DEVELOPMENT OF A LOW COST LED-PHOTODIODE BASED SPECTROPHOTOMETER FOR CONTINUOUS ON-LINE MONITORING USING OPTICAL FLOW CELLS

Report to the

Water Research Commission

by

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The publication of this report emanates from a WRC project entitled *Development of a Low Cost Led-Photodiode Based Spectrophotometer for Continuous On-Line Monitoring Using Optical Flow Cells* (WRC Project No. K8/865).

EXECUTIVE SUMMARY

This report describes the development of an LED-photodetector device for continuous on-line monitoring using optical flow cells as a low cost alternative to conventional spectrophotometry. Conventional spectrophotometers generally use tungsten or deuterium incandescent light sources, and have diffraction gratings, mirrors, filters and various other components that make up complex and expensive instruments. The development of light emitting diodes that emit at specific wavelengths in a narrow bandwidth offer several advantages for replacing the conventional technology: LEDs are robust, inexpensive, longer lasting, smaller, and stabilise within milliseconds.

The versatility of the system developed was demonstrated in two different applications: measurement of phenolic compounds in the UV light range (280 nm) during a chromatographic purification process, and monitoring of algal cell culture density in the visible light range (465 and 760 nm) during growth in a photobioreactor. The system was controlled and monitored using Labview software, and by using flow-through optical cells, it was possible to take continuous on-line measurements as opposed to periodic sampling and external measurement.

Phenolic compounds are ubiquitous in industry, e.g. pulp and paper, refineries, and many other chemical processes, therefore development of low-cost robust systems for measurement of such compounds in process streams is of obvious importance. We have demonstrated long-term operation for the monitoring of a chromatographic purification process to recover valuable phenolic antioxidant compounds from wastewaters generated by the olive processing industry.

In terms of bioprocess monitoring, algal cell culture was chosen as it presents more of a challenge than simply measuring optical density at 600 nm, as is common for microbial cell culture. Algal cells have chlorophylls and other pigments that interfere with optical density at 600 nm, therefore we measured beyond the pigment absorbance range (760 nm) and at chlorophyll peak absorbance (465 nm), in an attempt to quantify both cell density and chlorophyll content. Turbidity measurements were also performed on the algal cells after modification of the system,

however the high optical density of the algal culture limited the usefulness of approach.
Accuracy and precision of the system were determined and compared to that of a standard laboratory spectrophotometer, with results from the LED device being equal to or better than the laboratory spectrophotometer.

Limitations of the system were primarily that it is not possible to perform spectral scans or measure at multiple wavelengths as with conventional spectrophotometers; an LED is required to illuminate at each specific wavelength of interest. However, the use of multiple LEDs in one device can overcome this limitation.

The electronic components of the system have subsequently been transferred onto printed circuit boards (PCBs) to make the system more compact. The PCBs are to be incorporated into a new design that will be thoroughly waterproofed and will be rolled out in our lab for general bioprocess monitoring.

Future work will include:

- Multiplexing several detectors to run on one platform
- Dual and triple wavelength functionality
- Design of a low cost optical flow cell that incorporates the LEDs
- Low-cost fluorescence measurement
- Development of the system into a hand-held probe for in-situ measurements
- Signal telemetry for remote monitoring.

Besides for the applications described above, future applications of the system could include:

- Wastewater treatment
- Surface water quality
- Diverse chemical and industrial processes.

We anticipate developing the system such that it may be used for general commercial applications.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

The need for on-line monitoring of liquid streams in chemical and biological processes is of great importance. Typically, light absorption measurements at a specific wavelength can be used to measure different dissolved and colloidal species, however, this generally performed by periodic sampling and subsequent measurement in a spectrophotometer, which is tedious. These instruments are also expensive and thus cannot generally be used for dedicated measurement of one process or stream.

The purpose of this project was to develop a novel on-line monitoring system based on light emitting diode (LED) and photodetector technology, as a low-cost alternative to conventional spectrophotometeric measurement. LEDs offer several advantages compared to fluorescent and halogen based spectroscopic light sources as used in current commercially available instruments: they are robust, inexpensive, longer lasting, smaller, and stabilise within milliseconds. It was therefore the objective of this project to develop a LED-photodetector based device for on-line monitoring, and to evaluate and test it for different applications. Several such devices have been reported in the literature, for example Schmid *et al*. (2008) describe a UV detector developed for high performance liquid chromatography (HPLC) for measuring nanogram quantities of compounds that equalled or exceeded the capabilities of the original detector. In the medical field, pulse oximeters that measure heartbeat and blood oxygenation are also based on LED-photodetector technology (Webster, 1997). Dasgupta *et al*. (2003) provide a comprehensive review of different applications for which LED-based devices have been used for analytical purposes.

LEDs have developed radically over the past few decades, from the original dim devices of the 1970s emitting in the red wavelengths, to today's devices that have high intensity all the way down into the deep ultraviolet range (< 250 nm), and up to the infra-red range (> 1000 nm). As such, they can now readily be used as alternatives to conventional spectroscopic sources. LEDs do not allow for the scanning of spectra, however their emission bands are nowadays very narrow (< 20 nm), meaning that monochromators or other wavelength selection devices (filters) are not necessary at the detection side, and are thus ideally suited for absorption measurements at specific wavelengths. In addition, modern LEDs have high

efficiency, resulting in high light intensities with low power consumption, and their stability translates into low noise which means that limits of detection can be better than conventional devices. Similarly, photodetectors that convert incident light into current have developed in conjunction with LEDs; components are now available that have enhanced sensitivity and output across the UV, visible, and infra-red ranges.

This work grew out of a conceptual final 4th year electrical engineering project, for the measurement of phenolic compounds (Pikkering, 2008). It was further developed in another 4th year project where the system was used for monitoring of a chromatographic process for the purification of phenolic compounds (Austin and De Beer, 2009). The current project was proposed in order to build on these developments, such that the system may be used for routine on-line analysis, and to extend the range of applications for which the system may be used.

This report provides a description of the technology and the evolution thereof, and the theory upon which it is based. Thereafter the materials and methods used for subsequent measurements and evaluation of the device are described. The system was then used for detailed monitoring, analysis, and optimisation of a chromatographic process for the purification a valuable phenolic antioxidant from an agro-industrial waste stream. Subsequently it was used for the continuous measurement of cell density during an algal cell culture bioprocess. Algal cell culture is of interest in our laboratory for CO_2 sequestration and biodiesel production purposes. Lastly, in an attempt to quantify chlorophyll content during the algal growth process, a nephelometric (light scattering at right angles) method was investigated. This illustrated that conventional turbidity measurements are also possible using the developed system.

2. OBJECTIVES

Objectives described in the original project proposal were:

- Re-evaluate the electronic circuit developed in the fourth year project, simplify or even possibly omit it, depending on the capability of commercially available data capture cards.

- Design and manufacture of a cuvette flow-cell holder that can accommodate 2 LEDs of different wavelengths for simultaneous measurement.
- Test the LED-photodiode spectrophotometer extensively in laboratory-scale experiments, and assimilate the data on a computer with future process control in mind.
- Test the system across a range of applications.
- Investigate the possibilities of telemetry for remote monitoring.
- Develop the system into a compact, robust unit that can be evaluated in field trials.

3. THEORY AND DESCRIPTION

Many, if not most, molecular species absorb light at certain wavelengths, due to their shape, resonance, quantum energy, and the electrochemical bonds between atoms. This property can be exploited to measure different species according to the Beer-Lambert law (Equation 1), where the amount of light absorbed is proportional to the quantity (concentration) of a specific species at a specific light wavelength. This is the basis of light spectroscopy, which is extensively used in analytical chemistry, illustrated in Figure 1.

$$\log(I/I_0) = A = \varepsilon \cdot l \cdot C \qquad \text{…….. (1)}$$

Where	I_0 = incident light intensity	($W.m^{-2}$)
	I = exiting light intensity	($W.m^{-2}$)
	C = concentration	(M)
	ε = molar extinction coefficient	($M^{-1}.cm^{-1}$)
	l = light path length	(cm)
	A = absorbance	(dimensionless)

In a photodetector, the light intensity is proportional to current (or voltage) output, thus measuring the voltage output from an LED shining through pure water compared to the output when shining through a dissolved species is equivalent to the absorbance. Because the Beer-Lambert law is logarithmic, there is an upper limit to the concentration that can be measured for a specific path length. For accuracy,

spectroscopic measurements are generally performed at < 1 absorbance unit, thus dilution is often necessary. Above 1, less than 2% of the incident light is transmitted through the liquid, and thus accuracy and precision is lost, and relative standard deviation becomes excessive.

