ADDENDUM A

Method construction

Sequential injection analysis is dependent on precisely timed operations which takes place in a programmed sequence. The programming is done using the FlowTEK [1,2] program developed by Marshall [3]. The method used for the determination of total iron in multivitamin and water samples using a solid-phase reactor (Chapter 6) is used to explain the method construction and procedures in FlowTEK.

The analytical cycle used in the determination consisted of the following operations: aspiration of hydrochloric acid solution for reactor regeneration, the sample, the citrate buffer, the oxidation step itself, the complex formation of iron(II) and 1.10 Phenanthroline, the detection of the formed product (complex) and rinsing of the manifold (Fig. 6.1).

A Unicam 8625 UV-VIS spectrophotometere equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mülheim/Baden, Germany) flow-through cell for absorbance measurement was used as detector.

A FlowTEK [1,2] software package was used throughout for data acquisition and device control. This was achieved using a PC 30-B interface board (Eagle Electric, Cape Town) and an assembled distribution board (Mintek, Randburg). The FlowTEK was also used to send a signal

to the UV-VIS spectrophotometer when the product passed through the flow-through cell. Devices in the SIA system must be compatible with TTL or switch control signal [2] to be able to perform their functions. A device is defined as an analytial instrument or component which must be controlled by the software package. The first six device definitions are supplied with the software package. These devices can be viewed on the note pad menu (Table A1)

TABLE A1 Schematic representation of the FlowTEK notepad screen (page 1 for device description

Next Page Hard Copy RED Print MET Print PDR Print Quit

Board : PC30-B
Experiment time : 150
Zoom min time : 0.0
Zoom max time : 150
Start acquisition : 43
I/O port for GP : 1
I/O port for SV : 3
Save profile : Yes
Abridged profile : Yes
Regression on Height
Detector displ : Paged
Inject mode : Auto
Start up : (0)
Rescale Y-axis : Fixed
Min : 0.00 Max ; 10.0

F1 : Displ Analog input
F2 : Displ Digital input
F3 : 010000000000 (2)
F4 : 110000000000 (3)
F5 : 000000000000 (0)
F6 : 000000000000 (0)
F7 : 000000000000 (0)
F8 : 000000000000 (0)
F9 : 000000000000 (0)
F10 : Directory

Detector	1	2	3	4
A/D channel	1			
Transformation	None			
Auto Zero	None			
AZ time	0.0			
AZ offset	0.000			
Min Integ Lim	150.0			
Max Integ Lim	150.0			
Width Height	0.000			
Peak Time	@ Pk max			

Path : C:\ FLOWTEK \BOB\ IRON (II)
Main Procedure file : IRON (II).PDR
Method file : IRON (II).MET
Reduced data file : IRON(II).RED
Experimental Profile Root : Fe
Calibration file : DEFAULT.CAL

Name	AP	GP	IV	SV	AS	SW
Action	FWD REV OFF	FWD REV OFF	INJ LOAD	ADV HOME	NEXT	TRUE FALSE
Hotkey	F R O	F R O	I L	A H	N	T F
Output	01 (1) 10 (2) 00 (0)	10 (2) 11 (3) 00 (0)	01 (1) 10 (2)	10 (2) 01 (1) 00 (0)	1 (1) 0 (0)	1 (1) 0 (0)
Pulse	0.00	0.00	0.00	0.30	5.00	0.00

The function keys F1 to F10 are programmed to perform different functions. F1 is used to monitor and check analog inputs. When F1 is pressed one can adjust port on simulator and monitor reading on screen. The reading on the screen should be compared to a multimeter. F2 is used to check digital outputs. When F2 is pressed on the computer one uses jumpers to short out 0V to the digital inputs and monitor screen. To show that this is accomplished the inputs will go on and off. If all inputs on the screen are off, measure at the digital input on the 26 pin ribbon crimp connector for ±5V signal.

F3 - F9 are used to control the digital devices which are connected to one of the eight digital outputs on the distribution box. This setup is achieved on the setup menu page using the function key menu option. This digital outputs can be configured as switch. The voltage maybe set high (5V) or low (0V). In this work only F3 and F4 because only two devices were connected namely the SV and the GP. for example to programme F2 , 2 was typed which is equivalent ton 01 and for F3, 3 was typed which is equivalent to 11 (Table A1) F10 is used as directory to display or a list of defined procedures and methods.

