



**Bellingham  
+ Stanley**

**Technical**

**Bulletin No: R025**

**Title: RFM990 Micro Flowcell and Autosampler  
Performance Analysis**

**Background:**

The use of a micro flowcell with an autosampler is commonplace in many industries. The beverage industry often requires an automated system, particularly in audit labs, where a large number of samples are required to be measured in as short a time as possible. It is also a requirement of such a system to use as little sample as possible as other invasive tests are also required to be made with the limited trade sample.

In order to establish the performance of the RFM990 flowcell, a complete laboratory test was established and performed. The report particularly deals with the unique way in which the refractometer is elevated by a certain amount to ensure entrapped air is quickly removed from the sample chamber and deals with why samples are pulsed to help clean the prism and cell.

The following report forms the results of the laboratory test.

## **Advantages and Features - Auto samplers and Flowcells**

1. Rapid automated measurement of samples where high throughput is required.
2. Raising the refractometer to a predefined angle assists in removing unwanted entrapped air.
3. Introducing a set of 'coils' in the exit tube prevents air from being drawn back into the flowcell.
4. Pulsing samples through the system assists in cleaning the flowcell and prism.
5. Intelligent instrument monitoring automatically detects the flowcell and changes the instrument temperature control configuration without user intervention.
6. The RFM990 controls the temperature stabilisation of the sample and when configured to *SMART* takes into consideration the repeatability of the sample (in °Brix) and the temperature. A further use of *Intelligent Thermal Management*.
7. A barcode reader can be used to quickly program the 'LimsLite sample schedule'.

## ***RFM990 with Autosampler System***

An investigation was carried out into the performance of an RFM990 automated measurement system designed in conjunction with a leading beverage producer for use at their Audit laboratory. The system consisted of an RFM990 Refractometer fitted with a micro continuous flow through cell (Code 22-91) together with a peristaltic pump and autosampler. System design was based on the LimsLite / Autosampler package, several of which have been previously supplied for use within brewery applications in the UK.

The client had requested an investigation into how the performance of the RFM990 compared with an RFM300 Series Refractometer when measuring soft drinks. Details of these tests can be found in Technical Bulletin R021.

This report deals briefly with new aspects of the system design and provides an evaluation of the performance of the system as a whole.

### ***System Configuration***

- RFM990 Refractometer (Code 22-90)
- Micro continuous flow through cell (Code 22-91)
- Peristaltic Pump (Code 55-54)
- Autosampler (Code 55-22)
- PC with LimsLite Software (Code 97-002)

### ***System Constraints***

The system was designed to work with a maximum volume of 30ml of sample.

#### ***RFM990 Refractometer fitted with Micro Continuous Flow Through Cell (Code 22-91)***

For certain applications there is an advantage in having the prism face oriented towards the vertical plane in order to prevent air bubbles from coming into contact with the prism surface.

Experimentation using a camera to view the flow cell from underneath the prism highlighted problems caused by trapped air when the prism was horizontal. As the RFM990 instrument design does not lend itself to vertical mounting, an alternative solution involving inclining the instrument was appraised.

A satisfactory performance, with regard to the flow of entrapped air through the system, is obtained as the refractometer incorporates a special stand that elevates the instrument to an optimum 10° angle and positioning the inlet and outlet pipes of the flow cell so that the product flowed from bottom to top. Air bubbles entering the cell gathered near the outlet pipe in between flow pulses, and were then easily cleared from the system by the subsequent sample pulses.

The construction of an angled platform for the instrument to sit on will facilitate the correct positioning of the instrument and also allow for further accessories (to support the tubing for example) to be fitted if required. The platform is therefore an integral part of any RFM990 flowcell supplied by Bellingham + Stanley.

The refractometer was fitted with a micro continuous flow through cell (B+S Code 22-91). As a newly designed unit, it was subjected to pressure testing to ensure satisfactory operation with liquids at pressures up to 2BAR. Details of the tests can be found in B+S Laboratory Report 690.

