Development of a Portable Toxicity Detector for Water

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DEVELOPMENT OF A PORTABLE TOXICITY DETECTOR FOR WATER

FINAL REPORT

Contract Report for the Water Research Commission

by

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DEVELOPMENT OF A PORTABLE TOXICITY DETECTOR FOR WATER

The Steering Committee for this project consisted of the following persons:

Dr M J Pieterse	Water Research Commission (Chairman)
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EXECUTIVE SUMMARY

The technology described in this report was developed to satisfy the need for a low-cost, portable instrument for the measurement and processing of extremely low light levels emitted by certain bioluminescent compounds. The instrument is to be employed to detect hazardous materials in surface and drinking water supplies in the laboratory and/or field rapidly, simply and cost effectively.

Bioluminescent reactions, which are adversely affected by hazardous toxic compounds, have proved viable for the detection of such compounds in water. However, the instrumentation utilized thus far to detect the low light outputs of such biochemical reactions, depending as it does on the use of photomultipliers, is energy demanding, fragile and expensive. Such instrumentation is not suitable for use in the field.

Experimental development work carried out at the DWT and the Industrial Electronics division of Production Technology resulted in a prototype, single channel detector (LUCID 1) which was capable of providing reliable detection of these biochemical light emissions.

Subsequently an optimized, dual channel instrument incorporating a microcomputer which could be programmed to provide automatic indication of toxic hazard in water samples, was fabricated (LUCID 2). The development was funded by the Water Research Commission.

The instrument comprises a detector cell having two identical light-tight chambers and two channels for measuring the extremely low light levels emitted by the biological or biochemical samples contained in two small vials inserted therein. Temperature control is affected by circulating water from a temperature controlled bath through the detector cell.

Unique analogue electronic circuitry provided sufficient sensitivity, resolution and signal-to-noise ratio to achieve a meaningful correlation between the LUCID 2 instrument and a standard laboratory luminometer.

A dedicated microcomputer unit measures the processed light signals from both channels and displays the light output from both channels on a 16 character Liquid Crystal display panel.

The instrument is interfaced with a dot matrix printer which depicts peak height and peak area for the light output from each sample. The presence of hazardous substances is evaluated using a software expansion capability which compares the light output of a test and control sample.

The instrument is self-contained, battery operated with built-in battery charger and of a size and weight suitable for field use.

Laboratory simulation experiments investigating the effects of six toxicants upon the bioluminescent output of the bacterium Photobacterium phosphoreum have indicated that the instrument is capable of detecting, within 15 minutes, levels of toxicity equivalent to those of the standard 96-h fish bioassay.

LUCID 2, therefore, detects toxic effects successfully and its level of sensitivity compares well with standard bioassays presently being employed.

INTRODUCTION

Several biological tests have been developed for determining toxicity in aquatic environments using fish, protozoa, algae and other freshwater and marine organisms. However, most of these tests are relatively long and expensive and often require the time-consuming propagation of test organisms. As a result there is a general need to develop rapid, inexpensive and, at the same time, sensitive tests to determine and monitor the toxicity of an ever-increasing number of complex chemicals being discharged to aquatic environments. A study of the literature indicates that no adequate instrumentation is available nationally, and probably internationally, for use in the field to establish toxic hazard in aquatic ecosystems simply, rapidly and by unsophisticated technologists in Third World situations which prevail in many parts of Southern Africa.

The technology described in this report, the development of which was funded by the Water Research Commission, is aimed at correcting this lack of appropriate means of toxic hazard detection.

BACKGROUND INFORMATION

One of the most important mechanisms of toxic action within living material is the poisoning of enzyme systems. The inhibition of enzyme activity by waterborne toxicants adversely affects natural metabolic processes in biological organisms the detection of which forms the basis for a number of systems evolved to assess the degree of aquatic pollution.

One rapid screening technique that has recently received attention is the bacterial assay (described Microtox) developed by Beckman Instruments Inc., which measures the decrease in natural light emission from the luminescent bacteria *Photobacterium phosphoreum* in response to a toxic effect upon the enzyme luciferase. The decrease in light output is expressed as a 5-minute median effective concentration (EC 50), that is the concentration that effects a 50

percent reduction in light output. Data obtained thus far demonstrates that the luminescent bacteria test provides an extremely rapid, simple test of toxicity with a precision equal to or greater than traditional fish toxicity tests (Curtis *et al*, 1982; Qureshi et al., 1982).

