Portable capillary electrophoresis system with LED-absorbance photometric and LED-induced fluorescence detection

Design, characterisation and testing

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Abstract

Capillary electrophoresis (CE) has a wide range of applications in the field of analytical chemistry. In general the most expensive part in a CE system is the detector due to the fact that the detector must have a high sensitivity for small detection volumes and low concentrations. Building portable instruments is one way to make the instruments cheaper and has the advantage that they can be used virtually everywhere. However, downscaling of CE instruments puts some extra demands on the detector. This report describes the design and building of two homemade light-emitting diode (LED) based detectors; a LED-absorbance photometric detector (LED-AP) and a LED-induced fluorescence (LED-IF) detector. The main goal was to install them inside a portable CE and make a simple separation.

The performance of the two detectors had to be evaluated before the main goal could be achieved. p-Nitrophenol was used to create a sensitivity graph for the LED-AP detector, calculating the upper linearity to 5.6 mM when the sensitivity had dropped 10 % caused by non-linearity. The sensitivity graph also showed that the detector had an effective pathlength of 74.2 µm and a stray light of 4.5 % for a 75 µm i.d fused-silica capillary.

The LED-IF detector was evaluated by determining the limit of detection (LOD) for fluorescein, at a signal to noise ratio of 3. The LOD was 0.72 µM± 0.01 µM when immersion oil was used to limit the light scattering from the optic fibres in to the capillary and 0.58 µM ±0.02 µM when silicone oil was used.

Without doing any improvements only the LED-AP detector could be used in the portable CE. As a common application area for portable CE instruments is environmental analysis, indirect detection using p-nitrophenol as a probe for separating anions was done to test the system. All analytes were eluted in less than 4 minutes.

Contents

1 Introduction

Capillary electrophoresis (CE) is a simple method for separating analytes and it has a wide range of applications as both charged and neutral species can be separated. CE has the advantage over other liquid phase systems because of its high separation efficiency, short run time, minimum operation cost, instrumental simplicity and compatibility with small sample volumes. Despite its many advantages over the classical analytical instruments, CE remains relatively unapplied in life science laboratories, oftentimes because of the cost of equipment.

Downsizing to smaller instruments and chips is one way to make the instrument cheaper, simpler and more user-friendly. Portable instruments have a lot of advantages because they can be operated anywhere. This means that samples can be taken and analysed “in-situ”. The need for transporting and storing samples before the analytes may disappear and the risk of degradation or contamination of the sample can therefore be reduced. There is one commercial portable CE system available, the CE-P2 from CE resources in Singapore. This has so far not been very popular and its battery only lasts for 2 hours. Research of homemade portable CEs has been done before (1-3).

Presently in a CE system the most expensive part is the detector. Detection is usually performed on-column. The injection volume is small and the capillaries internal diameter is only 50-100 µm, therefore the detector must have a high sensitivity for small detection volumes and low concentrations (4). Various detectors such as absorbance, fluorescence, conductivity, amperometric and mass spectrometic can be used.

In commercial capillary systems UV-Vis absorbance detectors are the most commonly used, the light source here consists of a continuum source such as a deuterium or tungsten lamp (4). The light is directed through a monochromator and the wavelength selected passes through to the capillary. Many compounds absorb at a specific wavelength and those that do not can be visualised by using light-absorbing species (probe) in the background electrolyte creating a decrease in signal when the analytes pass the detector, this method is called indirect detection (4). Determination of the most suitable probe for a particular analysis depends on the type of analysis that is to be performed. An evaluation of probes for use in capillary zone electrophoresis has recently been presented (5).

Among all detectors available, fluorescence based detectors have the highest sensitivity. Non-fluorescing compounds are usually labelled with a fluorescent tag, but indirect detection using a fluorescent probe can also be done. One of the most common fluorophores used is fluorescein. Flourescein is water soluble, stable and relatively cheap. Research about fluorescence detectors has increased in the last few years, the main reason being that it is well suited for routine analysis in biochemistry, for example, the analysis of proteins and DNA (6-12). Lasers are most commonly used as excitation sources because of their brightness and spatial beam properties, meaning the light is very well focused on a small point (4). Laser induced fluorescence (LIF) has the advantage over UV-Vis detectors in that it has lower background noise, increased selectivity and higher resolution. However, lasers are generally expensive, have short lifetimes (≈3000 h) and they are relatively bulky.

The literature contains many references to in-house designed detectors both absorbance (13-15) and fluorescence (12, 16-19). There are also many references to designs that combine these two detectors (20-22). In recent years, light emitting diodes (LED’s) have become a more commonly used alternative to lasers and deuterium lamps. LEDs have a long lifetime (>10.000 h), reasonably high intensity and good output stability. Besides these characteristics, they are also small and cheap. They are very suitable for small, portable

systems because they can be powered with a battery. When using LEDs, the emission wavelength of the LED has to be carefully chosen in order to match the absorbance/excitation wavelength of the solution. Previously, the limited wavelength range of available LED's affected the scope of their usefulness in this application. Now-a-days, LED's are available in a wide range of wavelengths from 250 nm – 1000 nm.

In the past it has not been possible to use UV-Vis-absorbance or fluorescence detection in portable instruments, because the detectors were too large and power consumption was high because of the light source. The introduction of LEDs and small battery powered detectors has changed the situation.

This study presents a portable CE and two different in-house built detectors: LED absorbance photometric detector (LED-AP) and LED-Induced Fluorescence (LED-IF) detector. An evaluation of the detectors respective capacity in terms of limits of detection and linearity had to be made before the main goal, (getting the detectors to work inside the portable CE), could be achieved. The detectors also had to be user-friendly and standard operation procedures (SOPs) were therefore drawn up as the study developed. Portable instruments are useful for environmental analysis, therefore the choice was made to analyse anions using indirect detection with p-nitrophenol as the probe (5).

2 Basic principles of capillary electrophoresis

CE is an analytical technique that uses the differing migration time of analytes in an electric field to separate them from each other (4). Fig. 1 shows the basic configuration of a CE instrument.

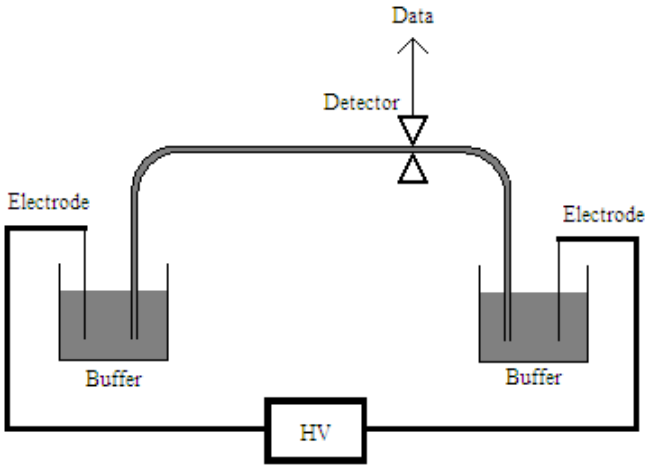

Figure 1: Schematic configuration of a capillary system.

The ends of a fused-silica capillary are placed in buffer reservoirs, the contents in both the reservoirs and the capillary are the same. The reservoirs also contain electrodes to generate contact between the high voltage power supply and the capillary (23). A detector is situated at the end of the capillary and the detection is usually done directly through an optical window on the capillary. Table 1 contains different detection methods and their advantages/disadvantages.

Table 1: Methods of detection (23).

Method	Advantages/Disadvantages
UV-Vis absorption	• Universal • Diode array offers spectral information
Fluorescence	• Sensitive • Usually requires sample derivatisation
Laser-induced fluorescence	• Extremely sensitive • Usually requires sample derivatisation • Expensive
Amperometry	• Sensitive • Selective but useful only for electroactive analytes • Requires special electronics and capillary modification
Conductivity	• Universal • Requires special electronics and capillary modification
Mass spectrometry	• Sensitive and offers structural information • Interface between CE and MS complicated.
Indirect UV, fluorescence, amperometry	• Universal • Lower sensitivity than direct methods

In CE only small volumes of samples are injected into the capillary. There are two types of injection that can be used (23); 1) Hydrodynamic injection is done by switching the inlet reservoir to the sample vial and applying pressure at the injection end of the capillary, vacuum at the exit end or siphoning by elevating the sample vial or lowering the end reservoir, 2) Electrokinetic injections are performed by switching the inlet reservoir to the sample vial and then applying high voltage over the capillary, the field strength when injecting is usually a bit lower than the voltage used for the separation (24).

When the sample has been injected the capillary is moved back to the buffer reservoir and a high voltage of up to ±30 kV is applied. When the voltage is applied electroosmotic flow (EOF) appears. Electroosmotic flow is the flow of the liquid inside the capillary (24). EOF appears because the negatively-charged wall in the fused silica capillary attaches positively-charged ions from the buffer creating a double-layer at the wall, Fig. 2 (4). When the voltage is applied the positively-charged ion layer starts moving towards the negative electrode carrying the buffer in the capillary with it. The EOF is stronger than the electrophoretic migration of almost all species, causing the species, (regardless of charge) to move in the same direction using the migration only to separate them (24). EOF is easily controlled and changed by the composition of the buffer. For example a cationic surfactant can be added to the buffer to reverse the EOF making it go towards the anode instead.

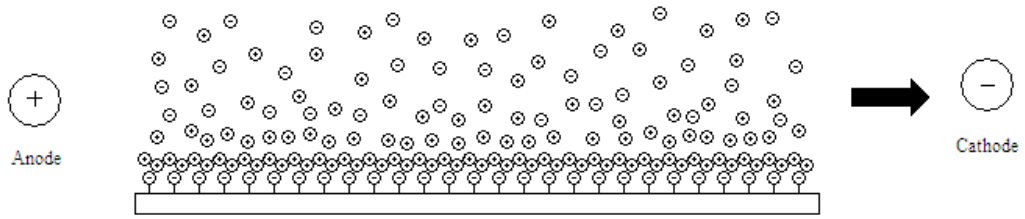

Figure 2: Electric double layer creating EOF (4)

3 Experimental

3.1 Instrumentation

3.1.1 Design of LED absorbance photometric detector

A black nylon holder, made to hold an Agilent alignment interface as previously described (13) was used as the base for the detector. The outline of the detector cell is presented in Fig. 3.