Calibration and cleaning of the instrument prism requires the removal of the flow cell. When calibrating, a cover should be employed to prevent the ingress of light.

### ***Peristaltic Pump***

The pump used was B+S Part number 55-54, comprising a Watson Marlow 313D Pump head, fitted with Tygon R3603 tubing (ID 3.2mm, OD 6.4mm, Wall 1.6mm). Step down tubing connectors were used to provide a connection to the micro flow cell inlet and outlet pipes via short lengths (6cm) of Tygon R1000 (ID 1.6mm, OD 4.8mm, Wall 1.6mm).

With the pump operating at maximum speed, the flow rate with Distilled Water was measured at 103ml / minute.

During the test, it was noticed that reverse operation of the peristaltic pump could result in air being reintroduced into the sample cell. Raising and coiling the outlet tube, prior to sending it to a waste liquid container, was found to be an effective method of preventing bubbles from returning into the flow cell. It also permitted sufficient product to stay within the tubing and prevent reverse pulses from reintroducing air to the flow cell.

### ***Autosampler***

The autosampler used was a Gilson Model 223, capable of using four racks of fourteen pots (pot capacity 50ml). Control of the autosampler was via serial port from a PC running B+S LimsLite Software. This allowed coordination of the sampling with pumping and measuring, and also provided settings which reduced the risk of sample contamination when moving the needle from one pot to another.

Various modifications had been carried out to the standard autosampler unit including the fitting of a B+S manufactured needle and guide.



## System Apparatus

### LimsLite Software

#### Autosampler Settings

The software package used to control the system was a modified version of the B+S LimsLite program.

**Autosampler Settings**

Autosampler

Needle up: 2100 Home (X/Y): 0000/0000

Needle down: 1000

Air flush: 0

Tray Descript.: 4 x Code 24 Racks Browse...

Pump schedule

Type: 2: 223 Contact Relays (Mode II)

Pump time: 5 Forward speed: 46

Pump pulses: 1 Reverse speed: 46

Pump interval: 5  Suck on up

Pump reverse: 3

Printout Options

Additional Header Data

Filename: C:\readings\calibData.txt Browse...

OK Cancel Help

**Figure 1** Example LimsLite Autosampler Settings Screen

In the example given above the total forward pumping time of ten seconds would be divided into twelve pulses with a two second pause between pulses.

In addition to the configurable autosampler settings shown above, a wash agitation feature was added to improve the flushing and cleaning characteristics. The purpose of this was to allow forward and backward movement of the sample during the pulsing sequence to maximise the efficiency of the flushing and obtain reliable results within the constraint of thirty millilitres of available product.

Wash agitation employed during these tests consisted of:

- No pause between the first two pulses - at this point fresh sample had not entered the flow cell.
- No reverse after the first, second and third pulses - to allow the cell to be purged of air.
- Reverse after pulse - forward pulse is double length and has a single length reverse. Provides improved flushing and cleaning of the cell.
- No reverse after the last pulses - to prevent air returning into the cell.

The reading is taken before the tube is removed from the pot, and "Suck on up" and "Spit after measurement" are used to ensure that the needle is clean before entering the next pot.

### Data Collection Configuration

Software was configured to obtain a reading from the RFM990 after completing the autosampler schedule. Any stabilisation criteria set within the RFM990 (in this case, a sixty second delay) are applied before taking the reading.