A measurable result of nearly all influences which affect the primary processes of photosynthesis is a change of the fluorescent light emission of a plant. This change of the fluorescence emission due to toxicants which affect or block the enzyme controlled photosynthetic pathways has been utilized to detect various levels of aquatic pollution. The fluorescent light emission test, due to the optical characteristics of photosynthetic pigments (chlorophyll fluorescence >660 nm), allows the measurement of the fluorescence of algae. The time from the dosage of a toxicant to a clear reduction in fluorescent light output from algae is very short (5 minutes) and the sensitivity of the test compares favourably with standard assays.

Techniques, therefore, based upon bioluminescence and algal fluorescence, have proved viable for the universal detection of toxic hazard in water. However, the instrumentation utilized thus far to monitor light output of such biochemical reactions, depending, as it does, on the use of photomultipliers and photo-electric cells, is both energy demanding and expensive, the Beckman Microtox instrumentation retailing at approximately R80 000 and 50c per test. Such instrumentation is not suitable for use in the field.

A new concept involving modern solid state electronic technology to detect light emissions has, therefore, been employed to solve this problem.

TECHNOLOGICAL DESCRIPTION

In order to utilize the enzyme inhibition effects, described above, it was required to measure accurately the extremely low light levels emitted and to detect fluctuations therein. Various photosensors,

normally used in applications such as this (including photomultipliers), were considered and rejected as unsuitable for portable field use because of their size, cost, fragile nature and their requirement for bulky, high voltage power supplies.

After experimenting with a number of solid-state photo-sensors, an OSI-5k type PIN photo-diode with integral transconductive amplifier was selected. Evaluation criteria included cost, size, supply voltage requirements, responsitivity and NEP (noise equivalent power).

A mechanical structure was devised (hereafter referred to as the detector cell), containing the photo-sensor (Figure 1, a) and a means of holding the liquid sample (b) in a light tight enclosure. A slotted disc (c), driven by a special low noise servomoter, chops the light emitted by the sample before it reaches the detector. This principle facilitates the processing of the extremely small resultant signal and improves the noise rejection characteristics. As can be seen in the accompanying function diagram, the signal is first amplified by a factor of approximately 10 000 employing two low noise stages (d). Next the signal plus noise is passed through a highly selective digital bandpass filter (e), which automatically tracks the signal within the noise. This is achieved through the use of a reference frequency extracted from the spinning chopper disc by means of a second photosensor (f) located in the cell. Referring to the functional diagram this reference frequency is passed through a pulse shaped circuit before being multiplied one hundredfold using a phaselocked frequency multiplier (h). The multiplier output serves as a clock signal for the digital filter thus continuously adjusting the filter's centre frequency to that of the chopped signal. The signal is filtered once more (i) and finally converted to a d.c. voltage proportional to its true R.M.S. value on a scale of 0 to 200 millivolts representing the intensity of the light emitted by the sample. This concludes the description of the detector and cell proper.

The detector's analogue output was connected to a multi-channel data acquisition unit incorporating a microcomputer which had been previously constructed for use in other experiments. Being programmable in BASIC language it was possible to configure the unit to continuously log the detector output and provide an automatic printout of the varying light intensity. Certain checks and operator prompts were incorporated so as to ensure a high degree of repeatability in the measurements. The accompanying flowchart shows the main programme features (Figure 2).

Laboratory tests showed the detector to have excellent sensitivity and noise characteristics (typical signal to noise ratio = 40dB) which were in fact beyond expectations. One serious problem remained, however. This had to do with static charge build-up on the window of the photo-sensor due to the rotating chopper disc. This had the effect of causing a spurious signal far in excess of the legitimate one and thus masking it to a large degree. This was solved most effectively by depositing a micro-layer of gold (thickness less than 1 micron) onto the photo-sensor window by means of a sputtering process in our Integrated Circuit production facility. The window remained transparent and showed negligible attenuation of the incoming light, while the low surface resistance of the gold layer completely prevented any static build-up.

LABORATORY SIMULATION TESTING

A prototype light emission detector unit (LUCID 1) was fabricated and interfaced to a data acquisition and control module in order to evaluate the concept under laboratory conditions and to compare its efficacy with that of a standard laboratory luminometer (LKB - WALLAC 1250).