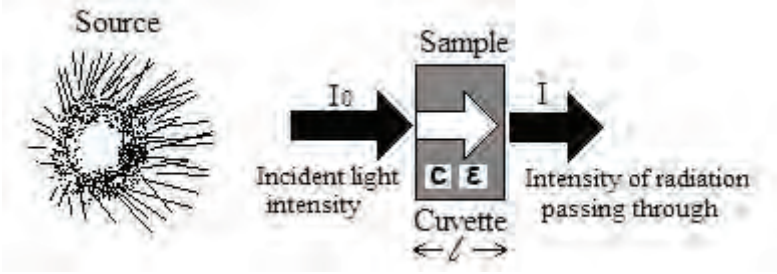

Figure 1: Passage of light through a cuvette containing a dissolved substance

As an alternative to dilution of high concentration liquids, it is possible to adjust the path length through which the light is transmitted: shorter for high concentrations and *vice versa*. This is a useful property that can be exploited for on-line measurement, as dilution is inconvenient during continuous measurement. Standard cuvettes have a path length of 1 cm. Flow-through cells are used for continuous measurements, and these come in a range of path lengths from 0.1-50 mm (Figure 2).

For colloidal or particulate matter, incident light is scattered. Measuring the amount of scattered light (typically at right angles) gives a proportional indication of the amount of colloidal or particulate matter, and is referred to as the nephelometric turbidity of a sample. This principle can be used to monitor microbial cell density during biological culture processes or in biological wastewater treatment systems.

4. MATERIALS AND METHODS

4.1 Equipment and electrical components

LEDs and photodetectors in the UV range were obtained from Roithner Lasertechnik GmbH (Vienna, Austria). Visible range LEDs of 400, 505, 605, and 760 nm were obtained from the same supplier. Visible range photodetectors were from Texas Instruments, supplied by RS components (Vorna Valley, South Africa). USB-6008

and USB-6009 data capture cards were obtained from National Instruments (Midrand, South Africa).

Figure 2: Examples of Hellma flow through optical cells

Flow through cuvettes of different optical path lengths were obtained from Starna (Essex, UK) and Hellma (Müllheim, Germany). Quartz flow cells were used for UV measurements, while less expensive glass units were used for the optical range. Disposable Fluorovette flow cells (ALine Inc., Redondo Beach, California) were also investigated as an alternative to the more expensive glass and quartz flow cells (see Figure 2). A microbore peristaltic pump was used for directing fluids through the flow cells. All other electrical components such as resistors, op-amps, capacitors, etc. were standard from an electrical workshop.

Figure 3: Short path length Fluorovette disposable flow cells as an alternative to more expensive glass and quartz units

4.2 System design and operation

The final electrical circuit for powering the LEDs and amplifying the photodetector output shown in Figure 4. A constant current chip was used to supply the LED in order to maintain a stable light output irrespective of extraneous factors. Current supply to the LED was adjustable (up to 100 mA) by judicious selection of resistors. This allowed for appropriate adjustment of light output as required. Similarly, the voltage output from the photodetector was adjustable *via* a variable resistor up to a maximum of 11 V. Power supply to the system was from a simple purpose-built transformer that converted 240 V AC mains supply to 12 V DC.

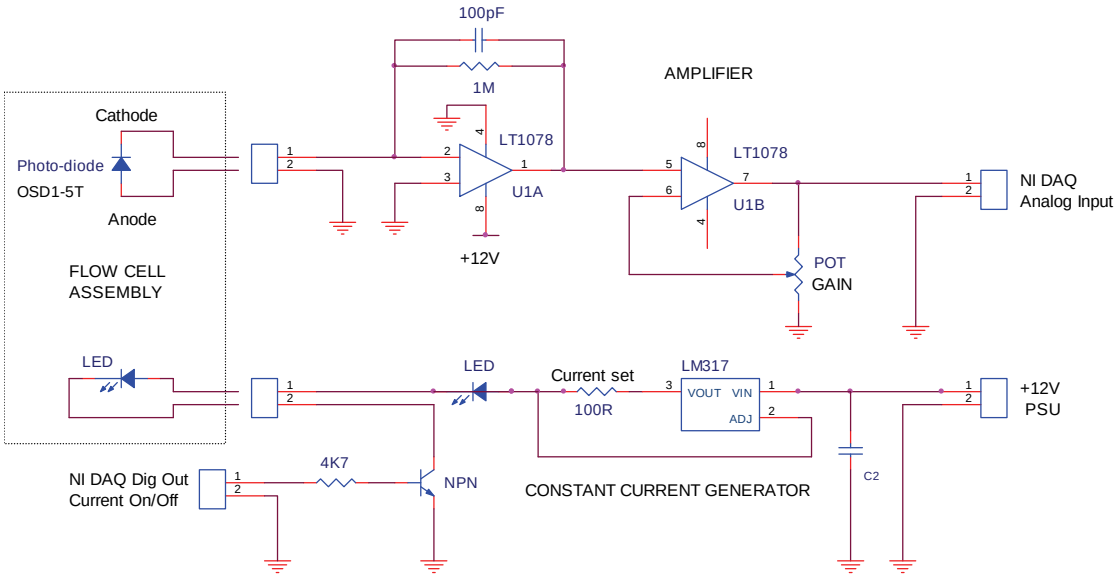

Figure 4: Electrical circuit for constant current LED supply and photodiode output amplification

The cuvette holder and mounting for the LED and photodetector were constructed from polyvinyl chloride, the design drawings are shown in Appendix A. Figures 5 A-C show the evolution of the system.

Initially, the electronic circuit was quite complex and over designed (Figure 5A). The system was thus reduced to a bare minimum of components (Figure 5B), and then mounted into the final format shown in Figure 5C.

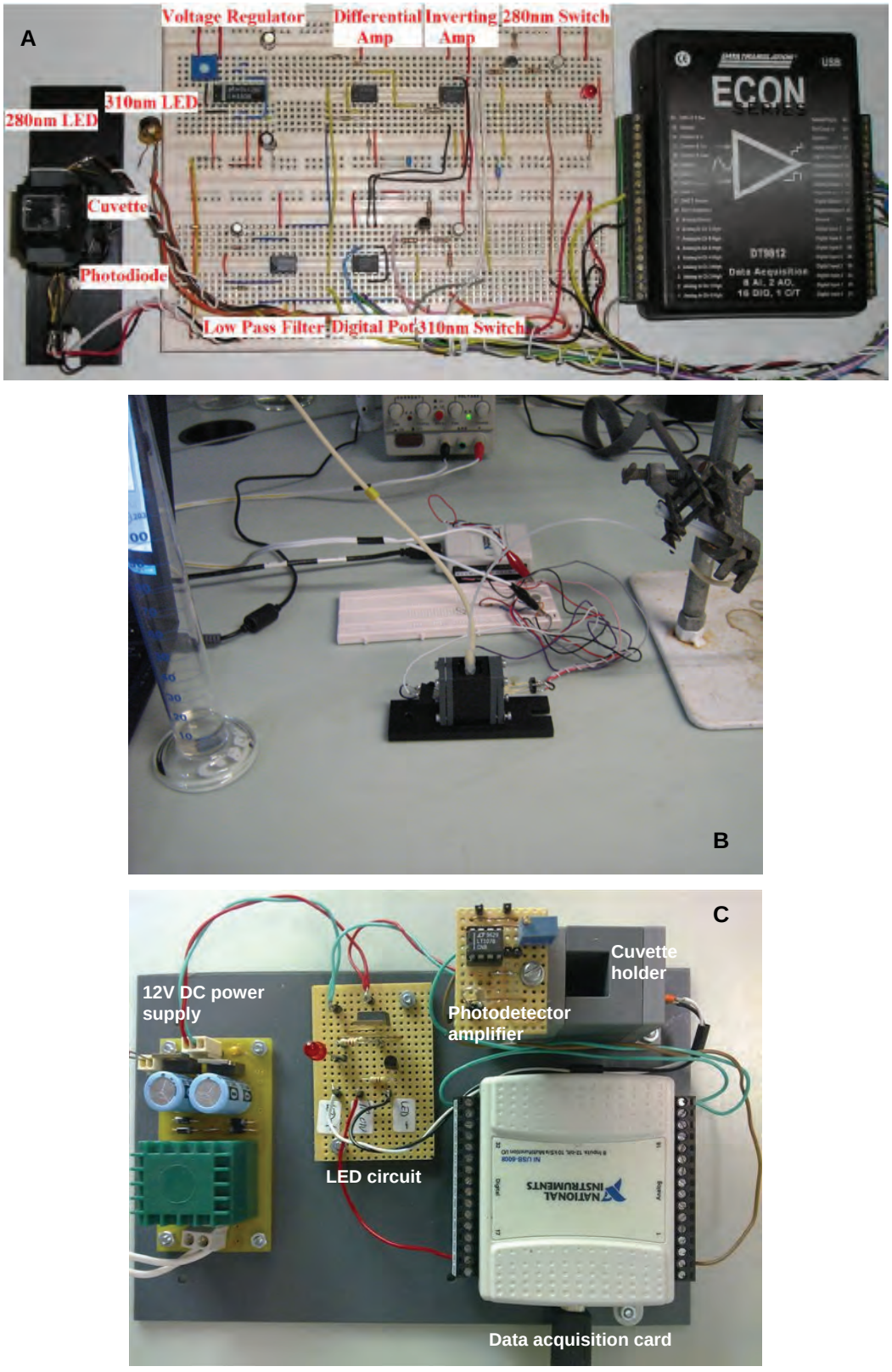

Figure 5: Evolution of the LED-photodetector and electronic circuitry

The voltage output from the photodetector was sent *via* the data acquisition card to a laptop computer and processed using Labview software. It was thereby possible to digitally process the signal (for noise reduction and averaging), negating the need for a low pass filter as in the original electronic circuit. Figure 6 shows the final Labview graphic user interface, while Figure 7 shows the back panel program developed to process the signal into a useful form. After calibration, it was possible to convert the voltage output from the photodetector directly into a concentration measurement using the Beer-Lambert law.