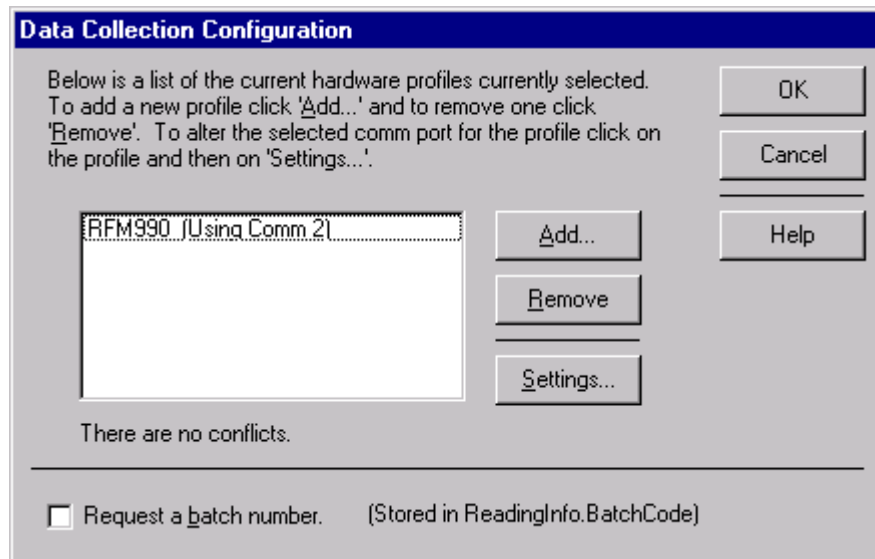


Figure 2 LimsLite Data Collection Configuration Screen

### Data Export

Results were logged to file to allow analysis of performance of the system

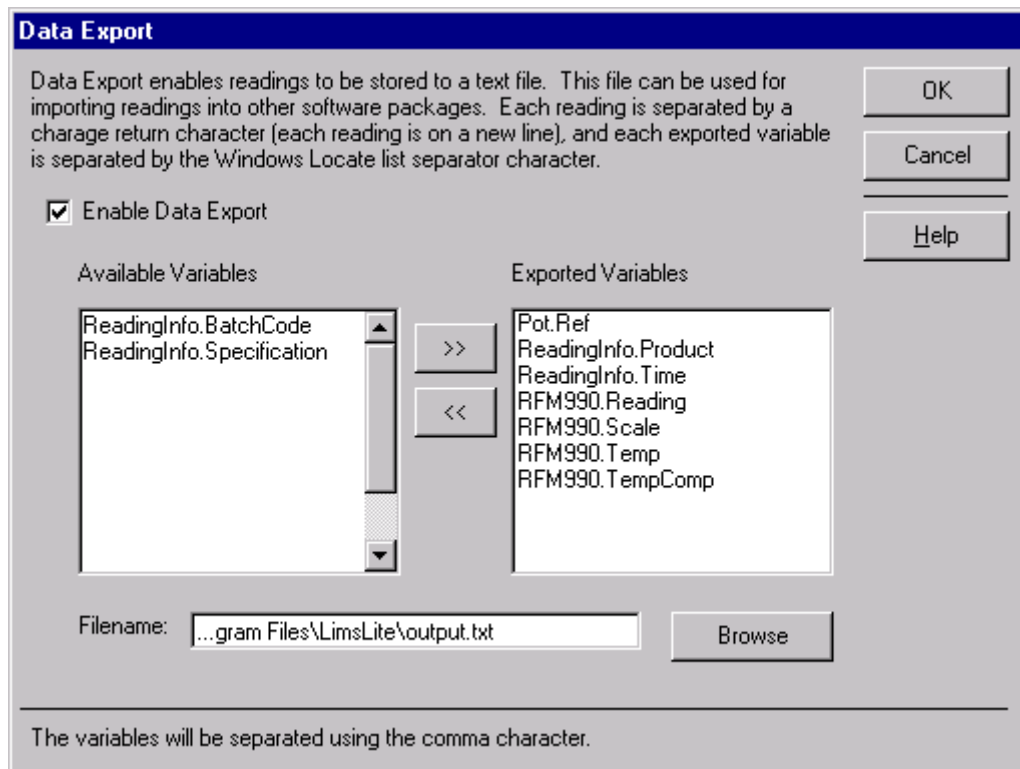


Figure 3 LimsLite Data Export Screen

### Autosampler Schedule for LimsLite Software

To demonstrate the system, a schedule was designed to show the repeatability of RFM990 readings during the changeover between three different products: Distilled Water, 15°Brix and degassed Cola.