An integrated photodiode and amplifier (OPT301, Burr-Brown, AZ, USA) was used as a detector. Using this photodiode, it is possible to detect a range of wavelengths from 250-1000 nm. Connections were performed according to the basic circuit connections in the datasheet enclosed with the photodiode (25). Two 0.1 µF decoupling capacitors (Maplin Electronics, Ireland) were located close to the input voltage pins to ensure that the voltage is kept stable and to minimise the noise.

A high power ultraviolet LED (HUVL400-510B, 400 nm 2800 mcd, Farnell UK) was used as a light source. The LED was placed in a small holder with a 2 mm hole drilled in the bottom. Both the LED and the detector were powered by a power supply capable of delivering ±15 V at 400 mA made by Prof. Peter Hauser (University of Basel, Switzerland). A 10 kΩ 22-Turn Cermet Preset Potentiometer (Maplin Electronics, Ireland) was placed on the cables to the LED to make it possible to change the current. All cables used were basic equipment wire 16/0.2 bought from Maplin Electronics (Ireland).

The output voltage from the detector is proportional to the incoming light *i.e.* the voltage (signal) increases with increasing light. The output voltage was fed to a recording system (e-corder, eDAQ, Australia) through a coax cable. A PC with e-chart software (ADInstruments, New Zealand) was used to process and record the data.

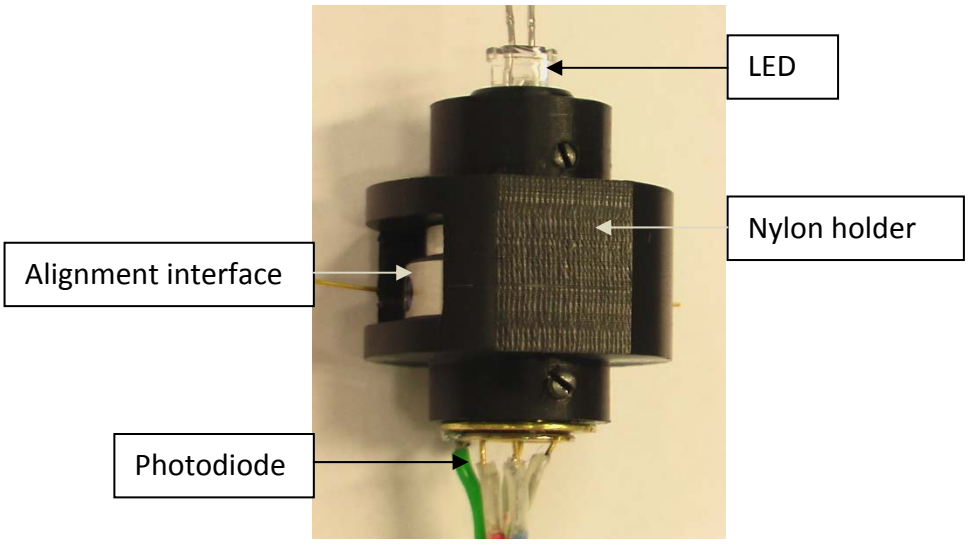

Figure 3: Photo of LED-AP detector cell.

3.1.2 Design of LED-induced fluorescence detector

The LED-IF detector was designed by Dr. Frantisek Foret (Institute of Analytical Chemistry, Czech Academy of Sciences, Brno, Czech Republic). The detector cell consists of two metal squares 5 cm × 5 cm held together with two screws (Fig. 4).
Inside there are 6 metallic holders for the capillary and the pick-up fibres. The pick-up fibres are optical fibres made of fused silica from Polymicro Technology, 300/330/370 µm (core/cladding/(buffer)coating) numerical aperture 0.22. There are two different holding tubes in the detector cell for the pick-up fibre, one is connected with a 45 degrees angle to the incoming light and the other with an angle of 90 degrees. To protect the capillary and the pick-up fibre, a square rubber gasket is placed in between the metal squares. Black tape is used to make sure no external light shines through the rubber to the detector cell.

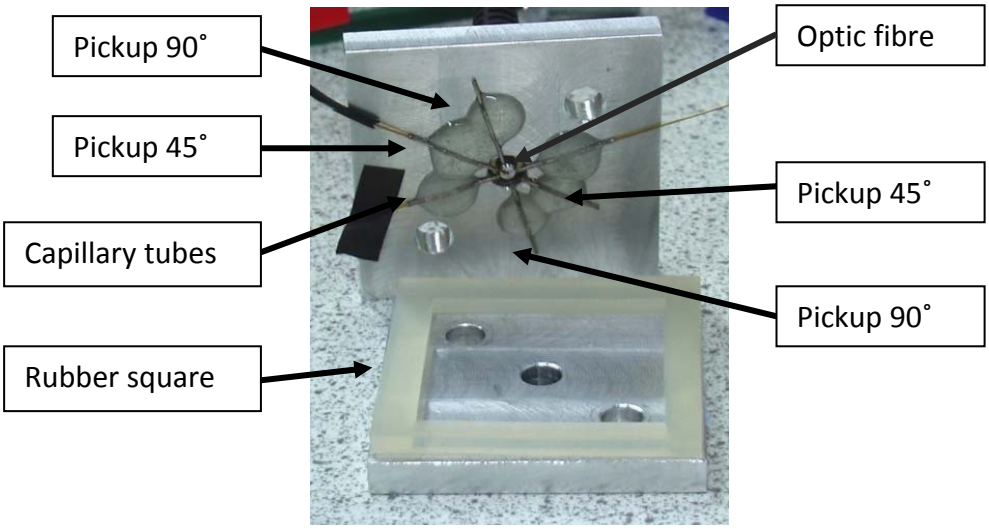

Figure 4: Photo of LED-IF detector cell

Emission light is brought from an LED to the detector cell through an optic cable (Polymicro technologies LLC, AZ, USA) using SMA Fibre Optic connectors (Timbercon, OR, USA). The LED (470 nm, 3000 mcd) is mounted on an electric board with a constant current of 30 mA. The pick-up fibre transfers the light to the detector box. This box was originally built to measure absorbance and therefore it doesn't have a log converter, which means the more fluorescence the lower the signal. The signal is transferred through a coax cable to an e-corder (eDAQ, Australia) and a PC with e-chart software (ADInstruments, New Zealand) was used to process and record the data.

3.1.3 Design of capillary electrophoresis instrument

The CE was delivered in parts from Prof. Peter Hauser (University of Basel, Switzerland) and the design has been described previously (1-3). A brief description of the instrument is given below. The instrument was housed inside a Perspex box with dimensions 310 mm × 220 mm × 270 mm (w × h × d) and the electronics were located in an aluminium case attached to the left side of the box. Photos of the instrument are shown in Figs. 5 and 6.

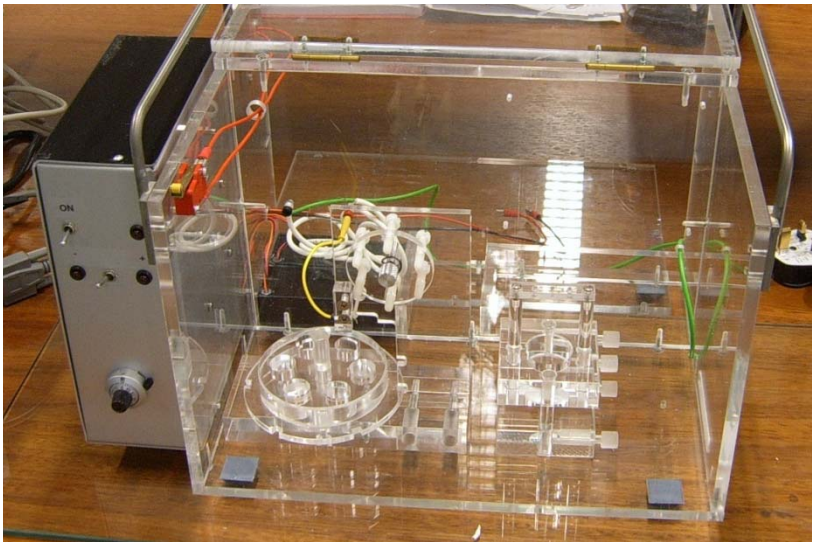

Figure 5: Side view of CE, without battery and detector.

Power to the instrument was provided by a 12 V lead battery (Yuasa NP 3.2-12, Yuasa Battery Ltd. UK), the battery can be recharged without being disconnected, using the connectors on the back of the box. The operating time for the battery was about 5 h. An external power supply capable of delivering 9-18 V can also be used. The supplied power was used to feed two high voltage modules (DX150, DX150N, EMCO High Voltage Corporation, CA, USA) capable of delivering a voltage of either +15 kV or -15 kV. Changing the polarity was easily done manually by switching between the modules. The separation voltage was adjusted with a 10-turn potentiometer. The high voltage modules and the battery were contained in a separate section in the back of the box. For safety reasons, a switch on the front lid interrupts the high voltage when the lid was opened.

In the front section of the box there was a sample turn-table with six vial positions, a support for the high voltage electrode, a spool to roll up the capillary and a detector stand containing one vial position for the buffer. The sample table could be moved manually and injections were preferably done electrokinetically but could also be done hydrodynamically. The high voltage electrode consisted of a 0.51 mm platinum wire (W219, Scientific Instrument Services, Inc, UK). The detector stand was made from two pieces, the lower to hold the buffer vial and the upper to fasten the detector. Both parts could be adjusted in height independently of each other, the lower part could be adjusted to the same buffer level as the sample tray to prevent siphoning and the upper part could be moved to change the distance from the detection site to the end of the capillary.