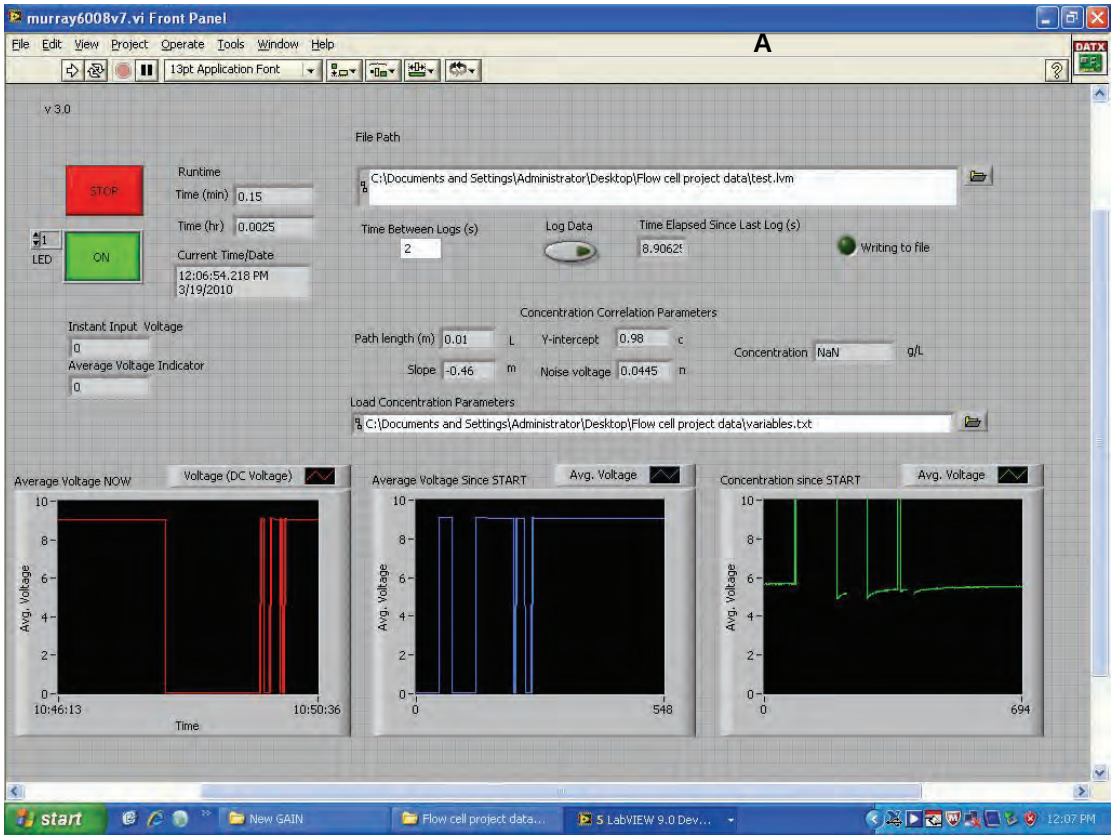

Figure 6: Labview graphic user interface showing the raw voltage signal (red), the processed signal (blue), and the concentration estimate (green) during a system test.

4.3 System calibration and evaluation

In order to calibrate the system it was first necessary to adjust various parameters, depending on the substance being measured and the optical density thereof. This involved taking slightly higher than maximum anticipated concentration and adjusting LED output, photodetector gain, and light path length such that at zero concentration (e.g. pure water) the processed output signal had a baseline maximum of around 10 V

without saturating the component (zero concentration = maximum light intensity, thus maximum voltage output), while at high concentration (high optical density, i.e. low light transmission) the photodetector was near its minimum output (between 0 and 1 V).

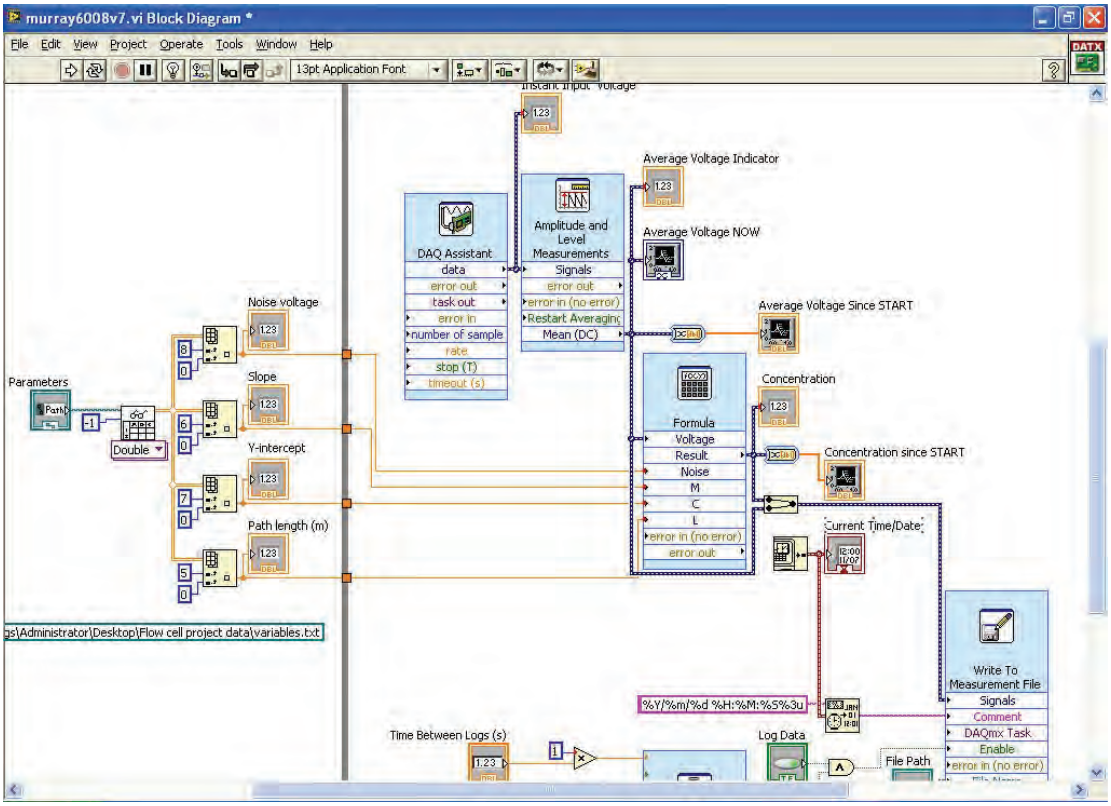

Figure 7: Labview back panel block diagram used to process photodetector output into a concentration estimate

Once this was achieved, a serial dilution was made up, and corresponding voltage outputs were measured for the different concentrations. This resulted in a logarithmic curve of voltage vs. concentration, which was then linearised according to the Beer-Lambert law. The point at which the curve deviated from linearity (high concentration, i.e. low voltage) was taken as the system maximum. Using this approach, it was possible to accurately measure very high concentrations indeed, around 2 $g.L^{-1}$ for both phenolic compounds and cell density. This is approximately ten times higher than what is possible to measure using a standard 1 cm cuvette in a spectrophotometer without dilution of the sample.

Accuracy and precision of the system were determined according to methods described by Wu *et al*. (2009). Accuracy and precision were defined according to Equations 2 and 3.

$$\text{Accuracy} = [(C_{obs} - C_{nom})/C_{nom}] \times 100\% \quad \ldots\ldots\ldots(2)$$

$$\text{Precision} = [\text{relative standard deviation} \div C_{obs}] \times 100\% \quad \ldots\ldots\ldots(3)$$

Where C_{obs} = mean value of observed concentration

C_{nom} = nominal concentration (according to least squares linear fit of data to Beer-Lambert law)

In order to determine these, standards were made up in triplicate at various concentrations, voltage measurements were taken and the data linearised and plotted against concentration. A best-fit line was then plotted through this data, and this was used to determine nominal concentrations, which could then be compared to observed concentrations (mean and standard deviation of triplicates). The same process was then repeated in a bench spectrophotometer to compare the accuracy and precision.