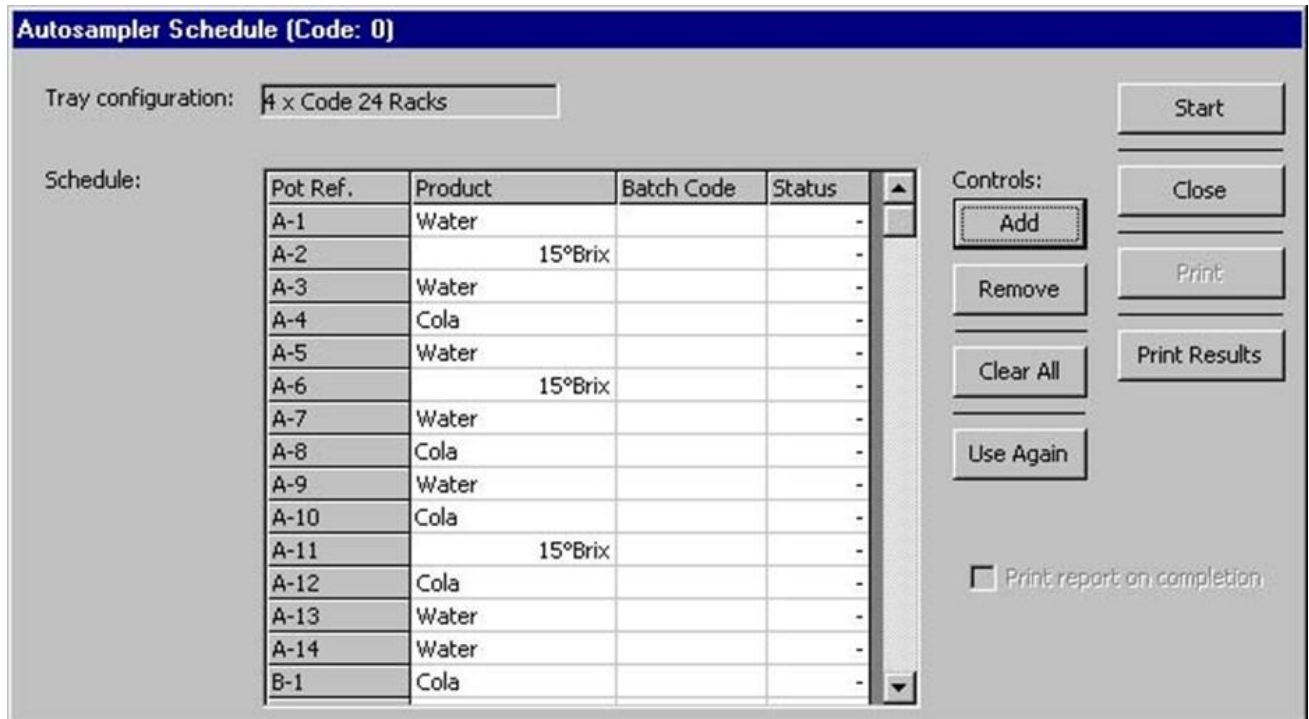


Figure 4 LimsLite Autosampler Schedule Screen (Pot Ref. A-1 to B-1)