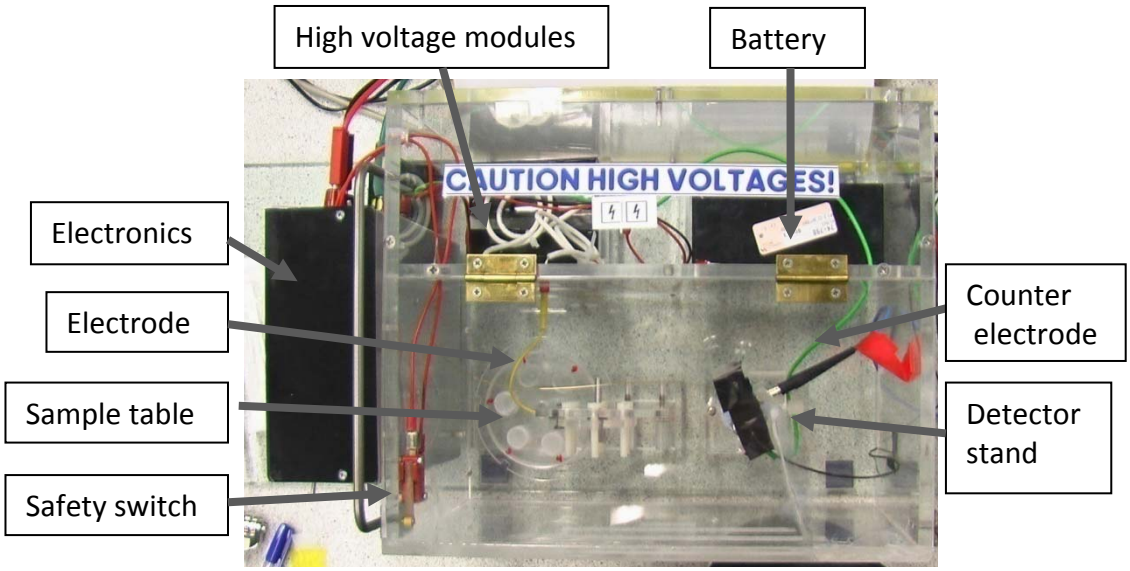

Figure 6: Top view of portable CE. The LED-IF detector is installed.

3.2 Chemicals

All chemicals used were purchased from Sigma Aldrich (Ireland) and were of analytical grade. If not stated otherwise the dilutant was water from Millipore, Milli-Q purification system (Bedford, MA, USA). All solutions were filtered through a filter (4 mm Syringe filter, 0.2 µm Nylon Membrane, Whatman, USA) before being injected. Manual flushing of the capillary with NaOH (5 min), Milli-Q (5 min) and buffer (10 min) was done daily.

3.3 Capillary electrophoresis method

The in-house made CE instrument was equipped with the LED-AP detector described below. Indirect detection was done using 9.5 mM p-nitrophenol and 38 mM diethanolamine as background electrolyte with tetradecyltrimethyl ammonium bromide (TTAB) added with a final concentration of 0.24 mM to reverse the electroosmotic flow. A fused-silica capillary with the total length of 50 cm, effective length of 43 cm and internal diameter of 75 µm was used. A detection window was formed in the capillary by burning off the protective outer polyimide coating.

Different anions (MnO_4^-, HCO_3^-, I^-, NO_3^-) at a concentration of 10 ppm were used as samples. Injections were done electrokinetically for 4 seconds at -15 kV. Potassium was the counter ion for all anions used. The baseline stability and the separation time for peaks were used to evaluate the performance of the CE.

When the CE was not used the injection of the different samples was carried out using a Lambda Multiflow Peristaltic Pump (Lambda Laboratory instrument, Zurich, Switzerland) at 0.15 µl/s, the measurements were made using the average function in the e-chart software on the signal when it had stabilised. To minimise carry-over errors the measurements were done from low to high concentrations.

3.4 LED-absorbance photometric detector measurements

For the LED-AP detector the measurements were done using a 100 mM p-nitrophenol stock solution (probe), 200 mM NaOH was used for dilution of the p-nitrophenol to ensure that it was in ionic form. A series of p-nitrophenol standards were prepared by serial dilution 1:1 with 200 mM NaOH. A series of solutions from 100 mM - 0.39 mM were prepared, giving a total of 9 samples. NaOH was used as a blank sample.

To be able to compare the detector with other absorbance detectors a few parameters had to be determined. One of the main parameters was the linearity of the detector. In this project a method using sensitivity graphs was explored (26; 27). The absorbances off the series of standard solutions were measured and the sensitivity (absorbance/concentration) was calculated. Results were shown as a graph where sensitivity was plotted against absorbance. From this graph it was possible to calculate the stray light (light that reaches the detector without passing through the sample) and the effective pathlength (effective average of all individual pathways the light can travel through the capillary). These values are a characteristic for the detector and can be used to evaluate the setup. The values are also independent of the absorptivity of the probe, but can give a good evaluation of the probe and its applicability in CE (26).

The output from the detector was given in Volt, and to calculate the absorbance the Beer-Lambert Law was used with modification so that the absorbance $A = \log(V_0/V)$.

3.5 LED-induced fluorescence detector measurements

The performance of a fluorescence detector is usually compared with its ability to measure small concentrations of analytes and the concentration range for linearity. For the LED-IF detector two stock solutions of fluorescein were prepared by diluting with 25 mM borate buffer (pH 8.18). 0.01 mM fluorescein was used to determine the limit of detection (LOD), but a 0.4 mM fluorescein solution was used for the linearity measurement. From the 0.4 mM stock solution, standard fluorescein solutions were made by diluting with 25 mM borate buffer (pH 8.18) 1:1 according to the same principle as for the absorbance detector giving a total of 9 samples from 0.4 mM to 1.6 µM. Borate buffer was used as a blank sample.

The linearity was evaluated by measuring the fluorescence for the series of standard solutions, which was then plotted against the concentration. A small drop of oil was used on the capillary's window and the tip of the optic fibre to help focus the light and minimize the light scattering. To investigate if the oil had any influence on the measurement, the LOD determination was done twice with two different oils; immersion oil for fluorescence microscopy and silicone oil for use with gas chromatography (GC). The limits of detection were estimated by calculating the concentration when the signal to noise ratio S/N equals 3.

4 Results and discussion

Standard operating procedures (SOPs) for the instruments can be found in Appendix 1, 2 and 3.

4.1 Evaluation of the LED-absorbance photometric detector

To reduce interference from the mains (typically 50 or 60 Hz) like ground loops, multiple pieces connected to mains power ground or unshielded power cables, the mains filter function in the e-chart software was used. The difference in noise can be seen in Fig. 7. To make sure the mains filter doesn't make the results too perfect, all experiments were made twice: once with the mains filter and once without.

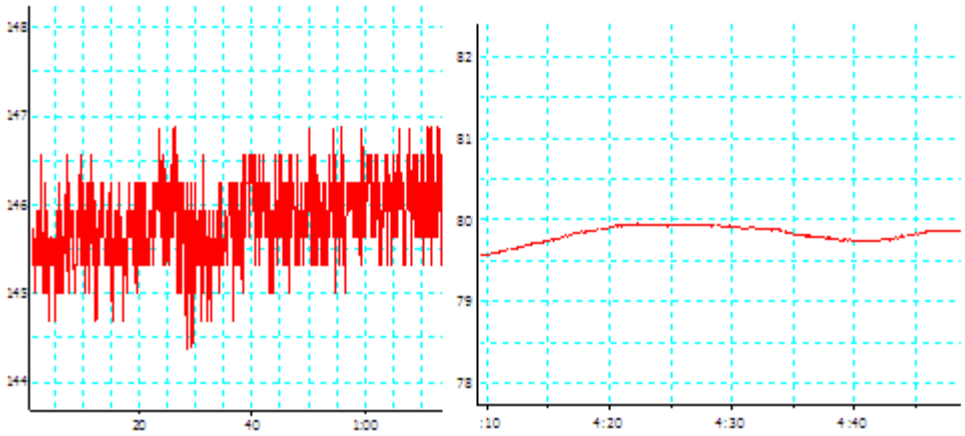

Figure 7: Left, baseline noise when no filter is used ≈1.5 mV. Right, baseline noise when main filter is used ≈0.5 mV. X-scale in [min:sec].

For commercial on-column absorbance detectors the noise is around 0.01-0.05 mAU. However, for this detector the noise is around 0.1 mAU when the mains filter was used and 4 mAU without the mains filter. Even with the mains filter the noise is ≈10 times too high. This is probably due to the fact that when doing photometric detection the baseline noise strongly depends on the intensity of the light source. In this case the 2 mm hole in the holder for the LED limits the light that reaches the detector and seeing as 400 nm is not the most sensitive wavelength for the photodiode, the signal gets close to zero and shows more noise than it should do at a higher wavelength due to dark errors. This photodiode gives the output in Volt and a calculation has to be done to get the answer in absorbance units. Commercial detectors have this built-in and the absorbance is given directly. As the absorbance is the ratio between V_o and V, the higher the background voltage V_o is the lower the noise gets. All these factors combined make the noise bigger. One extra disadvantage with the calculation is that it makes the detector somewhat less user-friendly but the calculation is relatively easily done using Excel.

Using the Lambda pump, injections of the standard solutions were made from low concentrations to high and repeated five times (Appendix 4). Air was let in between the sample to prevent mixing between the standards, it also had the advantage that it gave a clear line between each sample separating them, Fig. 8. Five absorbance calculations were made and then an average sensitivity graph was plotted Fig. 9.

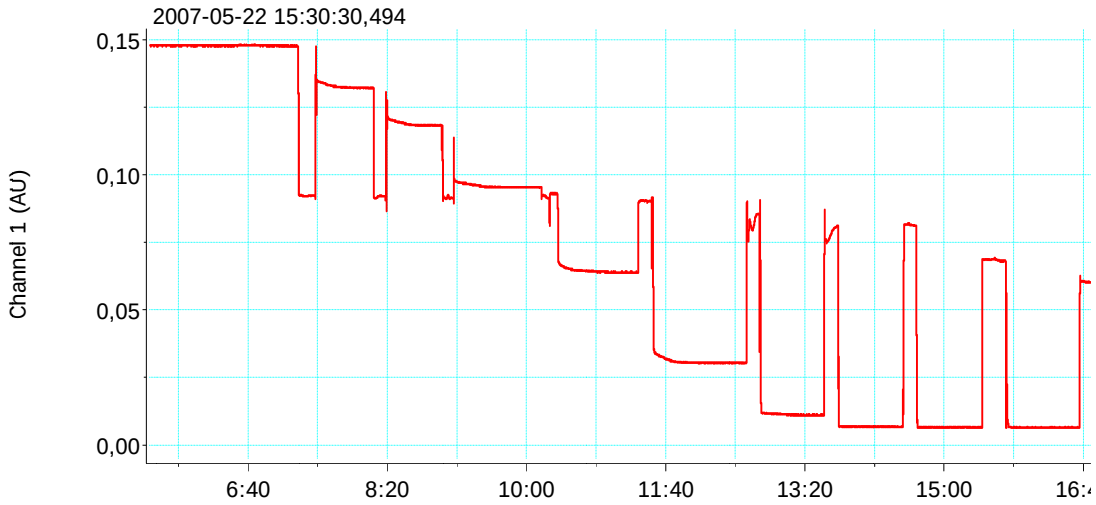

Figure 8: Example of absorbance measurement. X-scale is [min:sec].