LIGHT DETECTION

Enzymes are proteins which catalize specific chemical reactions under mild conditions. In certain cases the products of such reactions are

relatively easily identified, for example, firefly luciferase produces light by a bioluminescent reaction as follows:

$$E + LH_2 + ATP = E.LH_2AMP = PPi \quad (1)$$

$$LH_2AMP + O_2 = E.P. + AMP + CO_2 + light \quad (2)$$

where E = Luciferase; PPi = pyrophosphate
LH_2 = D(-) luciferin; ATP = adenosine triphosphate
P = Oxyluciferin; AMP = adenosine monophosphate

The amount of light produced is directly proportional to the concentration of substrate ATP.

The light detection capability of LUCID 1 was assessed utilizing an ATP Monitoring kit commercially available.

Picozyme F (United Technologies Packard) is an example of a highly purified firefly luciferase-luciferin mixture supplied in freeze-dried form, together with Trios-Mg buffer components and serum albumin. It is reconstituted by adding distilled water according to the manufacturers instructions. A pH of 7,7 is attained which is the optimum for luciferase.

In order to establish substrate dilution curves for ATP using Picozyme F, the latter was reconstituted in 100μl of distilled water and allowed to incubate at room temperature for 30 minutes. At the end of the 30 minute period, ATP in the range 10^{-6} to 10^{-5}M was rapidly added and the light output measured in a luminometer. Duplicate tests were performed on both a standard laboratory - model luminometer (LKB Wallac 1250) and on LUCID 1 (Figure 3).

Initial experiments showed that LUCID 1 was limited by a maximum output of 200 mV (Figure 4). This has been increased to 5000 mV (Figure 5) by a desentitization of the light recording device (such

that saturation is reached at 3,5 - fold higher light intensity) and the introduction of a scaling factor of 7 upon data output. This gives a final output (in millivolts) approximately equivalent to the LKB Wallac 1250 luminometer. Figure 5 indicates that the baseline output (electronic noise plus background light) and resolution have also automatically increased. Neither of the latter are significant, however, as the resolution is acceptable (0,5% of maximum output) and the baseline is easily corrected to zero upon data output.

The primary reason for the abovementioned change is an extension of the portable luminometers range. This will dictate using sufficient light - generating reagent to produce a suitably large light output, but will ensure a reduced error in detecting differences between control and inhibited enzyme - catalyzed reactions. Although every test for inhibition must be related to a matched control, LUCID 1 provides a system where the light output for the control is at least 1000 mV. In this way, even 10% inhibition of the reaction will give an output reduction of one to several hundred millivolts.

The light detection capability of LUCID 1 is, therefore, more than adequate for toxicity assessment and compares favourably with commercial luminometry systems.

TOXICANT ASSESSMENT

Bioassays were conducted by measuring the decrease in luminescence of the bacterium *Photobacterium phosphoreum* in response to a toxicant. Because the reaction is almost immediate, thus precluding the mixture of reacting substances outside the detector cell, a simple injection device was designed for LUCID 1. It involved sinking a threaded recess into the cap of the instrument. This recess communicates with the reaction vessel (cuvette) below, and with the outside (through a smaller cap) via fine-bore holes designed to take the needle of a syringe. A constant rate, semi-automatic syringe (Hamilton CR-700-200) was acquired for this purpose. It is sturdy, of medium weight and designed to delivery 10 - 200 μl. The syringe needle is pierced

through a silincone rubber septum (gas chromatography type) which lies in the abovementioned recess. In this way light is excluded from the recording chamber, but the reaction can be started and reagents rapidly mixed by injection of one or more of the substrates. The tests were conducted by injecting a set volume of bacterial culture into either a test or control solution whilst the data acquisition unit continuously logged the detector output printing results every 2 seconds. Six toxicants have been tested; Copper, Mercury, Cyanide, Arsenic, Phenol and Kelthane. The bacterial suspension (0,01 ml) was injected into either the test or control solution within the cuvette (100 µl adjusted to a salinity equivalent to 2% NaCl). At least four concentrations of each toxicant were employed and the concentration effecting a 50% decrease in light output after 5 minutes exposure calculated by interpolation. The effect of various concentrations of Arsenic (as Arsenate) upon bacterial light output is represented in Figure 6. The results obtained from these tests were compared with those using a Microtox toxicity analyzer system. (Curtis *et al.*, 1982; Qureshi *et al.*, 1982). The results expressed in Table 1 indicate that LUCID 1 performed better in this respect being more sensitive than the Microtox system, probably because the Microtox system employed reconstituted lypholized luminescent bacteria whereas we used fresh cultures in our tests.