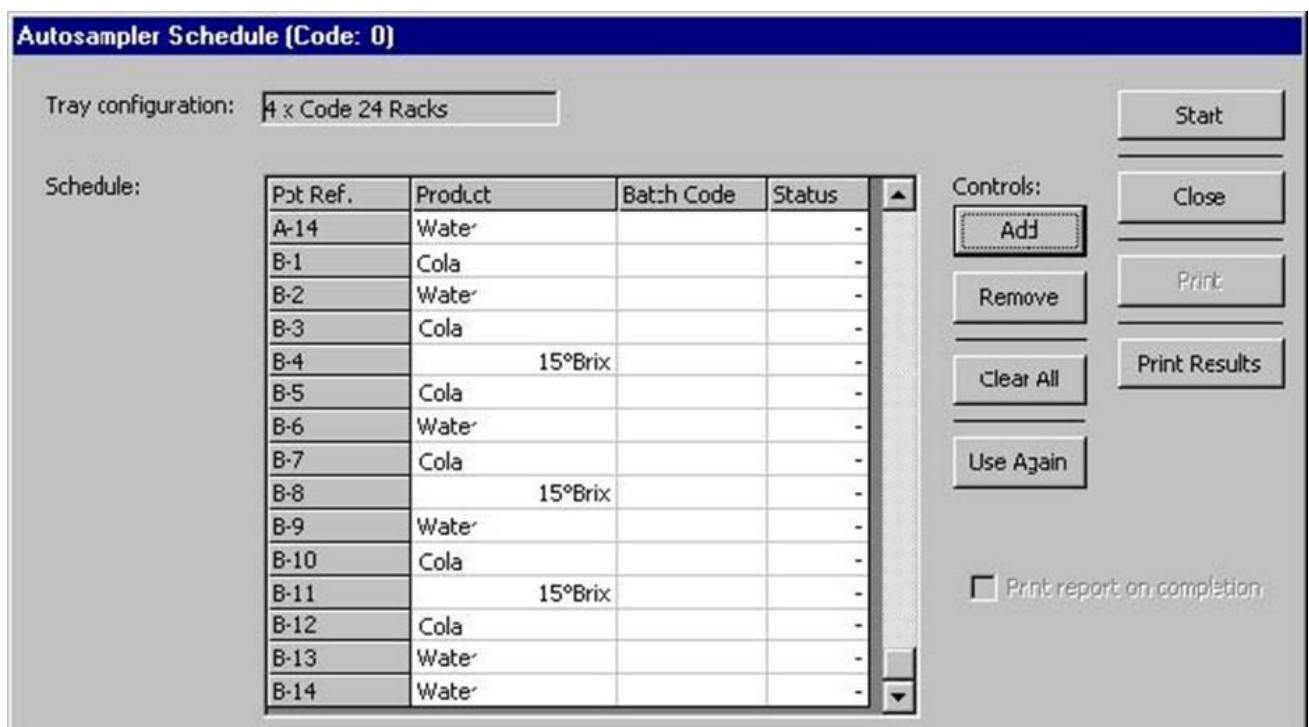


Figure 5 LimsLite Autosampler Schedule Screen (Pot Ref. A-14 to B-14)

## **Testing the System**

### ***RFM990 Stabilisation Time***

On completion of the introduction of a sample to the refractometer, it is necessary for the sample to stabilise on the instrument prism. The RFM990 has the facility for specifying a stabilisation criterion for the reading, and for the purpose of these tests a delay of sixty seconds duration was used. This was suitable for products close to the operating temperature of the instrument (20°C). Should the product temperature be different, then variation of the RFM990 stabilisation criteria and their parameters will permit a repeatable reading to be obtained.

### ***Sample Preparation***

For the purpose of the autosampler tests, three products were to be used.

#### ***Product Details:***

- Distilled Water
- Cola - degassed using Whatman 2V Filter Paper
- 15°Brix Solution - prepared in a one gallon demijohn using cane sugar and water

### ***Setting up the Autosampler***

Sample pots were filled in accordance with the autosampler schedule shown in figures 4 & 5. Each pot was filled to a level of 30ml.

### ***Configuring LimsLite***

LimsLite Software was configured as described above, with product alternating during the schedule to illustrate the flushing ability of the system.

### ***Running the Test***

The autosampler schedule was started and data export was used to log the results. Once the flow cell had been flushed with the new sample, a reading was taken from the RFM990 after a sixty second-stabilisation period.

### ***Verifying the Product Readings***

On completion of the autosampler schedule, the flow cell was removed and each product was measured on the RFM990. The prism was cleaned with distilled water between applications and samples were allowed sixty seconds to stabilise on the prism. To prevent ambient light from affecting the reading, a cap was placed over the flow cell collar before measuring the sample.

## Results

Two Autosampler racks, each consisting of 14 vials, were used to provide a schedule involving distilled water, 15°Brix and degassed Cola. Using the schedule given for the autosampler shown in figures 4 & 5 and allowing a sixty second delay as stabilisation criteria for the RFM990 the following results were obtained.