Limits of detection (at minimal concentrations) were not considered to be important for the current work, as species being measured were generally at high concentrations. More important was the upper limit of detection, this was determined by deviation of data from the Beer-Lambert law, i.e. the concentration at which least squares linear fit became $R^2 < 0.98$. Lower limits of detection are easily determined though, this being generally defined as when the signal-to-noise ratio becomes less than 3 (S/N < 3) at very low concentrations.

Gallic acid ($C_7H_6O_5$, M_w = 170.1) was used as a standard for phenolic compound calibration, as pure hydroxytyrosol (the phenolic compound of interest) is exorbitantly expensive (~US$ 2000/gram). The two compounds have similar molecular weight and molar extinction coefficients. Pure hydroxytyrosol standards were however used for subsequent HPLC analysis of results.

Algal cells of the species *Chlorella vulgaris* were taken from a high cell density culture (> 2 $g.L^{-1}$) for calibration purposes. The same species was used for optical density growth measurements and scattering experiments. Cell density was determined by filtration, drying and weighing; the same cell density was then serially diluted and used for calibration of the system. Calibration standards were also measured in a spectrophotometer for comparative purposes.

4.4 Measurement of phenolic compounds at UV 280 nm and chromatography for purification thereof

Phenolic compounds absorb strongly in the UV light range. Gallic acid and hydroxytyrosol both have absorbance peaks at 280 nm, and therefore this wavelength was chosen for detection. After calibration, the flow cell system was connected to a chromatography column as shown in Figure 8. The objective of the chromatography process was to purify hydroxytyrosol from a crude extract previously obtained from olive-derived wastewaters, as described in WRC report K8/814.

Column feed was supplied by a peristaltic pump. A 15 x 400 mm Omnifit column (Separations, Randburg, South Africa) was packed with Amberlite XAD-16 resin, which is selective towards low M_w phenolic compounds. Outlet from the column was directed through the flow cell for monitoring *via* Labview. Conductivity and pH of the eluent was monitored using hand-held instruments, in order to measure the concentration of salts and organic acids coming off the column. Salts and organic acids are the main impurities in the crude extract, from which the phenolics need to be purified.

After column equilibration with water, crude extract feed was loaded onto the column until phenolics were detected in the outlet (breakthrough), after which the column was rinsed with water. Once salts and acids had been rinsed off the column and were no longer detectable in the outlet, the feed was changed to a methanol/water mixture to elute the phenolic compounds. UV detection was used to monitor the elution of these and they were collected for further analysis. Yield (compared to the loaded quantity) and purity of the final product was determined. The column was then cleaned with 100% methanol, re-equilibrated, and was then ready for re-use.

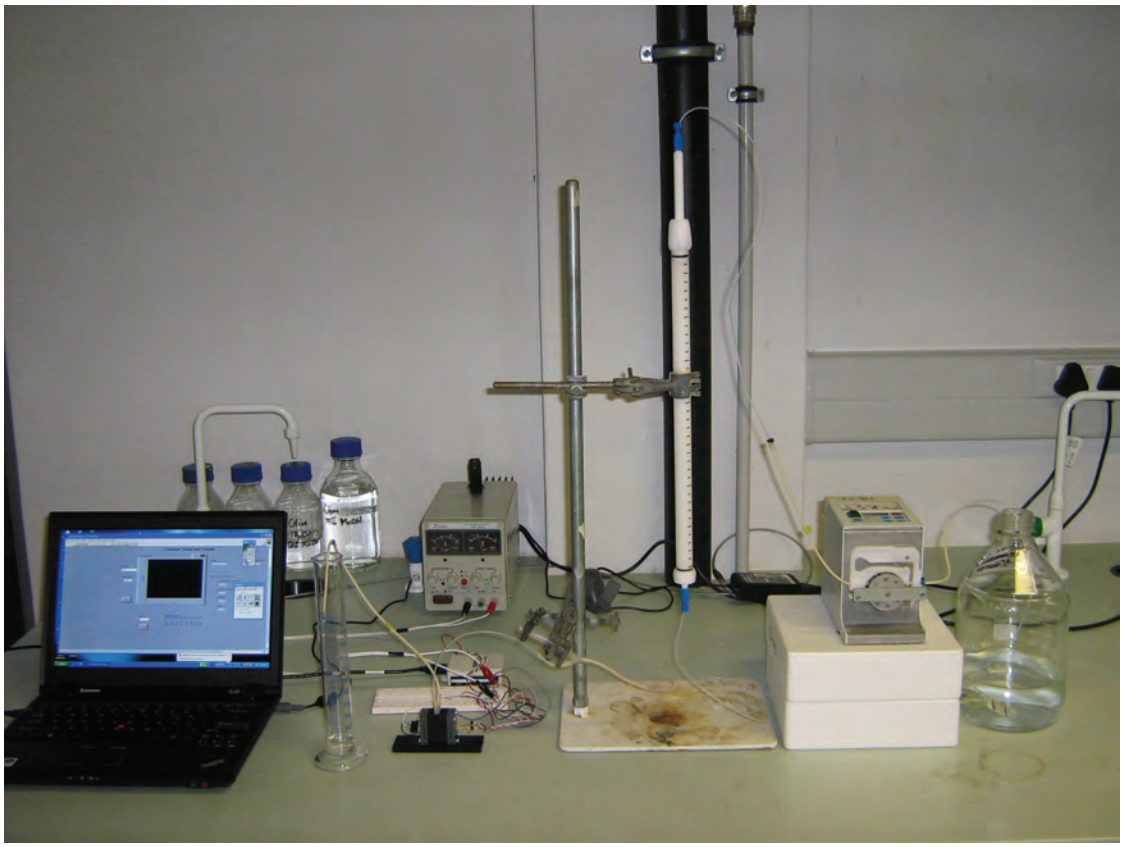

Figure 8: LED-photodiode detector attached to a column chromatography purification system

4.5 Optical density measurement of algal growth at 760 nm

During microbial cell culture, it is common to measure optical density at 600 nm (after calibration with cell dry weight) in order to determine cell density during the culture process. This works well for bacterial, yeast, and some fungal cultures, however, algae contains chlorophyll and carotenoids that have absorbance spectra across the visible range and would thus interfere with optical density measurements at 600 nm (see Figure 9). We therefore chose to measure optical density at 760 nm for the algae.

Cell density was continuously measured during an algal culture process in an aerated reactor by recirculating the growth medium through the flow cell using a peristaltic pump. Periodic samples were taken from the reactor to perform dry weight measurements, which could then be compared to the calibrated estimate from the LED-photodetector system.

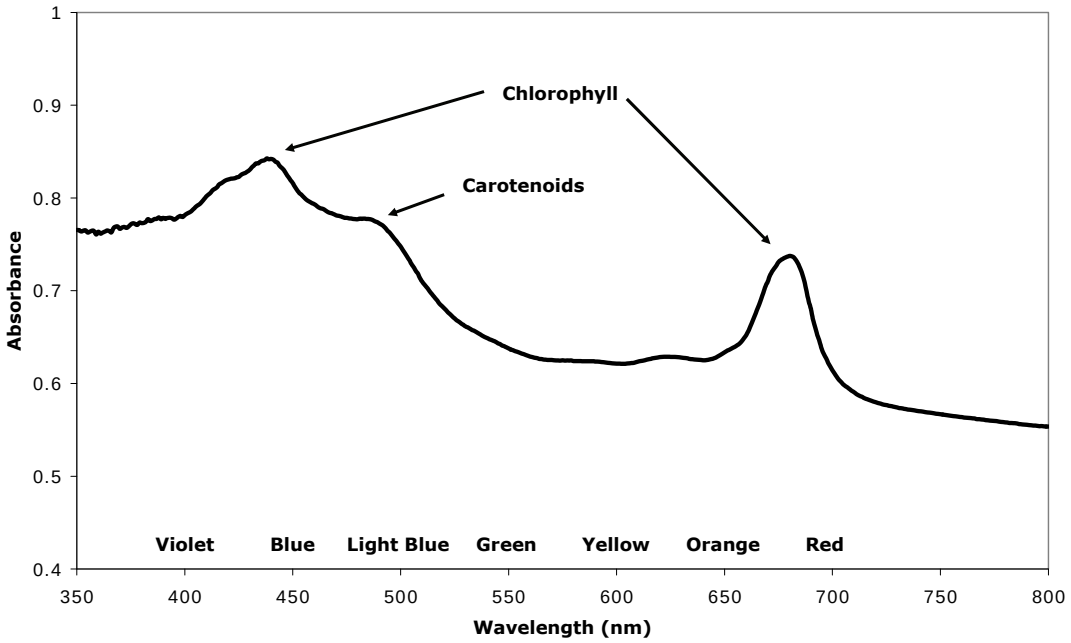

Figure 9: Averaged light absorbance spectra across a range of algal species

4.6 Nephelometry (scattered light detection) for simultaneous determination of algal growth and chlorophyll content

Nephelometric experiments of algal growth were performed in a similar manner to the optical density experiments, except that an additional photodetector was mounted in the flow cell holder at right angles to the original LED and photodetector, allowing for the measurement of scattered light. In addition, the LED was changed to 405 nm so that chlorophyll *would* interfere with optical density, i.e. the transmitted signal would be a function of cell density and chlorophyll content, while it was postulated that scattered light would be a function of cell density alone, i.e. independent of chlorophyll content. The difference between these two measurements should therefore give an indication of chlorophyll content.