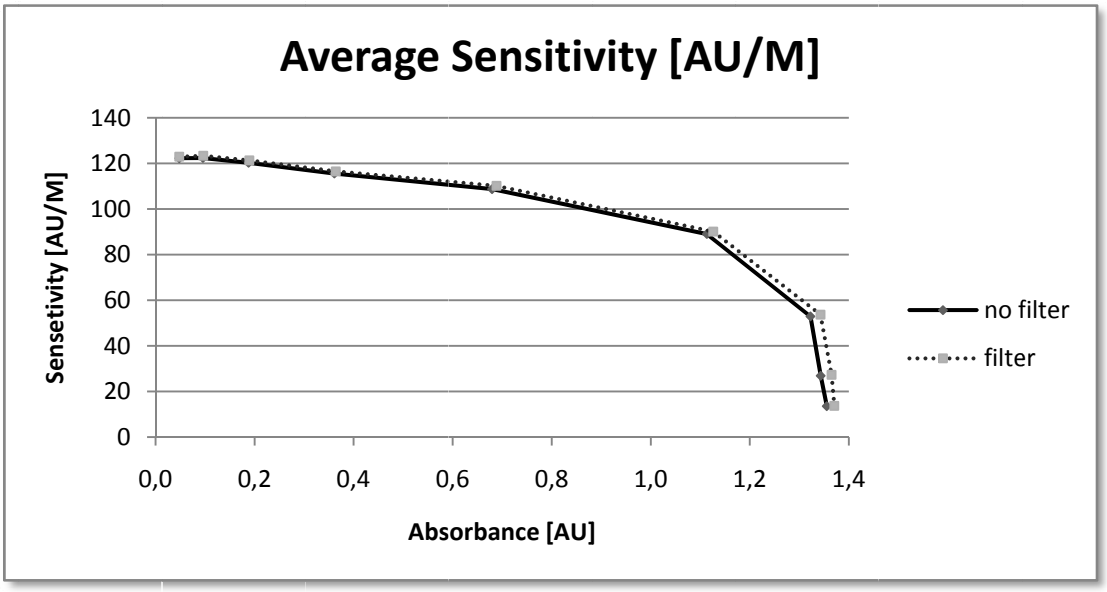

Figure 9: Average sensitivity graph for LED-AP detector with and without main filter.

Indirect detection can in many cases be more sensitive than direct detection but it adds one limiting factor, the concentration of the probe. The concentration should be in the linear range of the detector. The linearity range is evaluated from the sensitivity graph when the value falls 10 % below the maximum sensitivity value (27), for calculations see Appendix 5. In this case the detector is linear up to 0.61 AU without mains filter and 0.64 AU with the mains filter which equals a concentration of 5.6 mM and 5.8 mM respectively.

The effective pathlength is one of the best ways to determine if a detector is working properly or not. The effective pathlength is the average of all individual pathways the light can travel through the capillary and depends on the geometry of the light beam going through the capillary. When only the centre is illuminated the pathlength would equal the inner diameter of the capillary, the further away from the centre the shorter the distance the light travels through the capillary and so the pathlength is smaller. Practically this means that the pathlength is always going to be less than the actual diameter of the capillary but the higher the value is the more focused the beam is on the centre of the capillary. The effective pathlength (ℓ_p) was calculated by rearranging the Beer-Lambert

Law to give the ratio of sensitivity (s) to probe absorbtivity (ε = 16400 (5)) : $\ell_p = s/\varepsilon$ (Eq. 1) for calculations see Appendix 5. This detector has an effective pathlength of 74.7 µm when using the filter and 74.2 µm without the filter, in comparison to commercial instruments were the pathlength varies between 53.6 - 64.6 µm (26) for capillaries with i.d 75 µm. From this the conclusion is that the detector cell seems to have a very regular shape where the light passes through only the middle of the capillary. The alignment interface contains an optical slit which is matched to the inner diameter of the capillary and only allows this part to be illuminated giving optimised sensitivity, this is probably the reason to why effective pathlength is high.

The limiting factor for the maximum absorbance is the stray light. Stray light (I_o/I) is most commonly given in percentages and can be calculated using: $I_o/I = 10^{-A}$ (Eq. 2). By using the maximum absorbance (1.37 AU and 1.35 AU with and without the filter respectively) the calculated stray light is 4.3 % with the mains filter and 4.5 % without the mains filter (Appendix 5). The guideline is that the maximum absorbance should not be lower than 1 AU which equals 10 %. Considering that the total response area on the photodiode is larger than the area the light falls onto and it is impossible to see were the actual light beam is focused there is no way to determine at what angle the beam hits the detector area or if it reflects on the side of the package. All these factors increase the stray light. It is also possible that some of the light misses the area. The only way to optimise the absorbance is to move the photodiode inside the detector cell when the LED is shining on an empty capillary to try to get as high signal as possible. The diameter of the photodiode is smaller than the hole that it fits into in the black nylon holder, so to stabilise the photodiode small stripes of tape had to be wired around it. This means that even if a good position has been found it might not be preserved when tightening the screw to fasten the photodiode because the tape doesn't have enough resistance to keep the position. By improving the design and the optical system the stray light can be decreased, this might also help take away some of the noise. When comparing the results they are better when using the mains filter function but the difference is not big enough to be significant.

The alignment interface has a big advantage as it can accommodate capillaries with different inner diameters. The sensitivity measurement has to be redone but according to the technical information it is designed to accommodate all commercially available capillaries with outer diameters ≈365 µm.

4.2 Evaluation of the LED-induced fluorescence detector

Due to the broader emission light of and lower intensity of LEDs, LED-IF detectors are usually less sensitive for analytes than an ordinary LIF detector. In this setup there were even more factors that made the detector somewhat unstable. As the detector electronics were originally built for absorbance measurements the ground conditions were not optimal. For example the offset buttons could not be used as they could not be turned as much that would be needed to zero the signal. The offset buttons were therefore left in the same positions throughout all the experiments to have the same conditions. It is also important that the tip of the pickup fibre is situated as close to the capillary as possible without touching it to give the lowest LOD. This was problematic as it was hard to keep the pickup fibre in position when putting the detector cell together. When doing the LOD measurements the Lambda pump was used to inject the blank sample (borate buffer) followed by the 0.01 mM fluorescein sample. The experiment was done five times each for the different oils to give an approximation of the repeatability

(Appendix 6 and 7). One value from the immersion oil experiment had to be removed due to it being an outlier according to both Q-test and Grubbs test. When using the immersion oil the LOD is 0.72 µM ± 0.01 µM and for the silicone oil it is 0.58 µM ± 0.02 µM, for calculations see Appendix 8 and 9.

From the results of the LOD measurements with the two oils we can detect a difference between them and that the oil used did actually affect the LOD. The reason for this is that the oils don't have the same viscosities and thereby reduce the amount of light scattering differently. The light scattering properties depend on the oils refractive index. When the measurement was done it showed that during 1 hour the background level moved 0.07 AU for the immersion oil and 0.05 AU for the silicone oil, this is not a very large difference but it still showed that the signal was not stable over long periods of time. In further experiments only the silicone oil was used as this oil gave the lowest LOD.

The injections were done in the same way as for the LED-AP detector using the Lambda pump, from low concentrations to high and repeated five times (Appendix 10), the air that was let in between the injections made it simple to separate the different injections from each other, see Fig. 10. The graph of linearity in Fig. 11 shows that for fluorescein, the detector is linear up to a concentration of 0.2 mM with r=0.99, calculations see Appendix 10.

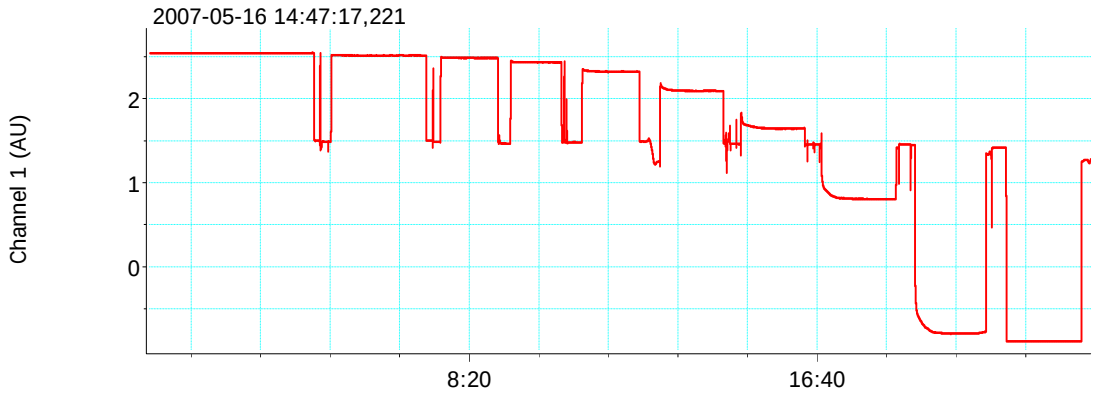

Figure 10: Example of linearity measurement. X-scale in [min:sec]

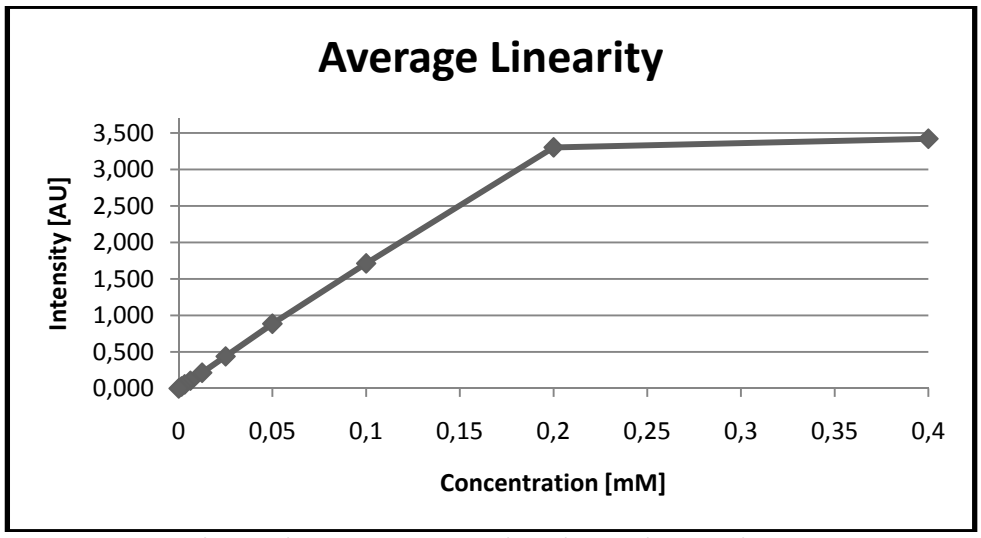

Figure 11: Linearity graph using the average intensity when silicone oil was used.

