittings as required. c) Close valve e) Clean valves. f) Check that valve clicks when operated. Call service engineer if valve faulty. g) Replace plunger. h) Check programming. Call service engineer if pump faulty.
ERROR 9: Low nitrogen pressure	a) Cylinder empty. b) Regulator faulty or incorrectly set.	a) Replace cylinder. b) Check regulator.

	c) Large nitrogen leak.	c) Trace and repair connection.
ERROR 10: Column over temperature.	a) Column heating control circuit faulty. b) Peltier element faulty.	a) Call service engineer. b) Call service engineer.
ERROR 25: Ninhydrin pressure higher than buffer pressure.	Buffer pump failed but did not shutdown on ERROR 8.	See ERROR 8.

Error 25 causes the reagent pump to be switched off. If the buffer pressure recovers the reagent pump will be started again at the next step where it is programmed.

7.2.2 Reagent Management Errors

ERROR NUMBER AND MESSAGE	POSSIBLE CAUSE	REMEDY
ERROR 100: Ninhydrin level below xxxmL	Ninhydrin level is below the warning level programmed in the Biosys setup	Place a new ninhydrin bottle and reset the liquid level in the reagent management.
ERROR 101 to 106: Buffer 1 to 6 level below xxxmL	Buffer level is below the warning level programmed in the Biosys setup	Top up the buffer bottle and reset the liquid level in the reagent management.

7.2.3 OpenLAB/EZChrom Errors

ERROR NUMBER AND MESSAGE	POSSIBLE CAUSE	REMEDY
ERROR 700: EZChrom not found	a) EZChrom/OpenLAB is not installed b) EZChrom/OpenLAB instrument is set to disabled in the Biosys setup	a) Install EZChrom/OpenLAB b) Select the instrument in the Elite tab in Biosys setup
ERROR 701: EZChrom: General failure	a) EZChrom/OpenLAB instrument is set to disabled in the Biosys setup	a) Select the instrument in the Elite tab in Biosys setup
ERROR 702: EZChrom: Instrument not found	a) An instrument has not been created in EZChrom/OpenLAB or the existing instrument has been deleted.	a) Create an instrument using the instrument configuration in EZChrom/OpenLAB.

	b) EZChrom/OpenLAB instrument is set to disabled in the Biosys setup	b) Select the instrument in the Elite tab in Biosys setup
ERROR 703: EZChrom: Could not connect to instrument	a) the instrument in EZChrom/OpenLAB is not configured	a) Configure the instrument in EZChrom/OpenLAB
ERROR 704: EZChrom: Run not waiting for trigger	a) Autosampler is switched off b) Alias manager software is closed c) Two Biosys sessions are opened at the same time d) The EZChrom/OpenLAB online window has been opened via EZChrom/OpenLAB software instead of Biosys	a) Switch on the autosampler. b) Reboot the instrument and PC. Open and KEEP the Alias Manager software opened at any time. c) Reboot the instrument and PC. When closing Biosys ALWAYS use the File\shutdown function. Closing the window will NOT close Biosys d) Reboot the instrument and PC. Always use the Open/Hide Elite button in the Biosys programmer to open the Online window.
ERROR 705: EZChrom: Run not triggered	a) Autosampler is switched off b) Alias manager software is closed c) Two Biosys sessions are opened at the same time d) The EZChrom/OpenLAB online window has been opened via EZChrom/OpenLAB software instead of Biosys	a) Switch on the autosampler. b) Reboot the instrument and PC. Open and KEEP the Alias Manager software opened at any time. c) Reboot the instrument and PC. When closing Biosys ALWAYS use the File\shutdown function. Closing the window will NOT close Biosys d) Reboot the instrument and PC. Always use the Open/Hide Elite button in the Biosys programmer to open the Online window.

ERROR 706: EZChrom: Method overrun, previous run truncated	The runtime in the EZChrom/OpenLAB method is greater than the lengh of the Biosys program.	Reduce the runtime in the EZChrom/OpenLAB method instrument setup so it is smaller than the Biosys program in use.