In order to calibrate this system, algal cells were bleached by extracting the chlorophyll using dimethyl sulphoxide (DMSO). This caused the bleaching of the cells without disrupting them, while the extracted chlorophyll could be re-suspended up to the original volume from which it was extracted to allow separate measurement thereof. Original cells, bleached cells, and chlorophyll could thus be used for

independent calibrations, which would help to evaluate the contribution that chlorophyll content made to transmission and scattering measurements respectively. The effects of bleaching the cells and extracted chlorophyll are shown in Figure 10. Spectral scans are shown for the original cells, bleached cells, chlorophyll resuspended in both water and DMSO, and the extracted components recombined.

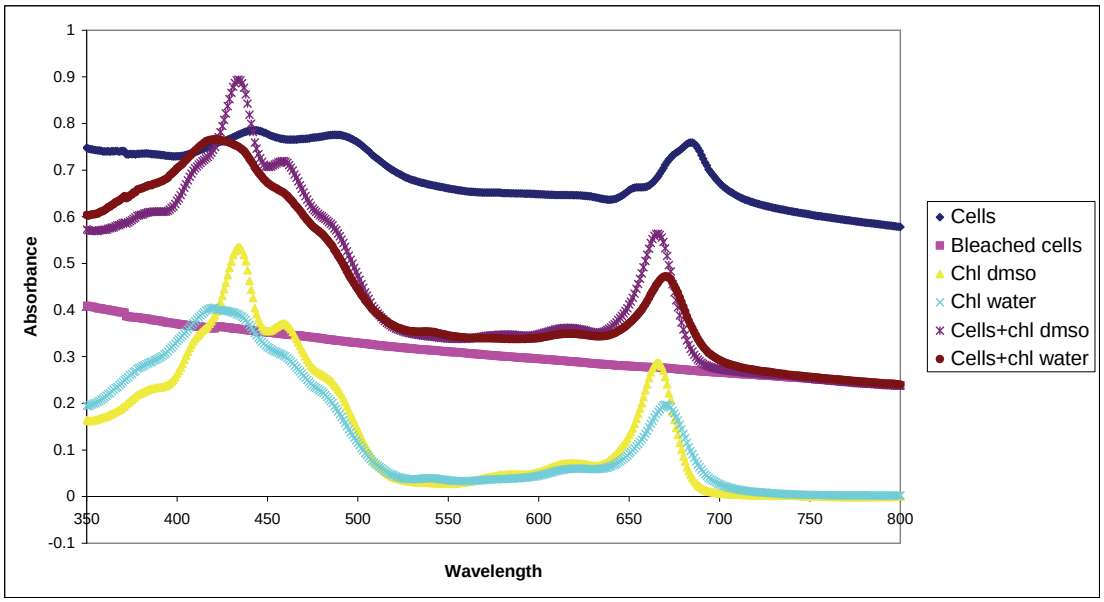

Figure 10: Effect of DMSO extraction of chlorophyll from algal cells upon absorbance spectra

It is evident from the above figure that chlorophyll was completely extracted, resulting in the pink curve of bleached cells showing an absence of chlorophyll peaks, while the chlorophyll extracts show no absorbance at 760 nm where optical density for the cells was measured.

4.7 Analytical procedures

De-ionised water from a Millipore system was used for all analyses. All experiments and analyses were performed at room temp of 25 ± 2^{o}C. All chemicals and reagents were supplied by Merck (Darmstadt, Germany), Sigma-Aldrich (Johannesburg, South Africa) or Fluka (Bellville, South Africa), and were of appropriate grade for the required purpose. Pure hydroxytyrosol was supplied by Cayman chemical (Michigan, USA). Total phenols were measured by spectrophotometric assay using the Folin-Ciocalteau reagent, using a modified method according to Garcia *et al.* (2001). Gallic

acid in the range 0-100 mg.L^{-1} was used as standard, and samples were appropriately diluted to fall within this range. A Helios Unicam UV/Vis spectrophotometer was used for assays and comparison to the LED-photodetector.

Phenolic compounds were analysed and accurately quantified by high performance liquid chromatography (HPLC) on a Beckman System Gold machine. The column was a reversed phase Phenomenex Luna C18(2) of 5 μm particle size and dimensions 250 x 4.6 mm. The mobile phase was $H_2O/CH_3OH/CH_3COOH$ in the ratio 80:20:2.5 at a flow rate of 1 ml.min^{-1}. UV detection was at 280 nm.

Organic acids were also measured by HPLC on the same system. The column was a Phenomenex Rezex ROA of dimensions 300 x 7.8 mm. The mobile phase was 0.005 N H_2SO_4 at a flow rate of 0.5 ml.min^{-1} with UV detection at 210 nm; relevant sodium salt standards were used for peak area quantification and retention times.

5. RESULTS AND DISCUSSION

5.1 Measurement of phenolic compounds and chromatographic purification thereof

UV calibration using gallic acid at concentrations up to 2 g.L^{-1} are shown in Figure 11. There is a distinct logarithmic curve up to this concentration, as predicted by the Beer-Lambert law. This data was obtained in a 0.1 mm path length flow cell, which is the shortest commercially available path length. In a standard spectrophotometer with a standard 10 mm cuvette it is only possible to measure these phenolic compounds up to a concentration of around 0.2 g.L^{-1}. The data shown is the mean of triplicate measurements with standard deviation.

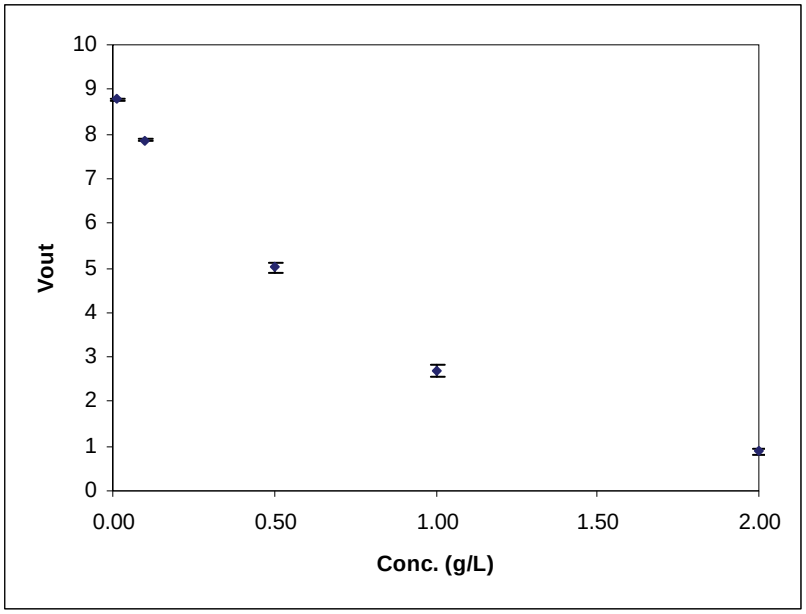

Figure 11: Logarithmic UV absorbance of gallic acid with increasing concentration shown as inverse absorbance (decrease) of photodetector voltage output

Linearisation of the voltage output ($\log_{10}$ V) according to Equation 1 results in the straight line shown in Figure 12. At 2.5 $g.L^{-1}$ there is significant deviation from linearity ($R^2 < 0.98$), indicating that 2 $g.L^{-1}$ is the upper detection limit for the system. This high concentration is adequate for monitoring of subsequent chromatographic purification.