Transporting the excitation light and the fluorescence emission to and from the capillary is not an easy task. One of the most common solutions to this is using optical fibres. This gives the advantage that the system becomes simple and flexible. The detector electronics can be moved away from the CE unit to avoid disturbances from the high voltage power supply. The diameters of the optics fibre have to be chosen carefully; if the diameter is too big this will increase the stray light and give non-linearity. However, it cannot be too narrow either as when the optical pathlength that is illuminated is decreased the limit of detection will be increased. Using optical fibres made the detector cell small and movable. The fibres did however restrict the detector cell due to the length of the fibres and the fact that they weren't supposed to be bent too much. If the fibres were moved during the measurements the signal became unstable.

The problem with the LED-IF detector did not occur when using the detector, it was getting the detector to work at all that was sometimes problematic. Getting the signal stable before the experiment took approximately one hour of taking apart and putting together the cell. This had to be repeated every morning or after the detector had been used for more than two hours.

Compared with lasers the beam from the LED is incoherent and not as focused. When using the LED mounted on the board it was not a problem to focus the light as it had SMA Fibre Optic connectors that made the design stable and gave the optimal optical condition. It was possible to change the LED but in that case the board couldn't be used and the light had to be focused in to the optic fibre using a collimating lens fixture (Ocean Optics, FL, USA). All LEDs examined gave very low or no difference when turned on/off. That combined with the sometimes unstable detector means that a few changes needs to be made on the detector before it can be successfully used for further experiments.

4.3 Evaluation of the capillary electrophoresis analysis

Due to the problems with the LED-IF detector and the fact that the LED could not be changed, only the LED-AP detector was used in the CE. Designing and building portable CEs would not be a difficult task to do as the technique is so simple, usually the problems that arise are related to the safety issues around the instrument and the fact that high voltages are needed for the separations. In our case the basics of the CE were already constructed and the instrument was equipped with a safety switch that was connected to the beginning of the electrical circuit and interrupted the current when the lid was opened. This safety precaution combined with the ground that prevents the CE to charge up can in our case be considered enough to ensure the safety of the operator.

Usually when things are not working with CEs one of the first things you check is the current over the capillary. In this setup it is not possible to measure the current. So if something goes wrong the operator has to look for the answer elsewhere and hope that the CE is working or that if something is wrong with the CE it is a visible problem. In this case the portable CE was working fine and the problems that occurred when using it had more to do with the detector and the buffer condition than the CE itself. For example without the mains filter it was hard to determine which were actual peaks and which were just ordinary noise so the mains filter had to be used in every separation.

Before any injections were done the capillary was filled with the background buffer and then the power supply was turned on at -15 kV for about one hour to make sure no air bubbles were left in the capillary and that the signal was stable. It turned out that during a longer time the baseline moved slowly upwards. When the injections were done the sample vial was put in position and the electricity was turned on at -15 kV for 4 s.

The vial was then changed to the background electrolyte and the separations were carried out at 15 kV. An example of a separation is shown in Fig. 12, and in Appendix 11. During the separations the baseline continued moving steadily upwards. The solvent for the samples was water this resulted in an electroosmotic flow (EOF) peak, which always appeared after ~10 minutes.

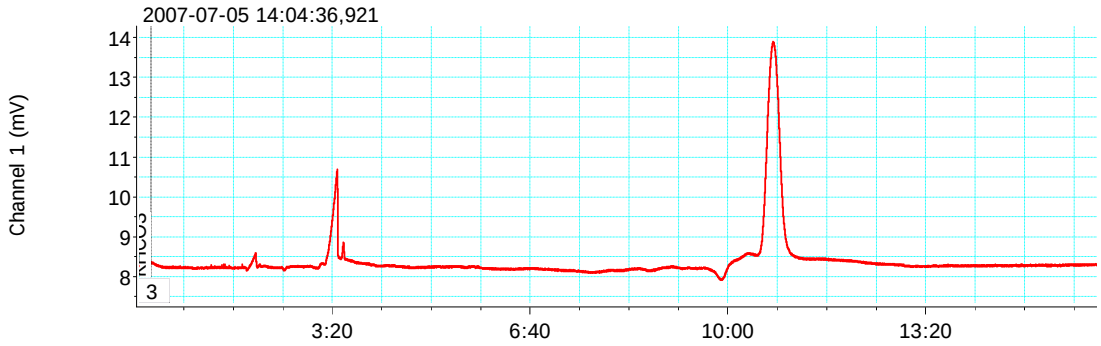

Figure 12: Detection of KCO_3. X-scale in [min:sec]. Anion peak shows after 3:24 and EOF peak after 10:45 min.

A moving baseline can be a sign that the probe adsorbs onto the surface of the capillary and the sometimes fronted peaks are most likely a result of the large difference in mobility between the sample and p-nitrophenol. The aim of this study was not about finding an optimised method for analysis of anions, it was about building two detectors to fit inside a portable CE and therefore nothing was done to solve the moving baseline or the fronted peaks.

The injection options for the CE were not the best available. Electrostatic injection has a known problem with discrimination and when using real samples this can be a crucial point. The way that the hydrodynamic injection is done, by lowering the upper part of the detector stand, is not good at all. Almost all detectors are sensitive to being moved and with this injection technique the detector had to be moved with every injection, thereby changing the conditions between every run.

The advantage of this CE is that any detector cell that fits inside the box can be used. This means that based on what types of samples that are going to be separated the detector can be changed to one that is suitable for that particular analysis. This gives the opportunity to use the instrument in a variety of situations in the laboratory and out in the field, if the detector can be made portable. The CE is also very easy to use and there are not that many buttons that need to be pushed to use it. The recording system can be changed to whatever the user has used before as long as the coax cable can be connected to a BNC plug on the recording system.

5 Conclusion

The goal of this study was to get a working portable CE system with two different detectors as a basis for further work. The main design of the portable CE and the LED-induced fluorescence detector (LED-IF) had already been made and only small changes were made here. However, the LED absorbance photometric detector (LED-AP) was made from scratch in the lab. During the building and testing of the equipment standard operating procedures (SOP) were written. The SOPs are very detailed to facilitate further work.

The goals of the study were achieved, both the LED-AP and the LED-IF detector work but they have some problems that need to be solved if they are going to be used in further experiments. The biggest issue is the noise of the LED-AP detector and the detector electronics for the LED-IF detector. The ground principles are good but a thorough evaluation of what can be kept and what should be changed has to be done.

For the LED-AP detector I think it might be a good idea to exchange the photodiode to a simpler one that has an external amplifier to enhance the signal. This means that the photodiode does not need electricity to work and if the power supply can be taken away, some of the noise should disappear and the mains filter could be removed.

Changing the electronics of the LED-IF will help with some of the basic problems with the detector. If the oil is changed to oil made especially for this kind of analysis the LED-IF detector has very good potential for further work. After the detectors have been optimised joining them would be very advantageous, making it possible to carry out absorbance and fluorescence measurements simultaneously.

The in-house made CE instrument works really well as long as the detector used is working and the separation conditions are optimised. One thing that might be good to figure out is to find a way to measure the current to check the performance in case of problems. Changing injection techniques is not an option as the CE is already built. The hydrodynamic injection has potential. One possibility is to redesign the injection table making it possible to lift the sample table higher during the injection instead of having to lower the detector stand.

6 References

1. **Kappes, T., Hauser, P. C.** Portable capillary electrophoresis instrument with potentiometric detection. *Anal. Commun.* 35, 1998, pp. 325-329.

2. **Kappes, T., Galliker, B., Schwarz, M. A., Hauser, P. C.** Portable capillary electrophoresis instrument with amperometric, potentiometric and conductometric detection. *Trends in Analytical Chemistry.* 20, 2001, no. 3, pp. 133-139.

3. **Kappes, T., Schnierle, P., Hauser, P. C.** Field-portable capillary electrophoresis instrument with potentiometric and amperometric detection. *Analytica Chimica Acta.* 393, 1999, pp. 77-82.

4. **Harris, D. C.** *Quantitative Chemical Analysis.* 7:th edition. New York : W.H. Freeman and Company, 2006.

5. **Balding, P., Boyce, M. C., Breadmore, M. C., Macka, M.** LED-compatible probes for indirect detection of anions in capillary electrophoresis. *Electrophoresis.* In press 2007.

6. **Freed, A. L., Audus, K. L., Lunte, S. M.** Investigation of the metabolism of substance P at the blood-brain barrier using capillary electrophoresis with laser-induced fluorescence detection. *Electrophoresis.* 22, 2001, pp. 3778-3784.

7. **Lu, M., Chiu, T., Chang, P., Ho, H., Chang, H.** Determination of glycine, glutamine, glutamate, and aminobutyric acid in cerebrospinal fluids by capillary electrophoresis with light-emitting diode-induced fluorescence detection. *Analytica Chimica Acta.* 538, 2005, pp. 143-150.

8. **Pobozy, E., Czarkowska, W., Trojanowicz, M.** Determination of amino acids in saliva using capillary electrophoresis with fluorimetric detection. *Journal of Biochemical and Biophysical Methods.* 67, 2006, pp. 37-47.

9. **Sluszny, C., He, Y., Yeung, E. S.** Light-emitting diode-induced fluorescence detection of native proteins in capillary electrophoresis. *Electrophoresis.* 26, 2005, pp. 4197-4203.

10. **Zhao, S., Wang, B., He, M., Bai, W., Chen, L.** Determination of free D-alanine in the human plasma by capillary electrophoresis with optical fiber light-emitting diode-induced fluorescence detection. *Analytica Chimica Acta.* 569, 2006, pp. 182-187.

11. **Zhao, S., Wang, B., Yuan, H., Xiao, D.** Determination of agmatine in biological samples by capillary electrophoresis with optical fiber light-emitting-diode-induced fluorescence detection. *Journal of Chromatography A.* 1123, 2006, pp. 138-141.