7.2.4 Hardware Errors

ERROR NUMBER AND MESSAGE	POSSIBLE CAUSE	REMEDY
ERROR 800: Analyser already connected	A second analyser is connected to to the PC.	Only one analyser can be connected to a PC at any one time
ERROR 801: Analyser Not Connected	The serial cable between the instrument and the PC is disconnected	Check the cable is connected to the COM1 port of the PC
ERROR 802: Analyser Port does not exist	The COM port in Biosys setup is set incorrectly	Check tha the COM port in the analyser setup is set to COM 1.
ERROR 803: Analyser Port already in use	The COM port in Biosys setup is set incorrectly	Check tha the COM port in the analyser setup is set to COM 1.
ERROR 804: Analyser not responding	The instrument is switched off	Switch on the instrument
ERROR 805: Analyser Busy	Unknown	Contact Service Engineer
ERROR 806: Unknown Analyser Error	Unknown	Contact Service Engineer
ERROR 807: Analyser Message not supported	Unknown	Contact Service Engineer
ERROR 808: Analyser Error	Unknown	Contact Service Engineer

7.2.5 Autosampler Errors

ERROR NUMBER AND MESSAGE	POSSIBLE CAUSE	REMEDY
ERROR 900 Autosampler already connected	A second autosampler is connected to the PC.	Only one autosampler can be connected to a PC at any one time

ERROR 901 Autosampler Not Connected	The serial cable between the autosampler and the PC is disconnected	Check the cable is connected to one of the COM ports of the PC
ERROR 902 Autosampler Port does not exist	The COM port in Alias Manager setup is set incorrectly	Check that the COM port number in the Alias Manager matches the COM port number set on the PC.
ERROR 903 Autosampler Port already in use.	The COM port in Alias Manager setup is set incorrectly	Check that the COM port number in the Alias Manager matches the COM port number set on the PC.
ERROR 904 Autosampler not responding	The autosampler is switched off	Switch on the autosampler.
905 Autosampler busy	Unknown	Contact Service Engineer
ERROR 906 Unknown Autosampler Error	Unknown	Contact Service Engineer
ERROR 907 Autosampler Message not supported	Unknown	Contact Service Engineer
ERROR 910 Autosampler Error: refer to status dialog	Unknown	Contact Service Engineer

Note: Errors 3,4 and 5 will shut down the instrument even if override is on. This is to protect the fluidics from overpressure.
Errors connected with failure of Autosampler components will also be reported; please refer to the Autosampler manual for details.
Call a Service Engineer, when a message displaying an error not listed above appears.

7.3 Fault Conditions

In addition to the faults described earlier, the following faults may occur without displaying an error message. The faults may be identified from their visible symptoms.

SYMPTOM	POSSIBLE CAUSE	REMEDY
Column temperature low	a) Incorrect programming b) Column heating faulty.	a) Check programming b) Call service engineer.
Only baseline on recorder	a) Reaction coil cold. b) Sample not injected due to: i) Empty vial ii) Autosampler fault c) No ninhydrin flow	a) Switch on coil heater. Call service engineer if faulty. i) Fill vial. ii) Call service engineer. c) See error 7.
No trace or does not not operate	a) Incorrect programming. b) Baseline offset incorrect.	a) Check programming. b) Check programming.
Baseline cannot be adjusted	a) Faulty electrical connection. b) Old or poorly prepared ninhydrin. c) Faulty photometer system. d) Flowcell dirty.	a) Check signal leads. b) Replace with fresh reagent. c) Call service engineer. d) Flush flowcell.
Baseline unstable	a) System recently restarted b) Flow rate varying due to: i) Leaks in fluidics. ii) Air in one pump. iii) Dirt in pump check valves. c) Column inlet frit dirty. d) Pre-wash column inlet frit blocked e) Air in flowcell or back pressure valve. f) Dirt in flowcell or back-pressure valve. g) Photometer lamp faulty.	a) Allow 30 mins to stabilise. i) Stop leaks. ii) Bleed pumps. iii) Clean check valves. c) Column needs servicing c) Column needs servicing e) Flush flowcell and back-pressure valve. f) Clean flowcell and back-pressure valve. g) Replace lamp.

	h) Buffer inlet filter blocked.	h) Remove and clean filter.
Poor separation	a) Incorrect program. b) Incorrect buffers. c) Headspace in analytical column. d) Sample loaded at incorrect pH. e) Analytical column resin contaminated.	a) Check program. b) Check relevant buffers are fitted in correct position. c) Column needs servicing d) Check: i) Loading buffer. ii) Regeneration and equilibration times. iii) Sample preparation method. e) Column needs servicing
High nitrogen usage	a) Leak at regulator connection to cylinder or the Bio 30+. b) Leak in nitrogen distribution system.	a) Check connections. b) Check all nitrogen connections for leaks.
Loss of communication	Various	Switch off the complete system for 30 seconds then reboot using the sequence: computer, instrument, autosampler.