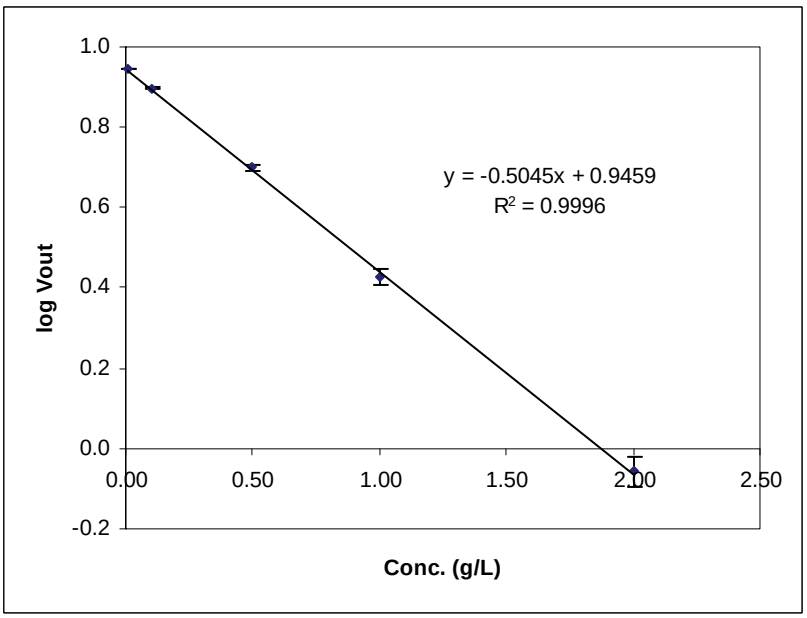

Figure 12: Linearised voltage output of gallic acid at increasing concentration

Figure 13 shows absorbance of the same gallic acid standards (at 1 in 10 dilution) measured in a spectrophotometer. It is notable that the linear regression coefficient was better for the LED-photodetector than for the spectrophotometer (0.9996 vs. 0.9975). Furthermore, accuracy and precision of the two instruments are shown in Table 1 for the different concentrations.

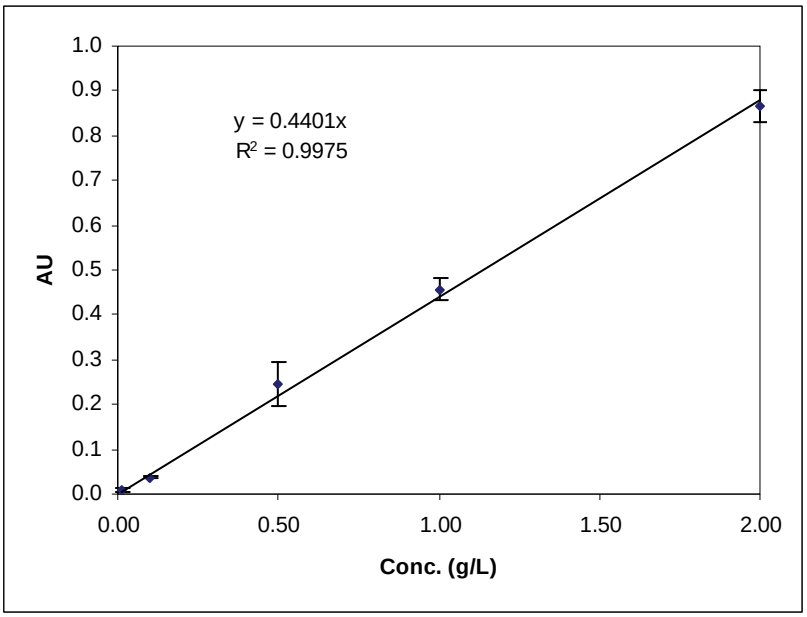

Figure 13: Spectrophotometer absorbance of gallic acid at increasing concentrations

Table 1: Accuracy and precision vs. concentration for different gallic acid phenolic standards

Conc. (mg.L^{-1})		**0.01**	**0.1**	**0.5**	**1**	**2**
Accuracy (%)	LED-photodetector	0.26	0.05	0.76	-3.15	-1.25
	Spectrophotometer	57.4	-16.8	10.6	3.70	-1.68
Precision (%)	LED-photodetector	0.05	0.27	1.32	5.01	-6.90
	Spectrophotometer	39.1	10.7	20.4	5.05	4.17

It is evident that in all cases both the accuracy and precision were better for the LED-photodetector than for the spectrophotometer. Accuracy and precision within ± 15% over the experimental range are considered to be acceptable (Wu *et al*. 2009); results shown above are well within these limits.

Next, the LED-photodetector was used for continuous monitoring of phenolic compounds (hydroxytyrosol) during a chromatographic purification process. Voltage

output from the photodetector was converted into absorbance units (AU) using the Beer-Lambert law based on the gallic acid calibration. Figure 14 shows an example of the phenolic profile of column output over time, while Figure 15 shows the pH and conductivity measurements during the same process.

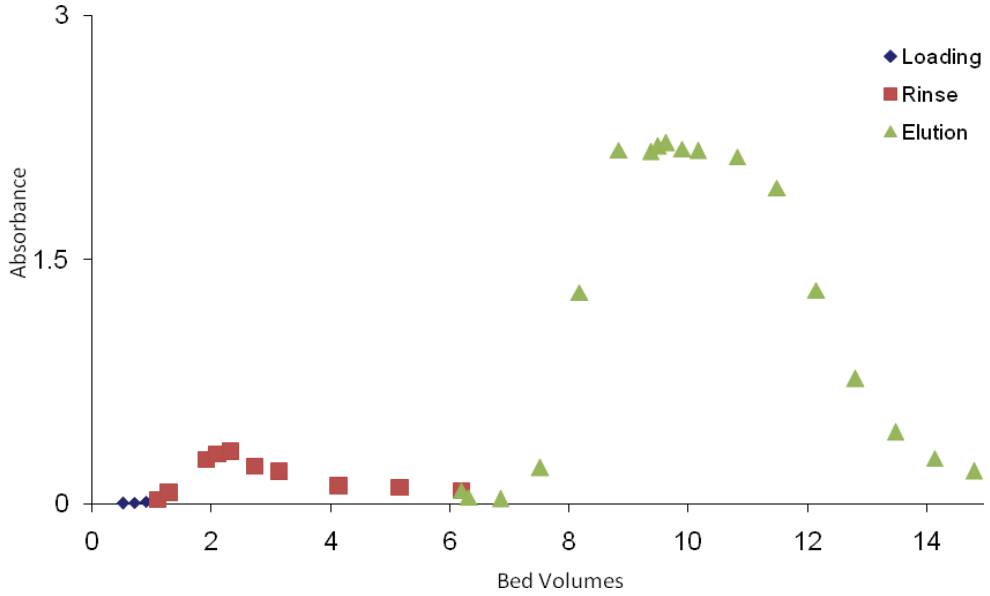

Figure 14: Hydroxytyrosol concentration profile in the outlet from a chromatographic purification process

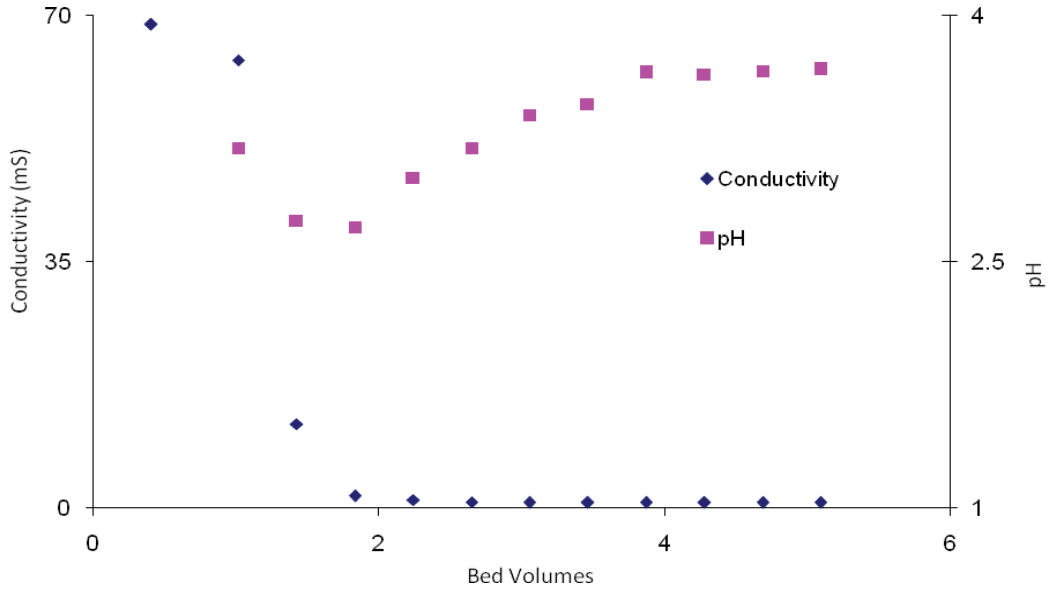

Figure 15: pH (acid) and conductivity (salt) profiles during the same chromatographic process

“Bed volumes” refers to the amount of liquid passed through the column equivalent to the chromatographic resin volume. It is a standard method of monitoring chromatographic processes, as it allows for comparison of the effects of different flow rates and other factors such as column size and dimensions.