12. **Casado-Terrones, S., Fernández-Sánchez, J. F., and Segura-Carretero, A., Fernández-Gutiérrez, A.** Simple luminescence detectors using a light-emitting diode or a Xe lamp, optical fiber and charge-coupled device, or photomultiplier for determining protein in capillary electrophoresis: A critical comparison. *Analytical Biochemistry.* 365, 2007, pp. 82-90.

13. **Cameron, J., Macka, M., Haddad, P. R.** Design and performance of a light-emitting diode detector compatible with a commercial capillary electrophoresis instrument. *Electrophoresis.* 25, 2004, pp. 3145-3152.

14. **Lindberg, P., Hanning, A., Lindberg, T., Roeraade, J.** Fiber-optic-based UV-visible absorbance detector for capillary electrophoresis, utilizing focusing optical elements. *Journal of Chromatography A.* 809, 1998, pp. 181-189.

15. **Boring, C. B., Dasgupta, P. K.** An affordable high-performance optical absorbance detector for capillary systems. *Analytica Chimica Acta.* 342, 1997, pp. 123-132.

16. **Arráez-Román, D. F., Fernández-Sánchez, J., Cortacero-Ramírez, S., Segura-Carretero, A., Fernández-Gutiérrez, A.** A simple light-emitted diode-induced fluorescence detector using optical fibers and a charged coupled device for direct and indirect capillary electrophoresis methods. *Electrophoresis.* 27, 2006, pp. 1776-1783.

17. **Hillebrand, S., Schoffen, J. R., Mandaji, M., Termignoni, C., Grieneisen, H. P. H., Kist, T. B. L.** Performance of an ultraviolet light-emitting diode-induced fluorescence detector in capillary electrophoresis. *Electrophoresis.* 23, 2002, pp. 2445-2448.

18. **Yang, B., Guan, Y.** Light-emitting-diode-induced fluorescence detector for capillary electrophoresis using optical fiber with spherical end. *Talanta.* 59, 2003, pp. 509-514.

19. **Zhao, S., Yuan, H., Xiao, D.** Optical fiber light-emitting diode-induced fluorescence detection for capillary electrophoresis. *Electrophoresis.* 27, 2006, pp. 461-467.

20. **Abbas, A. A., Shelly, D. C.** Optical properties of axial-illumination flow cells for simultaneous absorbance-fluorescence detection in micro liquid chromatography. *Journal of Chromatography A.* 691, 1995, pp. 37-53.

21. **Caslavska, J., Gassmann, E., Wolfgang, T.** Modification of a tunable UV-visible capillary electrophoresis detector for simultaneous absorbance and fluorescence detection: profiling of body fluids for drugs and endogenous compounds. *Journal of Chromatography A.* 709, 1995, pp. 147-156.

22. **Dasgupta, P. K., Eom, I., Morris, K. J., Li, J.** Light emitting diode-based detectors Absorbance, fluorescence and spectroelectrochemical measurements in a planar flow-through cell. *Analytica Chimica Acta.* 500, 2003, pp. 337-364.

23. **Heiger, D. N.** *High performance capillary electrophoresis - An Introduction.* 2nd edition . France : Hewlett-Packard Company, 1992.

24. **Beckman instruments, Inc.** *Introduction to capillary electrophoresis.* USA : s.n., 1994.

25. **Datasheet catalog**. *www.datasheetcatalog.com.* [Online] 2006. [Cited: 5 Augusti 2007.] http://www.ortodoxism.ro/datasheets/BurrBrown/mXtyrtw.pdf.

26. **Johns, C., Macka, M., Haddad, P. R., King, M., Paull, B.** Practical method for evaluation of linearity and effective pathlength of on-capillary photometric detectors in capillary electrophoresis. *Journal of Chromatography A.* 927, 2001, pp. 237-241.

27. **Macka, M., Adersson, P., Haddad, P. R.** Linearity evaluation in absorbance detection: The use of light-emitting diodes for on-capillary detection in capillary electrophoresis. *Electrophoresis.* 17, 1996, pp. 1898-1905.

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Prof Peter Hauser, the brain to the portable CE and the mastermind behind the idea of building the "starleaf" detector.

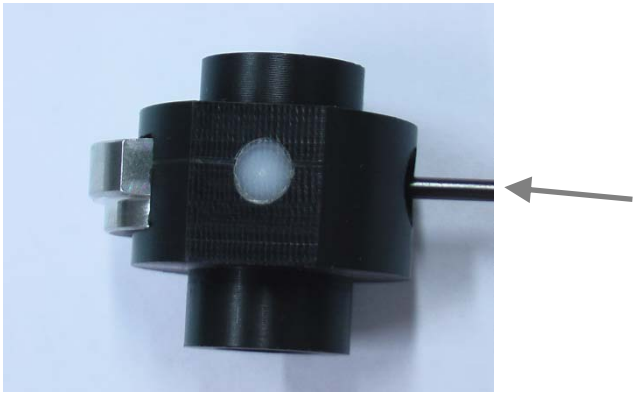
Dr Frantisek Foret, the builder of the LED-Induced fluorescence detector. It has some faults, but it took two days to build so it is impressive.

Appendix 1

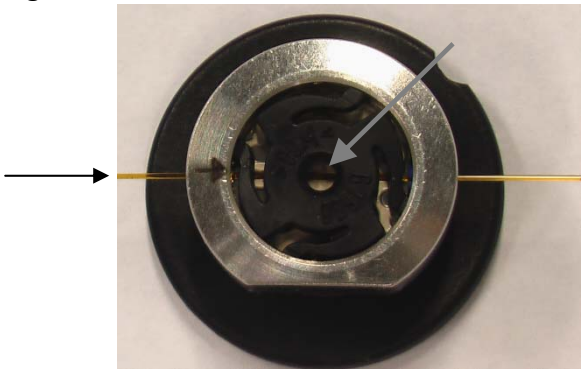
SOP, LED-AP detector

LED/PHOTODIODE/ CAPILLARY HOLDER

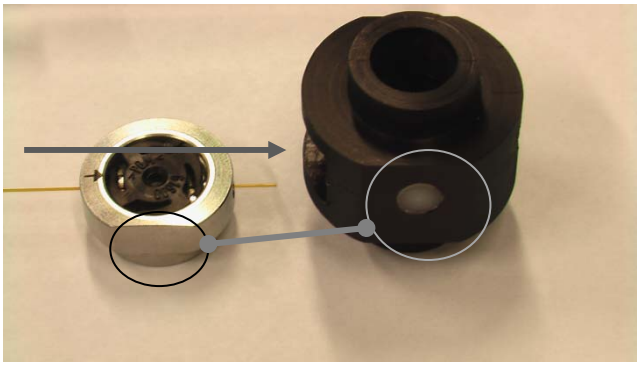
- Take out the capillary holder by pushing on it with, for example a screwdriver, through the small hole.



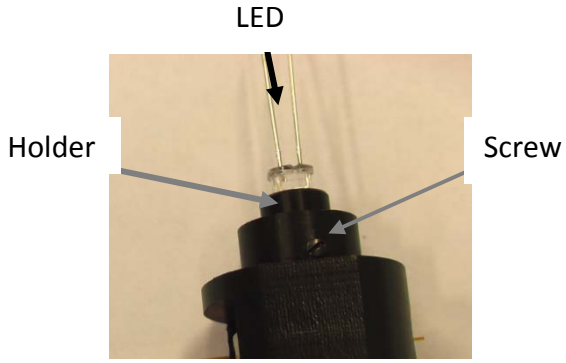
- The capillary is inserted in the holder by pushing the holder down on the black support “button” and gently inserting the capillary in the direction of the arrow. The capillary window should be approx 2 mm and situated in the middle of the holder. It can be seen through the hole in the middle.



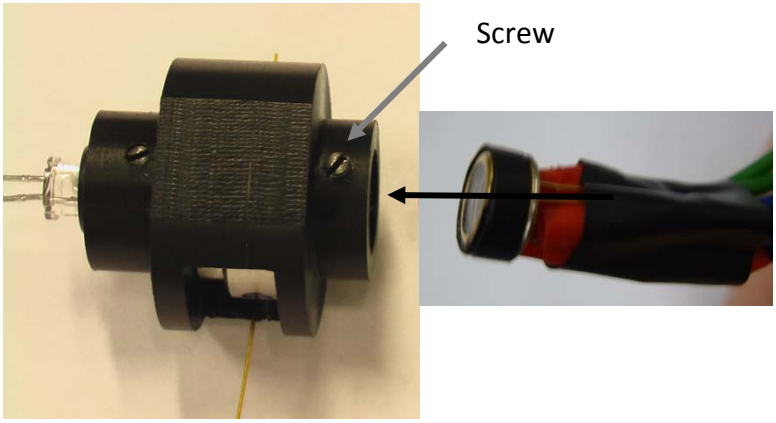
- The capillary holder has then to be pushed back in the big black holder. It should be easy to push the holder back in, if not, make sure it is not inserted the wrong way. Flat side should go to white dot in the direction of the arrow (same as for the capillary).



- The LED is then placed in the designed LED-holder (fits 5 mm LEDs) and placed in the hole at the top of the big holder. The distance to the capillary can be changed by loosening the screw and moving the black small LED-holder. For this detector to get the highest signal the holder has to be pushed close to the capillary holder and you may not be able to take out/insert the capillary holder without moving the LED-holder back out.



- The photodiode (OPT 301) is put in the black holder on the opposite side as the LED. It's fastened with the little screw. It is not necessary to take out the Photodiode. The distance from the capillary holder changes the sensitivity and the closer the better. **NOTE: DO NOT TOUCH THE GLASS WINDOW ON THE PHOTODIODE. FINGERPRINTS AND DIRT CAN GENTLY BE CLEANED AWAY WITH METHANOL.**



- The cables soldered to the photodiode are as follow:
  - Green = Ground
  - Red = +15 V
  - Black = -15 V
  - Blue = output signal (is also connected to the integral amplifier)
  - To the audio cable the green cable goes to the outside (ground) and the blue to the middle (output signal).

  NOTE: TO KNOW MORE ABOUT THE PHOTODIODE READ THE DATASHEET FOR OPT301.