The software package does however, provide for the configuration of another extra six devices as need may arise. These may further be viewed after typing the letter N to view the second page of the device configuration (Table A2).

TABLE A2 Schematic representation of the FlowTEK notepad screen (page 2 for device description)

Name Action	0	0	0	0	0	0
Hotkey						
Output						
Pulse	0.00	0.00	0.00	0.00	0.00	0.00

For the sequential injection analysis the device configuration data of the Gilson pump (GP) and the selection valve (SV), need not be programmed, since they are supplied with the software. From the main menu the letter **M** must be typed to obtain the method menu. The two devices needed can be selected using the option **Type (of) device**. The questions or commands which followed can be answered as follows:

Enter number of devices: **2**

Enter type device 1: **GP**

Enter digital point for GP: **1** (This represent the first position the device occupy on the interface board).

Enter digital point for SV: **3** (This represent the third position the device occupy on the interface board).

The screen is now divided into two panel, each containing a straight line in the middle of the panel. The position of these lines represent the 'OFF' position of each device. Programming of each device are now allowed. Device events are entered by choosing the option **Insert** in the

method menu. It is important to switch on **NUM LOCK** when using the **Insert** or **Delete** options. The programming for the different operations needed can be done as follows:

Insert

Device number:	**2**	(Selection valve)
Enter time of event:	**0**	
Hotkey (A H):	**H**	(Select HOME position-first port)

Insert

Device number:	**1**	(Gilson pump)
Enter time of event:	**1**	
Hotkey (F R O):	**R**	(Switch pump on in the reverse direction)

Insert

Device number:	**1**	(Gilson pump)
Enter time of event:	**6**	
Hotkey (F R O):	**O**	(Switch pump off)

Insert

Device number:	**2**	(Selection valve)
Enter time of event:	**11**	
Hotkey (A H):	**A**	(Select waste stream-second port)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **12**

Hotkey (F R O): **F** (Switch pump on in the forward direction to waste)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **23**

Hotkey (F R O): **O** (Switch pump off)

Insert

Device number: **2** (Selection valve)

Enter time of event: **24**

Hotkey (A H): **A** (Select sample stream-third port)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **25**

Hotkey (F R O): **R** (Switch pump on in the reverse direction)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **29**

Hotkey (F R O): **O** (Switch pump off)

Insert

Device number: **2** (Selection valve)

Enter time of event: **30**

Hotkey (A H): **A** (Select buffer stream-fourth port)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **31**

Hotkey (F R O): **R** (Switch pump on in the reverse direction)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **35**

Hotkey (F R O): **O** (Switch pump off)

Insert

Device number: **2** (Selection valve)

Enter time of event: **36**

Hotkey (A H): **A** (Select Orthophenanthroline stream-fifth port)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **37**

Hotkey (F R O): **R** (Switch pump on in the reverse direction)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **41**

Hotkey (F R O): **O** (Switch pump off)

Insert

Device number: **2** (Selection valve)

Enter time of event: **42**

Hotkey (A H): **A** (Select detector line-sixth port)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **43**

Hotkey (F R O): **F** (Switch pump on in the forward direction to pump stack of zones through detector)

Insert

Device number: **1** (Gilson pump)

Enter time of event: **150**

Hotkey (F R O): **O** (Switch pump off-end of analytical cycle)

Insert

Device number: **2** (Selection valve)

Enter time of event: **150**

Hotkey (A H): **H** (Valve return home to first port)

To delete any existing event, the option **Delete** on the method menu must be used. The time of the event needed to be erased must be typed in when asked for. The procedure to delete an event is the same procedure needed to insert an event.

To avoid storing redundant data, data acquisition could be started only a few moments after the valve was switched to select the detector line. This will result in the collection of only peak profile and relevant data. To accomplish this the option **Exp time** on the method menu must be selected and the time to start data acquisition must be typed in. The final method is represented schematically in Table A3.

After the method is constructed it is saved using the option **File** on the method menu and typing **S** for save under appropriate name. The method file used for the iron determination was saved as **iron (II).met.** When the method must be repeated a fixed number of times it is best to write a procedure to carry out the repetitions. Ten repetitions of the method were used during the final evaluation of the SIA system.