LUCID 1, therefore, detects toxic effects successfully and its level of sensitivity compares well with standard bioassays presently being employed.

TABLE 1: EC 50 results of the bioassays compared with Microtox tests

Toxicant	Compound	Test Results LUCID 1 (mg/1)	EC 50 MICROTOX (mg/1)
Copper	$CuSO_4.\ 5H_2O$	0,05	0,07
Mercury	$MgCl_2$	0,05	0,08
Arsenate	$Na_2H.AsO_4.H_2O$	0,09	0,04
Cyanide	KCN	0,01	0,01
Phenol	C_6H_5OH	0,11	0,22
Kelthane	--	0,26	0,45

Initial results with LUCID 1 were, therefore, promising and proved the viability of the concept. A pre-production field testing unit has consequently been designed and fabricated and is fully described as Appendix 1.

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QURESHI, A.A., FLOOD, K.W., THOMPSON, S.R., JANHURST, S.M., INNIS, C.S., and ROKOSH, D.A. (1982) Comparison of a luminescent bacterial test with other bioassays for determining toxicity of pure compounds and complex effluents. In: Aquatic Toxicology and Hazard Assessment: Fifth Conference ASTM 766. J G Pearson, R B Foster, W E Bishop Eds. American Society for Testing and Materials. pp 179-195.

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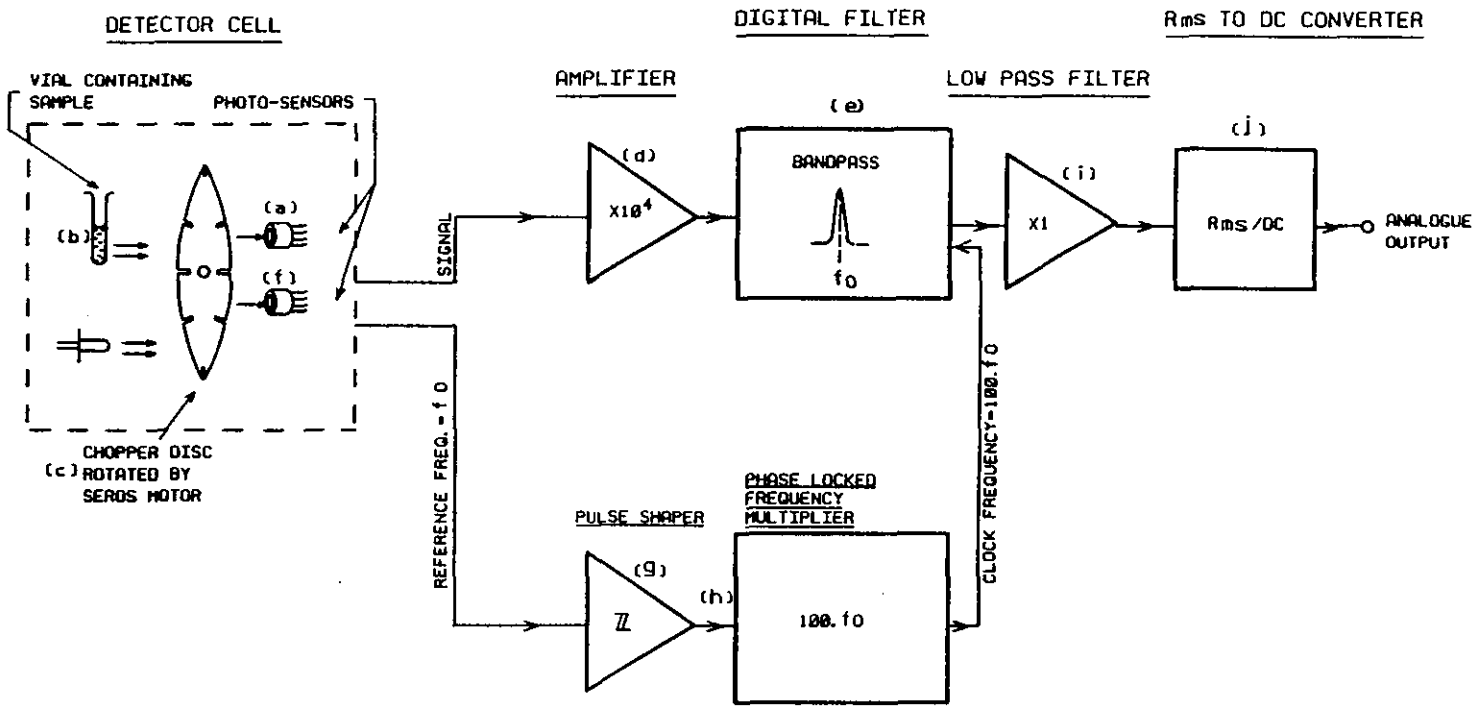


Figure 1. FUNCTION DIAGRAM OF DETECTOR CELL AND SIGNAL PROCESSING CIRCUITRY.