POWERBOX

- The power to the detector and LED comes from the powerbox made by Peter Hauser. To turn the box on connect it to the mains with the cable on the backside and push the ON/OFF button. When turned on the ON/OFF button shines. The box delivers ±15 V at 0.4 A
- The outputs give :
  - RED: +15 V
  - GREEN: Ground for photodiode
  - BLACK: -15 V
  - GREEN/YELLOW: Ground for the powerbox

NOTE: CONNECT THE CABLES BEFORE TURNING THE BOX ON.

USING THE DETECTOR

1. Connect the photodiode cables to the power box. Make sure it's red cable to +15 V, green cable to GND (ground) and black cable to -15 V.

   NOTE: Any other combination will damage the photodiode.

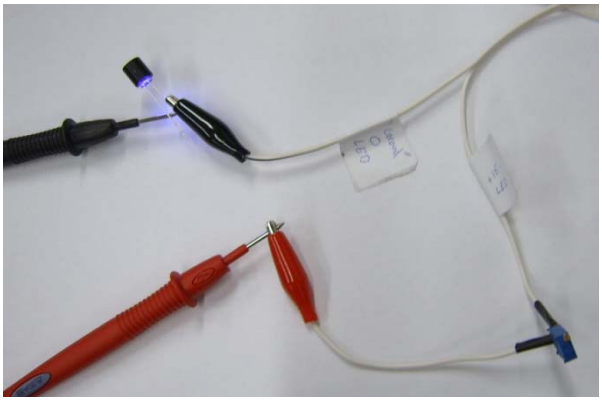
2. Plug in the cables to the LED. White cable (red plug) to +15 V (red output) and White/Black cable (black plug) to Ground (green output GND). The plugs to the photodiode have holes in them that fit the banana plugs to the LED.

   NOTE: The negative white/black cable should be connected to ground that is 0 V.

3. The blue little cube is a multi-turn and is used to change the current. This can be measured with a multimeter when the power is on:

   3.1. On the multimeter move the red cable to "A" output and change to measure A.

   3.2. Connect the cables in series with the LED. Red crocodile clip to red cable and black to the pin on the LED were the red crocodile clip was connected.



   3.3. Use a small screwdriver to turn the screw on the blue cube to change the current when watching the multimeter.

   NOTE: Don't have the multimeter connected too long. That might blow up the fuses in it. Always make sure the multi-turn is turned down to 15 mA if someone else is going to use the detector, to prevent accidentally blowing up LEDs.

4. Connect the audio cable to the e-corder.
5. Put the LED and the photodiode in the black holder as described previously.
6. Turn the e-corder and the power box on.
7. Gently move the LED in the holder to get the highest/best signal. The photodiode can also be moved but be extra careful not to scratch the window.
8. The detector is ready to use. No light gives a zero signal and the signal increases with increased light intensity.

   Low absorbance-> high current going through, high signal,

   High absorbance-> low current going through, low signal.

   The Invert option can be used in the e-Chart software.

Appendix 2

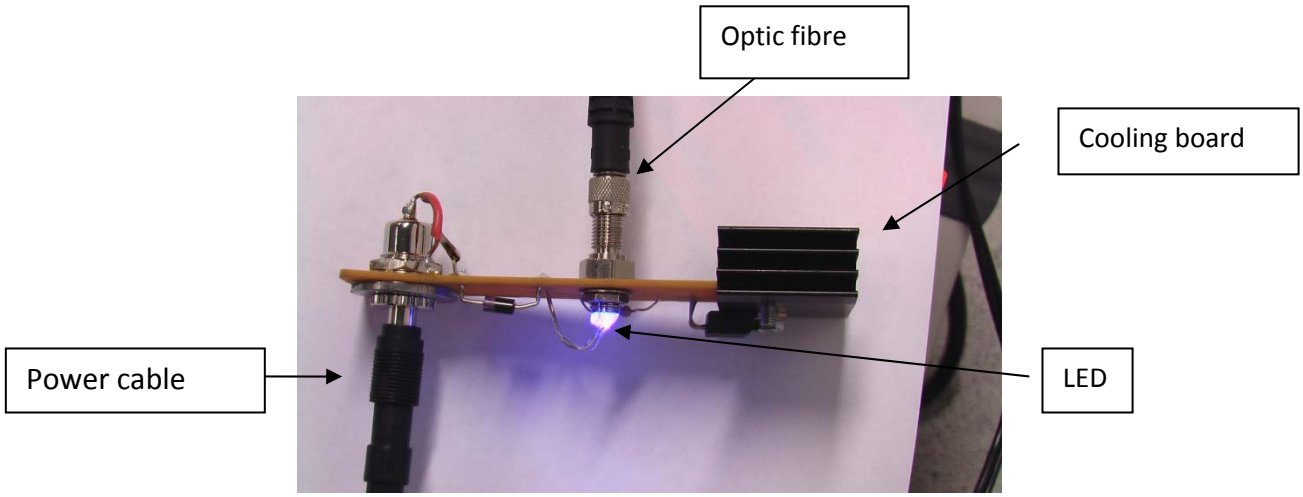
SOP LED-IF detector

LIGHT SOURCE

- The light comes from an LED mounted on an electric board driven by 6 V from AC/DC converter. The LED has a wavelength of 470 nm.

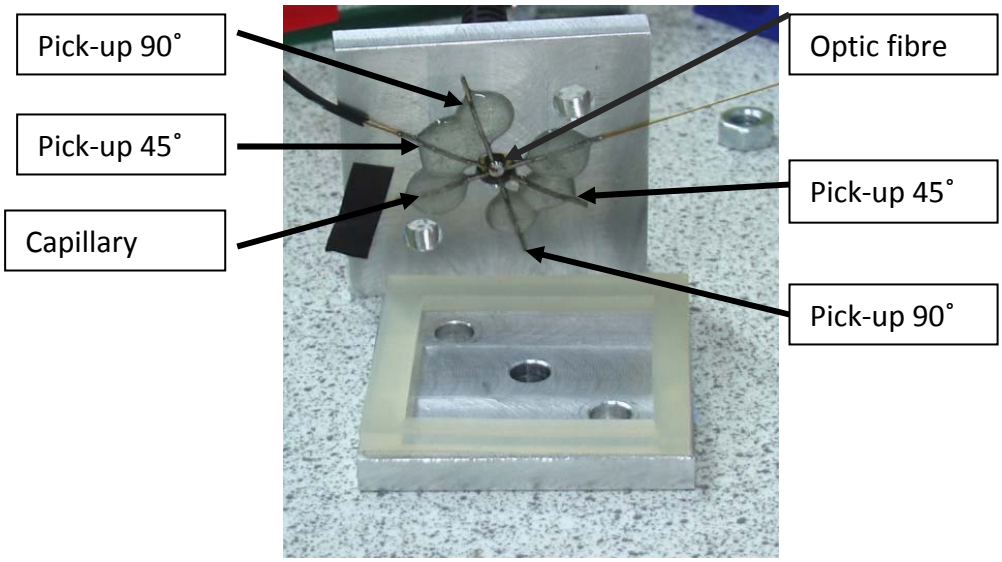
  NOTE: The converter should give negative in the middle and positive on the outside of the "plug" in to board from the power cable. Changing this will burn up the resistors on the board.

- The optic fibre can be taken away from the board by unscrewing the nut. Another light source can then be used shining in to the fibre.
- The black squared box is a cooling board (see picture below) and it is important to make sure the whole board is in a ventilated place so it does not get over heated.
- The other small things on the board are resistors that can be changed if they break.



DETECTOR CELL

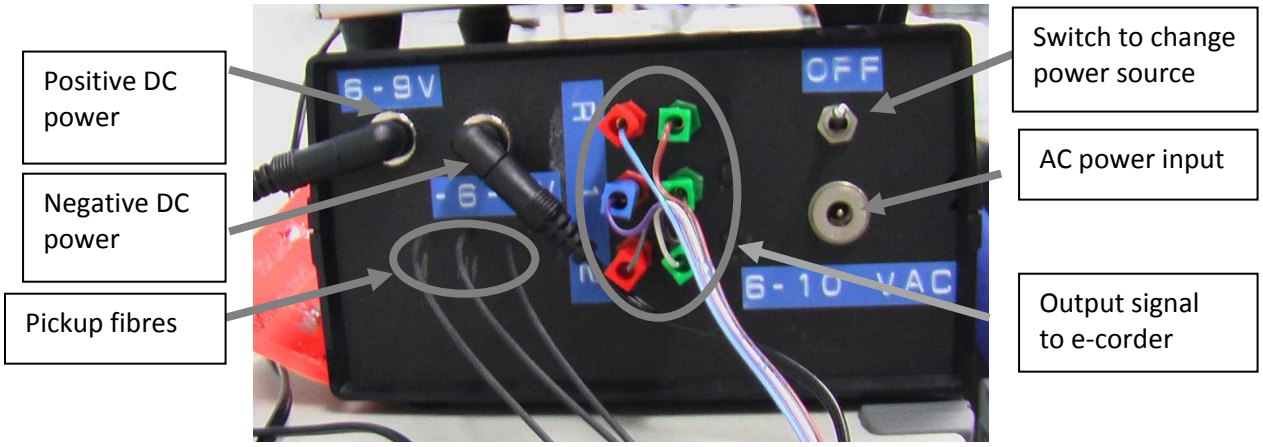
- The detector cell consists of two metal plates with a rubber square between them, that can be assembled with two screws and nuts placed in the holes in the corners.
- The hole in the middle is for the optic fibre. The optic fibre can be taken away by unscrewing the nut.
- Inside the detector are 6 metallic tubes situated opposite to each other. The tubes are needles that have been cut apart and glued to the metal. They are for the capillary and the pick-up fibre. The pickup fibre can have 45˚ or 90˚ angle to the capillary on both sides.
- The rubber square is to protect the capillary and the pick-up fibre from breaking and to make the cell as tight as possible when putting it together.