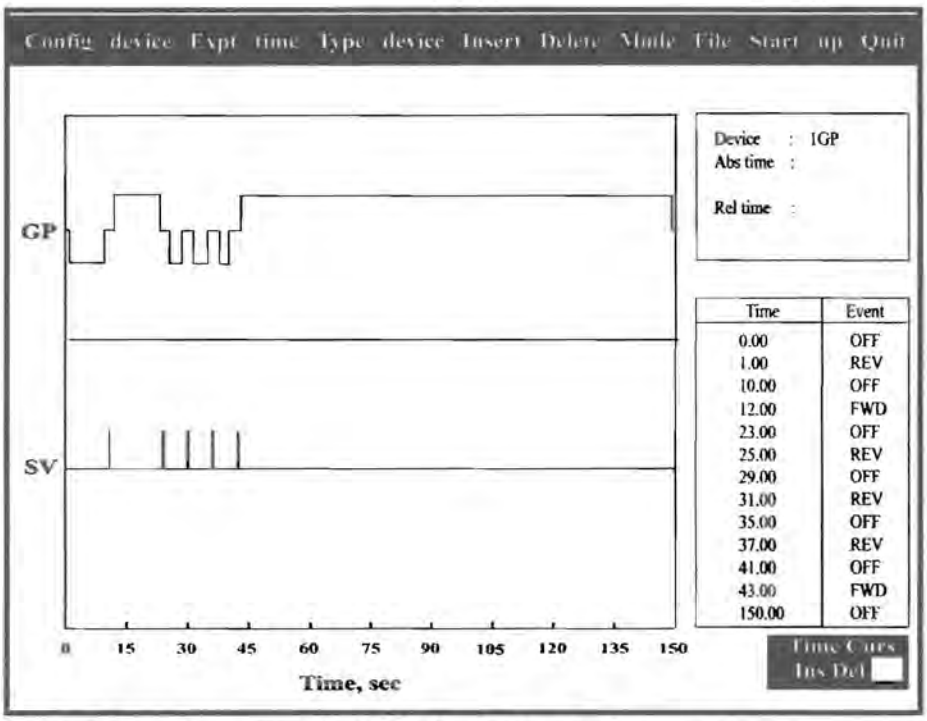

Fig.A1 Schematic representation of the flowTEK method screen.

To create a procedure, the option **Repeated** on the main menu must be selected. The option **Build Proc.** on the repeated menu is used to create a procedure. The procedure was named **iron (II). pdr**. It was necessary to specify whether a method file (.met) or another procedure file (.pdr) was used in this application. The following commands or questions must be answered:

Enter main procedure: **C:\ FLOWTEK\ BOB\Solid\iron(II).PDR**

Enter method or procedure file: **iron (II). MET.**

Enter number of repetitions: **10**

If more methods or procedures are to follow, the process must be repeated till all the methods together with their number of repetitions are listed. Otherwise **ESC** terminates the procedure definition. To use this main procedure file, the option **Main Proc** on the main procedure file must then be typed in.

The option **Red. Data file** on the **Repeated** menu selects a reduced data file for saving each experiment's peak parameter data and relative experiment identification information. The experiment number counter is reset to **1** when the reduced data file name is changed **or when the program was exited**. Every time the program is restarted a new reduced data file must be opened, otherwise the previous data would be lost. The option **Profile file** on the **Repeated menu** selects the file not name for storing the profile data. The file name extension gives the experiment number. If no profile root is chosen, profile files are not saved.

To execute the main procedure the option **GO!** on the **Repeated** menu must be chosen. The option **Once** on the Main menu is chosen if only one run is needed. The Main procedure can be aborted by pressing **ESC**.

Calibration can also be done using the FlowTEK program. Data used to calculate calibration constants are stored in a Calibration Response table. This table has 9 rows and 3 columns. The response for a p-articular calibration are stored on the rows of the table. Table A4 gives typical results for calibration from Chapter 6. Replicated values are placed in each column

the FlowTEK program keeps track of the number of replicates. When you measure more than

three replicates the oldest datum is replaced by the newest value. The regression is carried out on a simple arithmetic mean of the data for a particular concentration.

TABLE A3 A Response Calibration Table with some results of the calibration graph

Conc(ppm)	1st	2nd	3rd	Avg
1	0.3135	0.3079	0.3101	0.3165
10	1.1550	1.1501	1.1452	1.1501
20	2.2243	2.1779	2.1877	2.1960
30	3.2131	3.1618	3.2683	3.2000
40	4.3459	4.3190	4.3654	4.3434
50	5.4128	5.3298	5.2224	5.3216
60	6.1281	6.1745	6.2111	6.1711

Before a calibration calculation can be completed, you must specify the first and last standards in the calibration Response Table. This means that it is possible to store data for more than one calibration in the Calibration Response Table.