8 CHEMICAL KITS, PACKED COLUMNS AND SPARE PARTS

Contents

8 CHEMICAL KITS, PACKED COLUMNS AND SPARE PARTS

8.1 Introduction

The Bio 30+ is supplied with a shipping kit which contains the items required for its installation. A chemical kit is also supplied, which contains sufficient chemicals for the initial operation of the instrument.These chemicals are available for lithium systems. Spare parts and chemicals are available separately and also in kits.

8.2 Chemical Kits – Not Supplied With Bio 30+ Unless Otherwise Specified

8.2.1 Ultra Physiological Fluid Chemical Kit Part No 80-2117-77

Part Number	Description	Quantity
80-2038-15	Lithium Buffer 1, 2L	2
80-2038-16	Lithium Buffer 2, 2L	1
80-2099-83	Lithium Buffer 3, 2L	1
80-2097-18	Lithium Buffer 4, 2L	1
80-2037-69	Lithium Buffer 5, 2L	2
80-2038-20	Lithium Regeneration Buffer 6, 1L	1
80-2038-10	Lithium Loading Buffer, 2L	1
80-2104-80	Ninhydrin Filter	2
80-2117-65	Ultrosolve Plus 1.75L	4
80-2117-64	Ultra Ninhydrin solution	4

8.2.2 Physiological Fluid Routine Kit Part No 80-6000-06

Part Number	Description	Quantity
80-2038-15	Lithium Buffer 1, 2L	3
80-2038-16	Lithium Buffer 2, 2L	1
80-2099-83	Lithium Buffer 3, 2L	1
80-2097-18	Lithium Buffer 4, 2L	1
80-2037-69	Lithium Buffer 5, 2L	2
80-2038-20	Lithium Regeneration Buffer 6, 1L	1
80-2104-80	Ninhydrin Filter	2
80-2117-65	Ultrosolve Plus 1.75L	4
80-2117-64	Ultra Ninhydrin solution	4

8.2.3 Lithium Buffer Start-up Kit Part No. 80-6000-16

Part Number	Description	
80-2038-15	Lithium Buffer 1, 2L	2
80-2038-16	Lithium Buffer 2, 2L	1
80-2099-83	Lithium Buffer 3, 2L	1
80-2097-18	Lithium Buffer 4, 2L	1

80-2037-69	Lithium Buffer 5, 2L	2
80-2038-20	Lithium Regeneration Buffer 6, 1L	1
80-2038-10	Lithium Loading Buffer, 2L	1

8.2.4 Lithium Buffer Routine Kit, Part No. 80-6000-17

Part Number	Description	
80-2038-15	Lithium Buffer 1, 2L	3
80-2038-16	Lithium Buffer 2, 2L	1
80-2099-83	Lithium Buffer 3, 2L	1
80-2097-18	Lithium Buffer 4, 2L	1
80-2037-69	Lithium Buffer 5, 2L	2
80-2038-20	Lithium Regeneration Buffer 6, 1L	1

8.2.5 Ultra Ninhydrin Kit, 2L Part No 80-2118-30

Part Number	Description	Quantity
80-2104-80	Ninhydrin Filter	1
80-2117-65	Ultrosolve Plus 1.75L	1
80-2117-64	Ultra Ninhydrin solution	1

8.2.6 Ultra Ninhydrin Kit, 8L Part No 80-2117-76

Part Number	Description	Quantity
80-2104-80	Ninhydrin Filter	4
80-2117-65	Ultrosolve Plus 1.75L	4
80-2117-64	Ultra Ninhydrin solution	4

8.2.7 EZ Nin Reagent Kit, 2L Part No 80-6000-13

Part Number	Description	Quantity
80-2104-80	Ninhydrin Filter	1
80-6000-12	EZ Nin Reagent (2L)	1

8.2.8 EZ Nin Reagent Kit, 4L Part No 80-6000-14

Part Number	Description	Quantity
80-2104-80	Ninhydrin Filter	2
80-6000-12	EZ Nin Reagent (2L)	2

8.2.9 EZ Nin Reagent Kit, 8L Part No 80-6000-15

Part Number	Description	Quantity

80-2104-80	Ninhydrin Filter	4
80-6000-12	EZ Nin Reagent (2L)	4

8.2.10 Amino Acid Standards

Part Number	Description	Quantity
80-6002-80	Physiological Fluids Standard (Calibration Standard:2.5mM – ISO 17034 Reference Material)	1

8.3 Packed Columns

There is a range of packed columns available to suit your particular applications. Each packed column is supplied with top-up resin, a copy of the chromatography and a program which may need optimising to suit your instrument.
With careful use and good sample preparation a column can successfully analyse samples indefinitely.