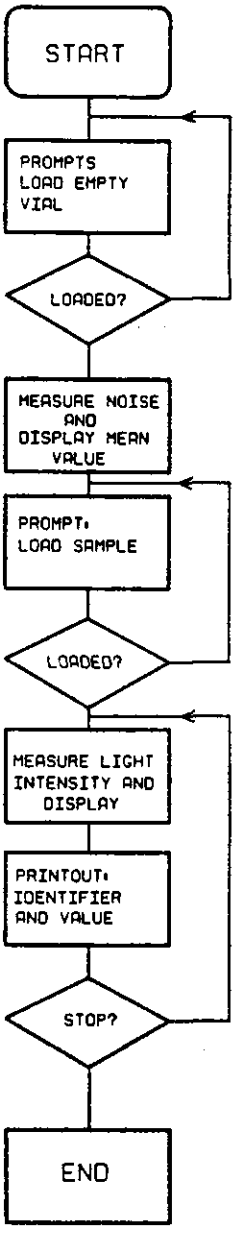


Figure 2. BASIC PROGRAM STRUCTURE

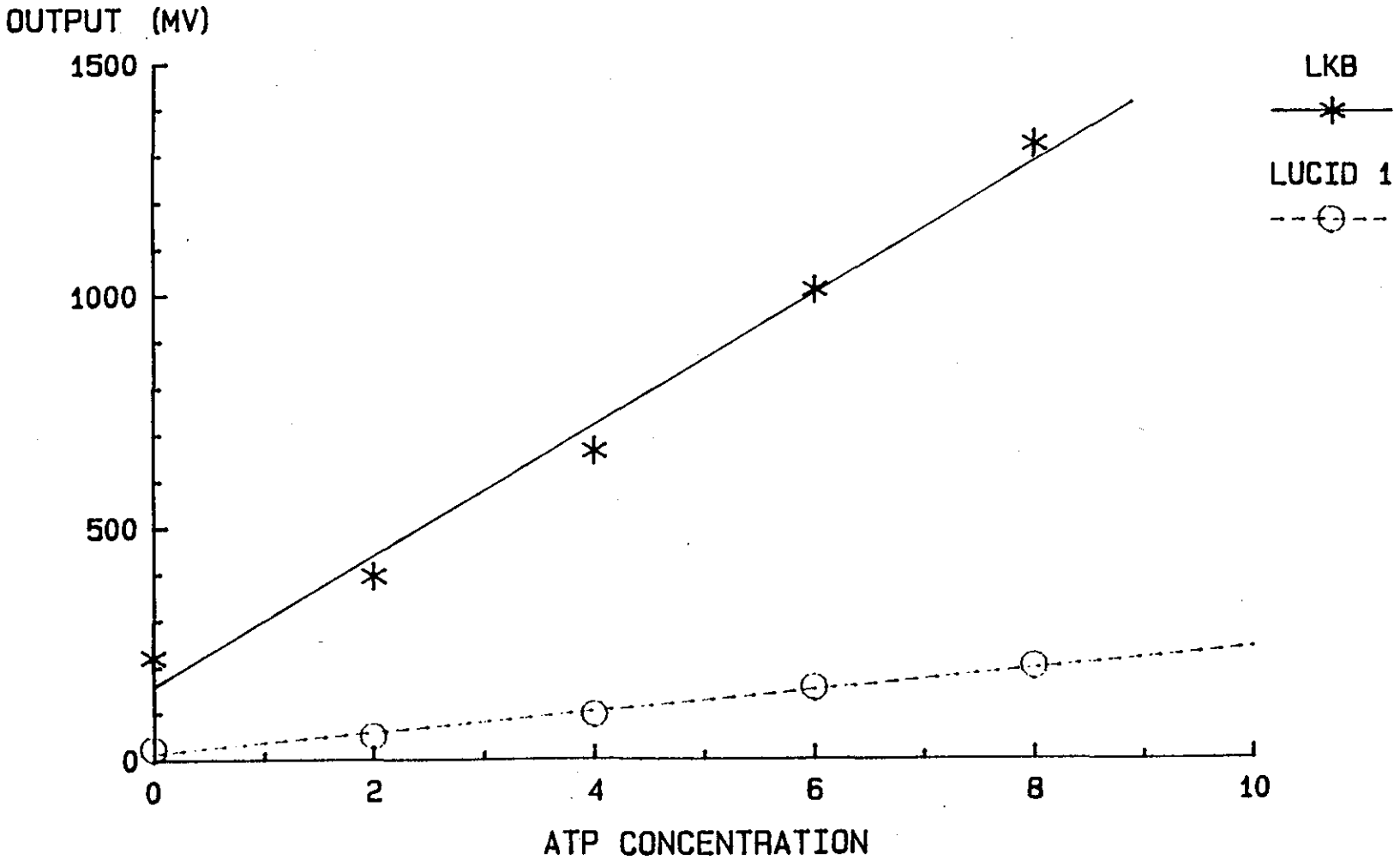


Figure 3. Substrate dilution curves (ATP) for **Picozyme F.**
Comparison of LKB Wallac and LUCID 1 luminometers