With reference to Figure 14, feed containing the product to be purified is first loaded onto the column (blue dots). The column rapidly becomes loaded and product is detected in the outlet (i.e. breakthrough occurs) at around 1 bed volume; this is because the feed contained a high hydroxytyrosol concentration. The feed is then changed to rinsing water (red dots), upon which there is initially a continued rise in concentration as unbound hydroxytyrosol elutes off the column. More importantly, during this period the salts and organic acids rinse out the column, as shown in Figure 17. After approximately 4-6 bed volumes of rinsing the column is cleaned of salts and acids, and the feed can be changed to a methanol solution (green dots) as a result of which the bound hydroxytyrosol desorbs from the column and is detected at the outlet. These fractions are collected and distilled, and the preparative chromatographic purification is complete, resulting in a pure or semi-pure product. The column is then cleaned of residual impurities and polymerised phenolics using 100% methanol, after which it is ready for re-use. Phenolics and organic acids in the various fractions were subsequently measured by HPLC and total phenols assay, in order to verify results.

Using the above procedure, it was possible to obtain a 70% recovery of the hydroxytyrosol loaded onto the column at around 60% purity. These experiments are ongoing, in order to try and improve both recovery and purity, and to minimise solvent use.

The LED-photodetector and flow cell system has thus been shown capable of being able to accurately and continuously monitor phenolic compound concentrations, and there is no reason why it could not be used to monitor other phenolic process streams, such as textile or pulp and paper effluents, or refinery process streams. Besides for incremental improvements, the system has given no problems during operation to date, illustrating the robustness thereof.

5.2 Continuous optical density measurement of algal cell growth at 760 nm

Calibration for *Chlorella* algal cells at 760 nm is shown in Figure 16. As with the phenolic calibrations, triplicate standards were used for each concentration. Least squares linear regression was once again very good (error bars are included in the graph), while accuracy and precision were even better than in Section 5.1. Note that the concentrations shown here are low for the algae (up to 0.25 $g.L^{-1}$). This because the particular experiment for which subsequent data is presented was for slow growth under limiting conditions, which we thought would be a good test for the accuracy and precision of the system.

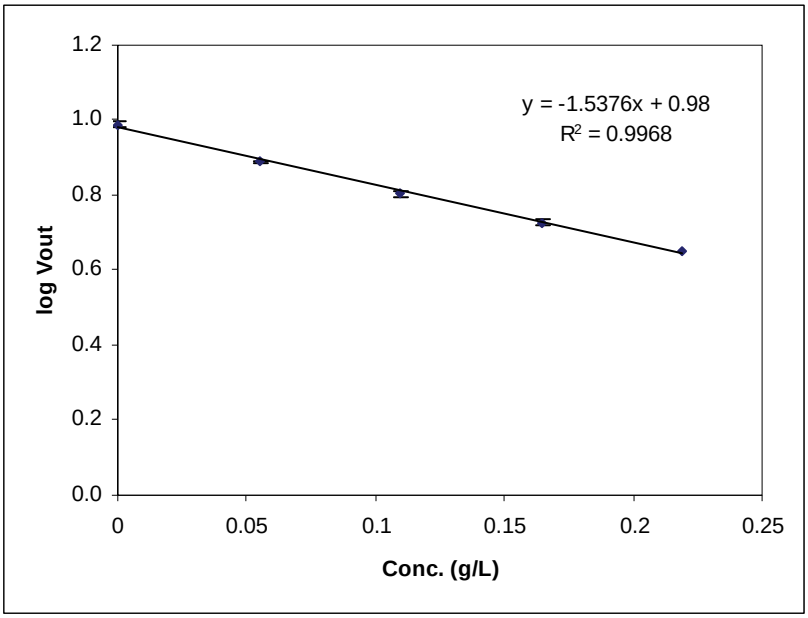

Figure 16: Calibration curve for *Chlorella vulgaris* at optical density of 760 nm

Figure 17 shows results over 5 days for the growth experiment. Data collected using the LED-photodetector is shown in blue, while the red markers show actual cell density measurements taken periodically. There is good agreement between the data, given that cell densities were so low. Typically, in optimal conditions this species will grow to concentrations in excess of 2 $g.L^{-1}$ over a shorter time. Unfortunately, the experiment was run over a period when several electricity failures were encountered (missing data). This also caused pump failure, resulting in settling of the algae within the flowcell, causing some erratic data. Nevertheless, the results are not unsatisfactory. Better data at the higher algal concentrations are expected, for which

smaller 1 mm path length flow cells will be used, along with the adjustment of the electrical circuit for intensity and sensitivity, and subsequent calibration.

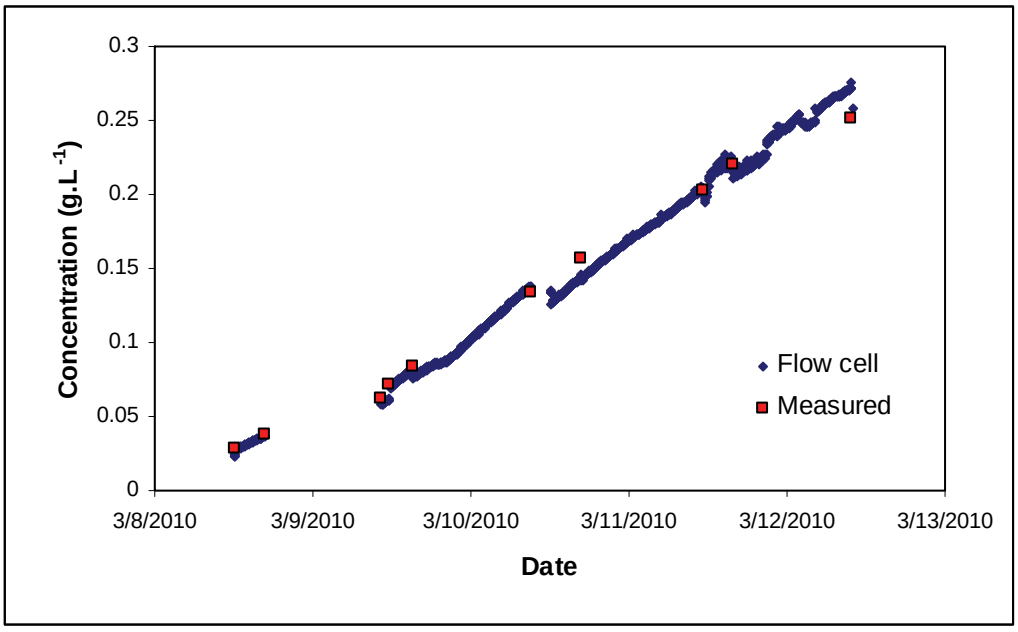

Figure 17: Growth of *Chlorella vulgaris* over 5 days with cell density measured both physically and using the LED-photodetector flow cell

There was no attachment of algal cells inside the flow cell during the course of experiments. Such attachment would cause erroneous concentration measurements. The experiments performed using Fluorovettes for algal measurement, on the other hand, were not so successful. The Fluorovettes have a very narrow flow channel leading to the optical window. This channel got blocked with algae, causing leaks in the system and obviously incorrect measurements, and thus the experiments were discontinued.

5.3 Nephelometry (scattered light detection) for simultaneous determination of algal growth (turbidity) and chlorophyll content

In order to attempt to determine cell density using scattered light another photodetector was fitted to the system, at right angles to the incident LED light. The LED was also changed to a wavelength at which chlorophyll absorbs, i.e. 405 nm (see Section 4.6). Hence, by measuring the difference in signals between the scattered light and the transmitted light, we attempted to obtain a measurement of chlorophyll

content in the cells. The experiments were performed with algal standards at different concentrations, and also with bleached cells and the chlorophyll extracted therefrom, made up to the same concentrations as the whole algal cells from whence it came. A 2.3 $g.L^{-1}$ algal culture was used as stock for these experiments; this was taken to be 100% concentration.

These experiments were largely unsuccessful for several reasons. Firstly, it is apparent that there are significant differences in extinction coefficients between chlorophyll and the algal cells themselves. Figure 18 shows light transmission measurements (as with all the above work). It is evident that chlorophyll accounts for nearly all the absorbance at this wavelength: the measurements for chlorophyll and whole cells containing chlorophyll are virtually the same, whereas the bleached cells have a much lower absorbance (i.e. higher relative voltage output from the photodetector). We were hoping to obtain a more cumulative situation where the absorbance from the bleached cells plus that from chlorophyll (semi-proportionately or even non-linearly) equalled the absorbance from the whole cells. This was not the case. We would need a larger difference (of extinction coefficients) between the whole cells and the chlorophyll to make the concept work.