Pot	Description	Time	Reading	Scale	Temp.	TC
A-1	Water	09:33:38	0.00	bx	20.0	sucomp
A-2	15°Brix	09:35:44	15.00	bx	20.0	sucomp
A-3	Water	09:37:50	0.01	bx	19.9	sucomp
A-4	Cola	09:39:56	10.84	bx	20.0	sucomp
A-5	Water	09:42:02	0.01	bx	19.9	sucomp
A-6	15°Brix	09:44:08	15.00	bx	20.0	sucomp
A-7	Water	09:46:14	0.01	bx	20.0	sucomp
A-8	Cola	09:48:21	10.85	bx	20.0	sucomp
A-9	Water	09:50:27	0.01	bx	19.9	sucomp
A-10	Cola	09:52:33	10.85	bx	20.1	sucomp
A-11	15°Brix	09:54:39	15.00	bx	20.0	sucomp
A-12	Cola	09:56:45	10.86	bx	20.0	sucomp
A-13	Water	09:58:51	0.01	bx	19.9	sucomp
A-14	Water	10:00:57	0.01	bx	20.0	sucomp
B-1	Cola	10:03:04	10.85	bx	20.0	sucomp
B-2	Water	10:05:10	0.01	bx	20.0	sucomp
B-3	Cola	10:07:16	10.85	bx	20.0	sucomp
B-4	15°Brix	10:09:23	15.00	bx	20.0	sucomp
B-5	Cola	10:11:30	10.85	bx	20.0	sucomp
B-6	Water	10:13:37	0.01	bx	19.9	sucomp
B-7	Cola	10:15:44	10.85	bx	20.1	sucomp
B-8	15°Brix	10:17:51	15.00	bx	20.0	sucomp
B-9	Water	10:19:58	0.01	bx	20.0	sucomp
B-10	Cola	10:22:05	10.85	bx	20.0	sucomp
B-11	15°Brix	10:24:12	15.00	bx	20.1	sucomp
B-12	Cola	10:26:19	10.85	bx	20.0	sucomp
B-13	Water	10:28:26	0.01	bx	20.0	sucomp
B-14	Water	10:30:33	0.01	Bx	20.1	sucomp

**Table 1** LimsLite Data Export for Two-Rack Schedule

To verify the correct reading for each of the samples, the flow cell was removed and each product involved in the schedule was measured with the sample cover applied and allowing sixty seconds for the sample to stabilise. The prism was cleaned with distilled water between measurements, and all readings were taken in °Brix with sucrose temperature compensation applied.

Sample	Date	Time	Reading	Temperature	Quality
Distilled Water	25/05/04	11:49	-0.01	20.0°C	103
15°Brix	25/05/04	11:51	15.00	20.0°C	101
Degassed Cola	25/05/04	11:56	10.85	20.0°C	101

**Table 2** Verification of Product °Brix Readings

## **Conclusion**

### ***Performance of the system***

Repeatability of the measurements obtained using the autosampler system were:

Water 0.01°Brix  
Degassed Cola 0.02°Brix  
15°Brix 0.00°Brix

Accuracy of the measurements obtained using the autosampler system (compared to static measurements on the same instrument) were:

Water  $\pm 0.02^\circ\text{Brix}$   
Degassed Cola  $\pm 0.01^\circ\text{Brix}$   
15°Brix  $\pm 0.00^\circ\text{Brix}$

With the configuration tested here the combined sampling, flushing and reading time for a sample was 126 seconds.

The system coped with the introduction of air through the pumping process. This was proven by both visual inspection of the flow cell and by practical experimentation. Performance to the above specification was achieved when flushing with a sample volume of 30ml.

### ***Operational Notes***

Samples should be degassed thoroughly prior to introduction to the system. This ensures that small air bubbles do not form on the prism surface.

It is recommend that the instrument is zeroed daily, which means the removal of the flow cell insert. Intermediate Zero verification and correction can then be performed automatically using distilled water from an autosampler pot.