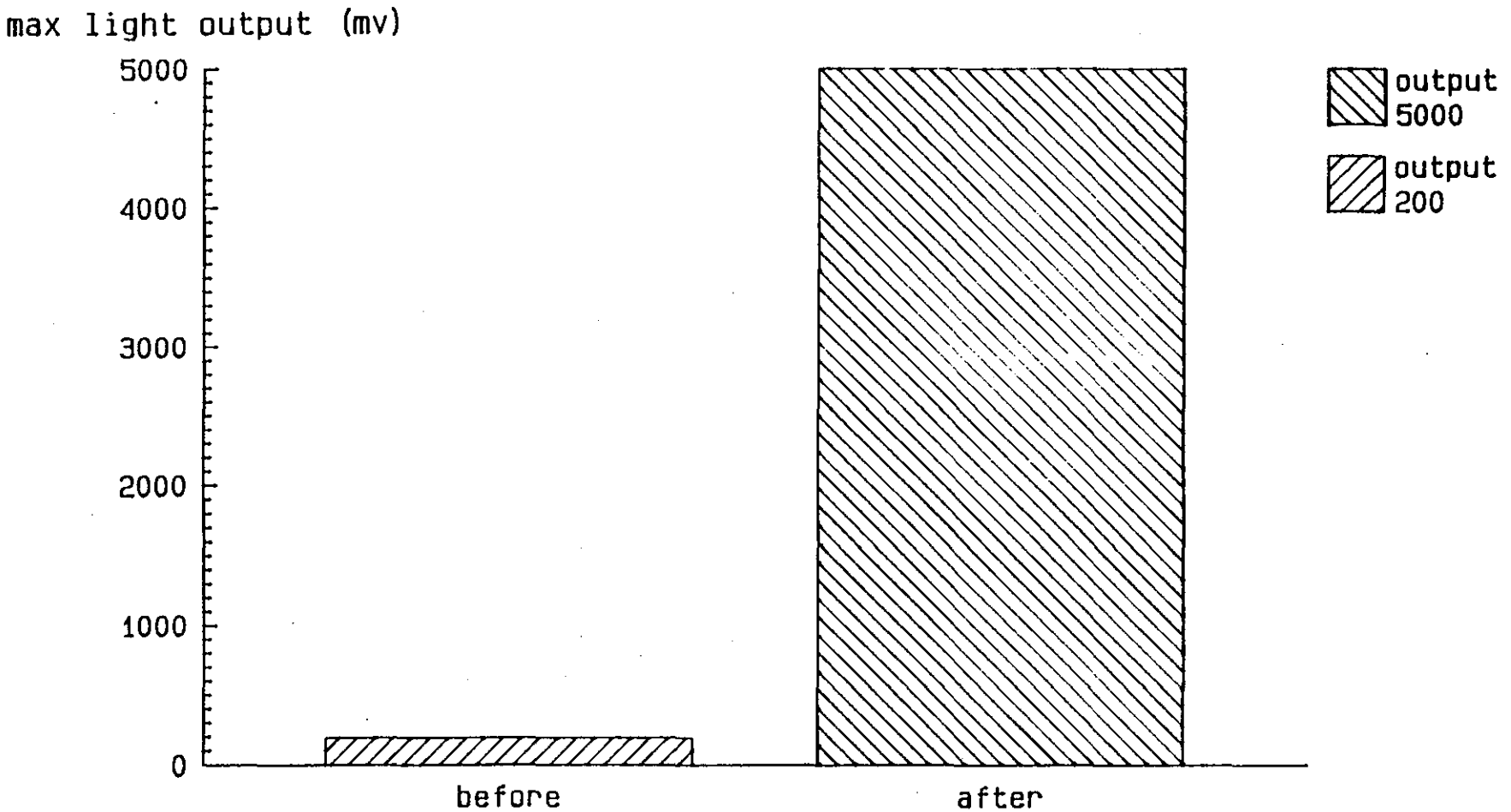


Figure 4. Comparison of LUCID 1 maximum output before and after modification

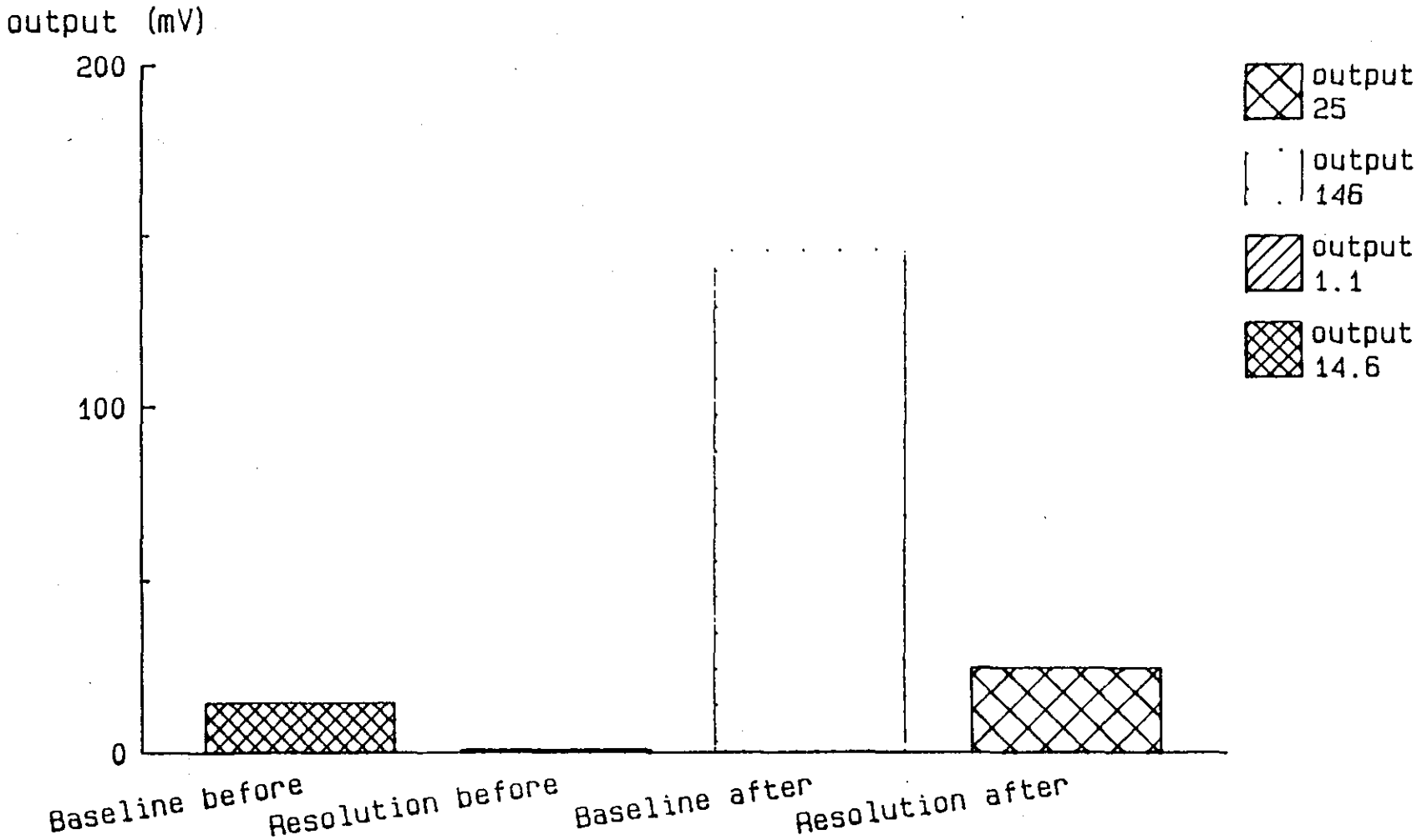
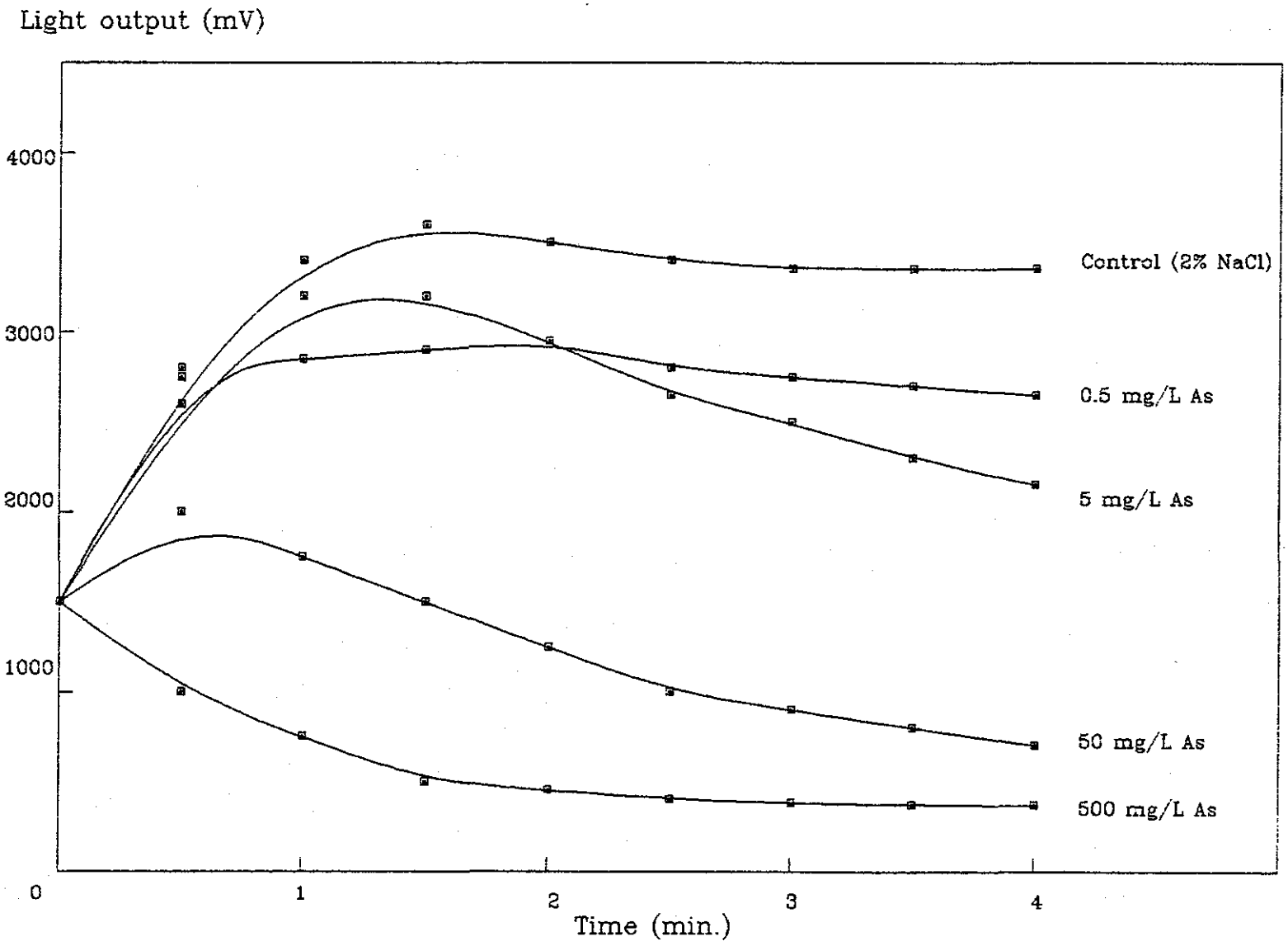


Figure 5. Comparison of LUCID 1 baseline output (electronic noise and background) before and after modification

Figure 6 The effect of different concentrations of arsenate upon light output of Photobacterium phospheoreum



APPENDIX 1

The development of electronic hardware and software for a low cost luminometer system for the detection of hazardous toxic substances in water.

For information on the electronic hardware and software contact Dr W S G Morgan, Division of Water Technology, CSIR, P O Box 395, Pretoria, 0001