Part Number	Description
80-6002-47	Li PEEK Column 200x4.6 HR
80-6002-48	Li PEEK Column 200x4.6 HP
80-6002-49	Li PEEK Column 200x4.6 Accelerated
80-6002-50	Li PEEK Column 100x4.6 Prewash

8.4 Spare Parts

80-2107-57	Lamp
80-2104-24	Flow Cell
80-2111-81	Back Pressure Valve
80-2114-85	Check Valve Cartridge
80-6001-61	Sample Needle (Alias)

8.5 OpenLAB CDS EZChrom Edition Data Handling

Part Number	Description
OpenLAB CDS EZChrom Edition	
80-6000-47	For 1 chromatography system, incl. 35900E A/D box, latest version of OpenLAB CDS EZChrom Edition, automation and system suitability software.
80-6000-49	For 1 chromatography system, latest version of OpenLAB CDS EZChrom Edition, automation and system suitability software. No A/D box

80-6000-48	For 1 chromatography system, desktop computer with Windows 10, 64bit professional Edition loaded with latest Biosys version and latest version of OpenLAB CDS EZChrom Edition, automation and system suitability software. No A/D box. Monitor not included.

9 APPENDICES

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APPENDICES

9.1 APPENDIX A: Buffer Storage.

It is recommended that buffers are stored at a temperature of 15°C to 25°C, out of strong direct sunlight and kept well sealed. The shelf life unopened is 3 years. Once opened the buffers should be used within 6 months.

9.2 APPENDIX B: Standard Programs

Standard programs for use with Bio 30+ are listed below. These programs are stored in the “Standards” directory of the software; see section B1 for details of loading instructions.

Because different batches of resin and different instruments have slightly different characteristics these programs may require optimisation to suit your system (see Appendix E).

_Physiological Accelerated

Program for Physiological Fluid analysis using the Accelerated column.

_Physiological Accelerated Regeneration

Regeneration program for Physiological Fluid analysis using the Accelerated column.

_Physiological High Performance

Program for Physiological Fluid analysis using the High Performance column.

_Physiological High Performance Regeneration

Regeneration program for Physiological Fluid analysis using the High Performance column.

_Physiological High Resolution

Program for Physiological Fluid analysis using the High Resolution column.

_Physiological High Resolution Regeneration

Regeneration program for Physiological Fluid analysis using the High Resolution column.

9.2.1 Loading a Standard Program

The programs listed above are found in the **Standards** subdirectory of BioSys.

To use any of the Standard Programs, insert them into the Sample List as follows:

Click **Insert\Program Filename** then select the Standards subdirectory. Any program can now be selected and added to the Sample List.

Standard programs can be edited in the Program Editor.

To keep any of the Standard Programs in the Programs subdirectory, remove the _ character from the filename or rename it and save the program in the usual way.

9.3 APPENDIX C: Chart Recorder

Connecting a Chart Recorder

The recorder remote 15 way connector is wired to fit the **REC 102** recorder.

On/Off control signal for remote control of recorder chart drive.

This facility makes it possible to start and stop the paper chart drive on some recorders with a remote start/stop circuit.

Some recorders require an active low or an active high signal for the chart drive circuit, this can be selected from the REC switch on the electronic unit, at the top of the recorder output connectors.

The connections on the Recorder Remote 15 way connector are as follows:

Pin 15:	0V
Pin 12:	Active Low or Active High

When selecting Recorder, pin 12 goes high or low and the recorder chart drive starts.

The settings for the **REC 102** recorder are:

Chart speed	2mm/min (for Lithium system)
mm/s - /min switch	down
rec. off - on switch	down
Range	0.1V (both channels)

zero suppr.	0
pen up-down switch	down

Zero for both channels should be set on the right hand side of the chart.

9.4 APPENDIX D: Use Of an Integrator

Connecting an integrator to the Bio 30+.

If it is required to connect an integrator to Bio 30+, other than the default integrator system (OpenLAB CDS) supplied by Biochrom, the table below describes the signals present on the integrator signal output connector.

Pin	Function
Pin 1	440nm signal
Pin 2	SUM signal (570nm + 440nm)
Pin 3	570nm signal
Pin 4	Common to 570nm, 440nm and SUM channels
Pin 5	Start Of Run (SOR)

The start of run signal is a 2 second active low pulse and it is automatically produced every time a sample is loaded.