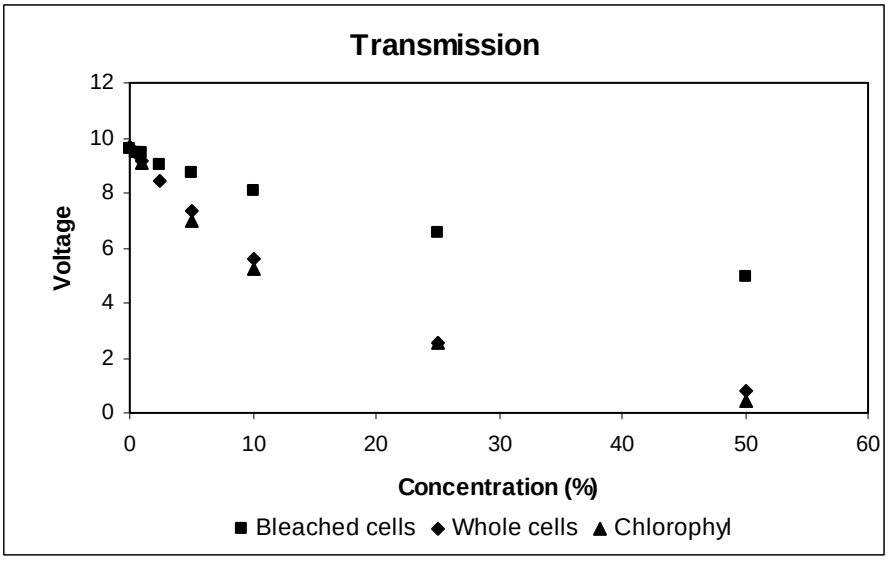

Figure 18: Absorbance of whole cells, bleached cells and chlorophyll at 405 nm

Secondly, chlorophyll also interfered with the scattered light signal, as illustrated in Figure 19. For the bleached cells, there was a near-linear response to of voltage

output with increased concentration. Note that the scattered light signal *increases* with increasing density (or turbidity): if there are no cells or particulate matter in the liquid then no light is scattered (*q.v.* Tyndall effect). However, when chlorophyll is present in the cells it absorbs light that has previously been scattered from other cells, and the signal attenuates at higher concentrations (> 25%). We were hoping to achieve the same amount of scattering irrespective of whether chlorophyll was present in the cells or not.

Chlorophyll on its own does not cause any scattering because it is not particulate (Tyndall effect again), in fact it caused less light to reach the scattering detector at the higher concentrations. This is explained by the fact that the LEDs are not perfectly unidirectional and polarised in their light output, and thus even at zero concentration there is some light reaching the detector and hence a non-zero voltage output; as the chlorophyll concentration increases this signal is also attenuated.

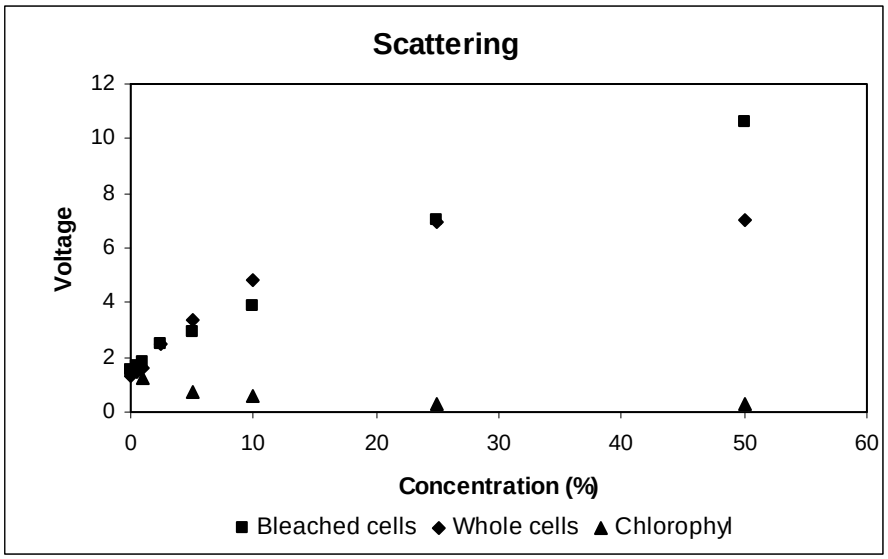

Figure 19: Scattered light intensity for whole and bleached algal cells, and chlorophyll

Despite the failure of these experiments, it has been shown that standard turbidity measurements using the system are indeed possible if the detector is mounted at right angles to the LED, as illustrated by the scattering obtained with the bleached algal cells. This work is being pursued, and will incorporate the use of proper nephelometric standards (formazin), as well as subsequent data analysis and curve fitting thereof.

In order to measure chlorophyll content in the algal cells we have decided that a different approach is warranted and will be investigated soon. Given that it is possible to measure cell density without the interference of chlorophyll at 760 nm, while chlorophyll itself absorbs strongly at 405 nm, we postulate that by using 2 LEDs alternately emitting at these wavelengths, we could take the two different responses and subject them to non-linear multi-component analysis in order to differentiate between the cells and chlorophyll. Using increasing concentrations of whole and bleached cells and chlorophyll for calibration as above should then allow for measurement of unknown concentrations and the relative proportions thereof.

6. COST OF THE SYSTEM

Cost of the system, including estimated manufacturing cost, is shown in Table 2. Clearly this is much lower than commercially available bench top spectrophotometers, which cost tens to hundreds of thousands of Rands. As such bench top spectrophotometers cannot generally be dedicated to one particular purpose, which was the rationale for developing this system. The most expensive components are the flow cells and the data acquisition card, both of which would also be necessary for continuous measurements using a conventional spectrophotometer. Here they are included in the total cost of a dedicated, purpose-built system.

Table 2: Cost estimate for an LED-photodetector based spectrophotometer

Component	**Cost (Rands)**	
	Visible range	UV range
LEDs	100	500
Photodetectors	200	1000
Cuvette holder and mounting	100	
Electronics	100	
Flow cell	2000	
Data acquisition card	2000	
Total	**R4500**	**R5700**

It is anticipated that the costs shown above could be reduced even further. LEDs and photodetectors become cheaper all the time as the technology develops. UV range components in particular are currently quite expensive compared to the visible range.

Furthermore, we have plans to construct our own custom-built flow cells that will incorporate LED and photodetector mountings; this could reduce the cost of this component by an order of magnitude.

7. CONCLUSIONS AND FURTHER WORK

The following conclusions can be drawn from the work presented in this report:

- Through an evolutionary process spanning two and a half years, we have successfully designed, constructed, and tested a low-cost LED-photodetector device for continuous on-line monitoring as an alternative to conventional spectroscopy.
- The device has high accuracy and precision and has been shown to be robust through extended laboratory use with few problems.
- The system has been shown to be capable of both UV and visible wavelength measurements, demonstrated by the monitoring of a chromatography process and the measurement of algal growth in photobioreactors.
- The system has been shown to be capable of nephelometric turbidity measurements as well as transmission (absorbance) measurements.
- Although the device is not capable of performing spectral scans, its utility at specific wavelengths is made possible by the wide range of LEDs capable of emitting light at a specific, narrow band. LEDs are inexpensive and it is no problem to change the wavelength to the desired value by replacement of the component. Photodetectors, on the other hand, have a broad spectral response and it is not necessary to change these.

Although this report marks the end of WRC-funded project K8/865, we have found such utility for the device in our laboratory that we plan to continue using and developing the system. The following developments and applications are imminent:

- The current system still needs to be packaged into a waterproofed unit. This was not achieved in the current project as we were still experimenting with

configurations and making other incremental improvements and modifications.

- We plan to roll out the system throughout our laboratory for continuous monitoring of bioreactor cell culture (algal, bacterial and yeast), as an alternative to the tedious periodic manual sampling and measurement that is currently performed. Negating the need for sampling should also improve the sterility of these processes. As the first step in this roll out, we plan to connect the system up to a parallel bank of 10 airlift photobioreactors used for algal culture. We will attempt to connect all the reactors to a single data capture card through a process of multiplexing in Labview. Components for this have already been acquired thanks to the funding from the current project.
- Using Labview, we plan to use the current system for feedback control of the chromatography process, and also for the membrane purification process described in WRC project K8/814
- We wish to design and construct a flow cell that is capable multiple wavelength measurement through the use of several LEDs in one device. This should enhance the capability of the system and make more advanced measurements possible, e.g. for the measurement of chlorophyll and cell density at the same time.
- We plan to investigate other low-cost, high-tech applications of the system, for example in the beer brewing process: monitoring of yeast cell density, liquid turbidity, and colour, at the same time using only one device.
- We are in the process of transferring the electronic circuit to printed circuit boards (PCBs). This will result in even lower cost electronics and smaller devices.
- Lastly, as the next evolutionary step, we would like to investigate fluorescence detection.

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Appendix A – Design drawings for cuvette holders and photodiode and LED mountings