To calculate the regression constants for the first data, specify the first standard as 1 and last standard as 3. To calculate the regression constants for the second data specify the first standard as 4 and the last as 6. You can have any number of standards for a particular calibration set

References

1. G. D. Marshall, **Analytical Instrumentation, 20 (1)** (1992) 79.

2. **FlowTEK Reference Manual, Device Control and Data Acquisition software, ver. 1.1,** Mintek, 1993.

3. G. D. Marshall, **Sequential-Injection Analysis**, PhD-Thesis, University of Pretoria, 1994.

ADDENDUM B

Publications and Presentations

Publications

1. Determination of Manganese in tap water and effluent streams using a solid-phase Lead (IV) dioxide reactor in sequential injection systems.

   E. B. Naidoo and J. F. van Staden. **Fresenius J. Analytical Chemistry, 370 (6)** (2001) 776.

2. Super serpentine reactors - a comparative and precision study.

   E. B. Naidoo and J. F. van Staden. **Instrum. Sci. and Techn., 29 (2)** (2001) 77.

3. Determination of Iron as Fe(II) in multi-vitamins, Haematmics and effluent streams using a solid-phase (cadmium reductor) reactor incorporated in a sequential injection (SIA) system.

   J. F. van Staden and E. B. Naidoo. **South Africa J. Chemistry** (2001).

4. An improved technique for the determination of oxidised nitrogen in water using a solid-phase reactor with a sequential injection analysis (SIA) system.

   E. B. Naidoo and J. F. van Staden. **Waters SA, 27 (3)** (2001) 355.

5. An alternative enhanced method for the determination of chromium in electroplating and natural waters with a sequential injection analysis (SIA)

system.

E. B. Naidoo and J. F. van Staden. (submitted).

Presentations

1. The assay of S-enantiomers of enalapril, ramapril and trandolapril using an amperometric biosensor/sequential injection analysis system.

   J. F. van Staden, R. I. Stefan, H. Y. Aboul-Enein, E. B. Naidoo and L. V. Mulaudzi.

   ISCD 12, **The International Symposium on Chirality, Chamonix, Mount Blanc, France. 24-28 September 2000**

2. Determination of Manganese in tap water and effluent streams using a solid-phase Lead(IV) dioxide reactor in sequential injection systems.

   E. B. Naidoo and J. F. van Staden

   Euroanalysis XI. Working Party on Analytical Chemistry at the Federation of European Chemical Societies and the Portuguese Chemical Society, Lisbon, Portugal. 3-9 September 2000

3. Super Serpentine Reactors in SIA - a comparative response and precision study

   E. B. Naidoo and J. F. van Staden

   Euroanalysis XI. Working Party on Analytical Chemistry at the Federation of European Chemical Societies and the Portuguese Chemical Society. Lisbon, Portugal. 3-9 September 2000

4. Determination of S-Pentopril using an amperometric biosensor/SIA system.

R. I. Stefan, L. V. Mulaudzi, E. B. Naidoo, H. Y. Aboul-Enien and J. F. van Staden.

Flow Analysis VIII, Polish Academy of Science, Polish Chemical Society. Warsan, Poland. 25-29 June 2000

5. The assay of S-enalapril using an amperometric biosensor/SIA system.

J. F. van Staden, R. I. Stefan, E. B. Naidoo and H. Y. Aboul-Enein.

Flow Analysis VIII. Polish Academy of Science, Polish Chemical Society. Warsaw, Poland. 25-29 June 2000

6. Determination of Manganese in tap water and effluent streams using a solid-phase Lead (IV) dioxide reactor in sequential injection systems.

E. B. Naidoo, and J. F. van Staden.

Flow Analysis VIII. Polish Academy of Science, Polish Chemical Society. Warsaw, Poland. 25-29 June 2000

7. Super Serpentine Reactors in SIA - a comparative response and precision study.

E. B. Naidoo and J. F. van Staden.

Flow Analysis VIII. Polish Academy of Science, Polish Chemical Society. Warsaw, Poland. 25-29 June 2000.