The maximum signal level from pins 1, 2 and 3 is +1.0V.

9.5 APPENDIX E: Optimisation Of Chromatography

9.5.1 Physiological Fluid Optimisation

The physiological fluid programs supplied with Bio 30+ may require slight changes in the program to achieve optimum separation of the amino acids in the physiological fluid standard. The Bio 30+ amino acid analyser uses five buffers and a regenerating solution (0.30M Lithium hydroxide) in a stepwise elution program. The parameters which affect the separation of the amino acids in physiological fluids are:

9.5.1.1 pH and molarity of the buffers:

As in the sodium system, the higher the pH and/or the concentration of the lithium ion, the faster the amino acids will elute. The pH and molarity of the buffers supplied by Biochrom are designed to give optimum separation of the amino acids.

9.5.1.2 Time of buffers:

The time of buffer 2 in the program may require a slight increase or decrease to optimise the separation of the pH sensitive amino acids like Cystine and Cystathionine. The elution of Cystine and Cystathionine is delayed when the time of buffer 2 in increased and elute earlier when the time of buffer 2 is decreased. For special applications, buffers can be pumped alternatively for short periods of time (pulsed chromatography).

9.5.1.3 Column Temperature:

The optimum separation of the amino acids in the physiological fluid is achieved by using the following four temperature levels throughout the program:

a) **T1**: This temperature is designed to achieve optimum separation of Asn/Glu/Gln. To optimise the T1 level, it is necessary that the physiological fluid standard used for this optimisation contains Asn, Glu and Gln. As the amino acid Glu is more sensitive than Asn and Gln to column temperature, a high level of T1 elutes Glu earlier and as a result it may coelute with Asn, while a low level of T1 will elute Glu later and as a result it may coelute with Gln. The optimum level of T1 temperature (30-36ºC) is that level which elutes Glu in central position between Asn and Gln. To optimise T1 increase or decrease as required, changing the T1 level by 1-2ºC at a time.

   The length of time that the column temperature stays at T1 affects the separation of Citrulline, Alanine and α-Aminoisobutyric acid. Citrulline is more sensitive to temperature than Ala or α -Aminoisobutyric acid. When T1 changes to T2 early, Citrulline coelutes with Ala and when the change to T2 is delayed, Citrulline coelutes with α -Aminoisobutyric acid. The optimum time for the change from T1 to T2 is when Citrulline elutes centrally between Ala and α -Aminoisobutyric acid. To optimise Citrulline, increase or decrease, as required, the time of T1, by steps of 1 to 2 minutes.

b) **T2**: This temperature (60-70ºC) is designed to achieve optimum separation of Tyr/β-Ala/Phe. As both Tyr and Phe are more sensitive than β-Ala, a high level

of T2 will elute both Tyr and Phe earlier and as a result Phe may coelute with β-Ala. When the T2 level is low, both Tyr and Phe will elute later and as a result Tyr may coelute with β-Ala. The optimum temperature for T2 is the temperature which elutes β-Ala between Tyr and Phe. An increase or decrease of 3-5ºC at a time may be required to change the elution position of Tyr and Phe with respect to β-Ala.

c) **T3**: This temperature (70-75ºC) is designed to separate Tryptophan between Histidine and 3-Methylhistidine or between 3-Methylhistidine and Anserine, after expanding these areas using pulsed chromatography.

d) **T4**: This temperature (75-85ºC) is used to speed up the chromatography and achieve good regeneration of the analytical column.

9.5.1.4 Summary of the Physiological Fluid Optimisation

Tau/Pea:	Increase T1 level at the steps of buffer 1 before injection
Thr/Ser:	Lower T1 level Increase equilibration time
Asn/Glu:	Lower T1
Glu/Gln:	Increase T1
Ala/Citr:	Delay introduction of T2
Citr/α-ABA:	Introduce T2 earlier
Val/Cys:	Increase time of buffer 2
Cys/Met:	Decrease time of buffer 2
Met/Cysth:	Increase the time of buffer 2
Cysth/Ile:	Decrease time of buffer 2
Ile/Leu:	Lower T2 level
Tyr/ β-Ala:	Increase T2 level
β-Ala/Phe:	Decrease T2 level
Phe/ β-AIBA:	Increase T2 level
Amm/Hlys:	Increase time of buffer 4
Lys/1-Mhis:	Decrease time of buffer 4
His/Trp:	Lower T3 level
Trp/3-Mhis:	Increase T3 level

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