DETECTOR BOX

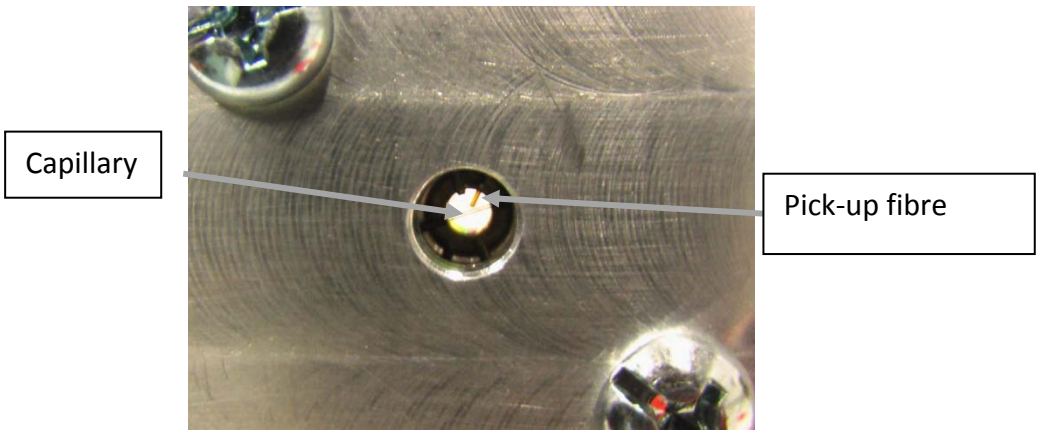
- The box can get power in two different ways changed by the switch on the back.
  - When the switch is in OFF position, the box uses 2 AC/DC converters: one negative and one positive. The voltage should be between 6 and 9 V. Positive = positive tip, negative is negative tip if you are uncertain this can be controlled with a multimeter.

    NOTE: Do not change polarity this will shortcut the box.
  - When switch is changed to ON the box can be powered through 6-10 V AC.
- The box has three different pick-up fibres R, I and II. The fibres are all the same. All fibres go in to the box on the backside and the signal shows on respectively display on the front. Each pick-up fibre has its own offset button.
- The signals can be transferred to the e-corder from the back of the box, by the small coloured cables. The ones that have green sockets (Brown, Black and White) should be connected to the outside of the audio cable (black crocodile clip), they are ground cables and are internally connected. The cables with red and blue sockets (Blue, Purple, Grey) are signal outputs and should be connected to the red crocodile clip. The audio cable is then connected with the BNC connector to the input on the e-corder.



USING THE DETECTOR

1. Turn the detector box ON by plugging in the 2 AC/DC main adapters. The box will automatically turn on when power is connected. The displays should all show numbers around 3.34.
2. Connect the output signal to the e-corder or other recording system with the audio cable with BNC connector and crocodile clips. The red crocodile clip should be connected to the signal output from chosen pick-up cable (see 7) and the black crocodile clip to one of the ground cables.
3. Take the detector cell apart.
4. Put the optic fibre in place if it is disconnected.
5. Put some immersion oil on the window of the capillary and clean most of it off and do the same with the top of the optic fibre.
6. Install the capillary in the tubes marked with a scratch and red dots. Use a small piece of tape to fasten the capillary. It's easy to move it unintentionally when putting the detector together.
7. Choose pick-up fibre and place it in one of the tubes. The pick-up fibre should be as close to the capillary as possible.
8. Put the detector cell together. Use the hole opposite to the optic fibre to make sure the pick-up fibre didn't move. See picture.



9. Cover the hole with a piece of black tape.
10. Connect the optic fibre to the LED (light source) and turn the LED ON by plugging in the adapter. The number on the display, for chosen pickup fibre, should now show a number around 2.3-2.8 AU and be stable. The light transmission depends on the fibre bending and should not be bent to a radius of less than 5 cm and should not move during the measurement.

NOTE: If too much oil is left on the capillary the signal will be either stuck on -0.886 which is the lowest signal possible or unstable and moving towards -0.886.

11. Put black tape around the detector, the signal might go up somewhat when doing this. Use the e-corder to monitor the signal for ≈10 min, if the signal is moving a lot or is outside the signal interval [2.3-2.8 AU] turn off the LED, take the detector apart, clean the optic tip and the capillary window and try again.

12. The offset buttons can be used to change the signal but DO NOT use them if it's a large change that needs to be done. Small changes of max 0.05 units can be done, but it's still not recommended since the change will effect the signal difference (reason unknown).
13. The detector is now ready to use. It works like an absorbance detector that means that no fluorescence gives high signal (no light absorbed => everything shines through) and when the solution fluoresce it gives low signal (light absorbed => less light comes through).

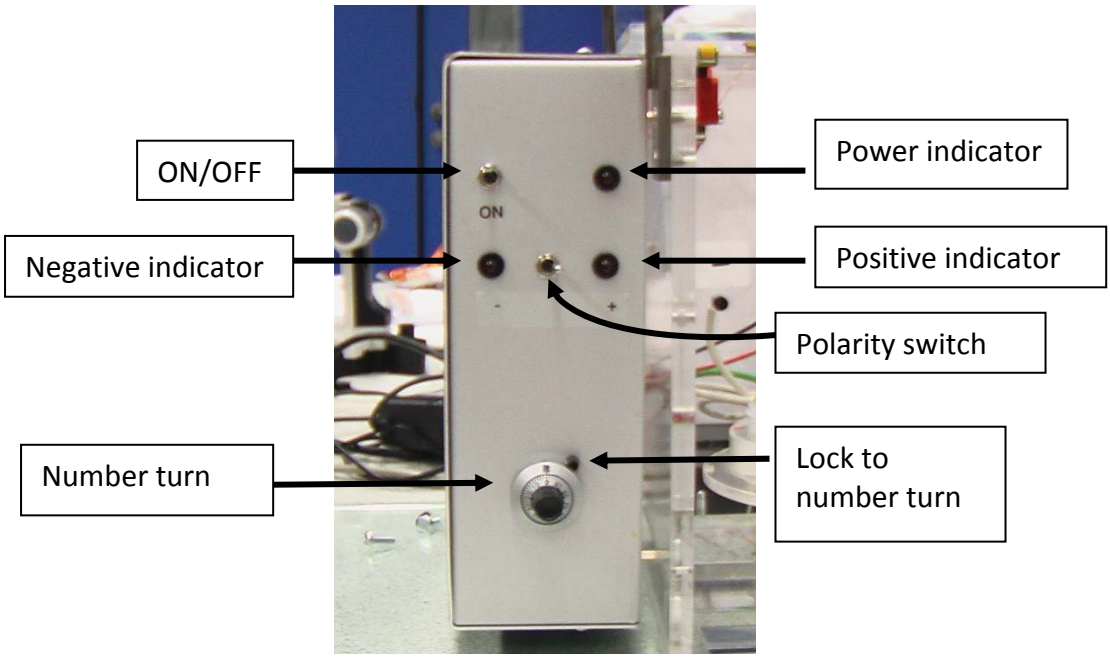
Appendix 3

SOP, Portable capillary electrophoresis instrument

- The CE box consists of two main parts: the control panel and the “separation” box itself.
  - The separation box is made from Perspex and can easily be taken apart by unscrewing the screws.
  - The control panel is made of metal and can also be taken apart, but this is not recommended. Before doing this take a look at the electrical scheme and make sure the power is not connected. Be extra careful if battery is used.

CONTROL PANEL

- The CE system is controlled from the front panel.
  - ON/OFF switch. This is used to turn on and off the system. A red light indicates that the power is on.
  - Polarity switch. This is used to change the polarity of the system. (To use this see below: Using the CE). A red light indicates which polarity is set.
  - Number dial. Controls the voltage to the system. A setting of 0 equals 1.6 kV and 10 equals 15 kV, In this range, the voltages are linear. On the left side of the dial is a switch to lock it.



BACK OF CONTROL PANEL

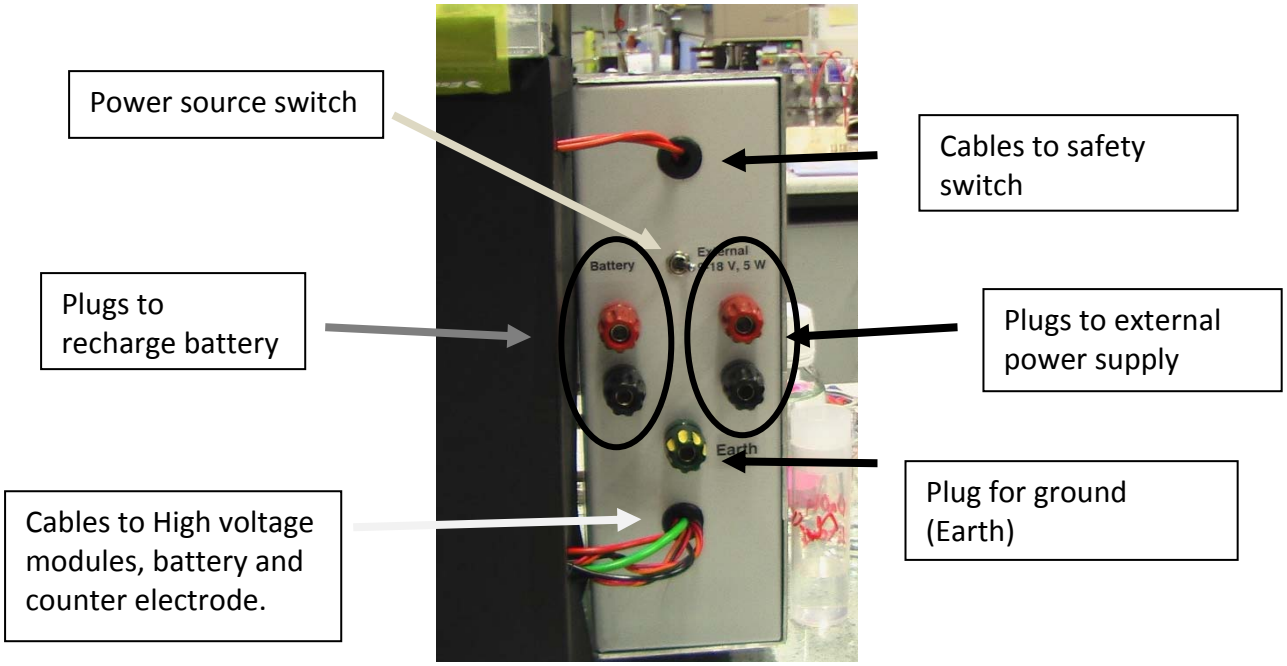
- ***NOTE: Always make sure the ground is connected, if not the box can charge itself up due to leakage from the high voltage supply.***

- The power to the CE system is connected to the back of the control panel. The switch is used to control the source of the power. The system can be run on an external power supply delivering between 9 and 18 V, or the 12 V battery. The switch is used to choose which power source to be used.

- If power supply is used the cables are connected: red to red (positive), black to black (negative) and green to green (ground).

- If battery is used no cables except for the ground should be connected. In the field, a metallic spike pushed in to the soil can be used as ground.

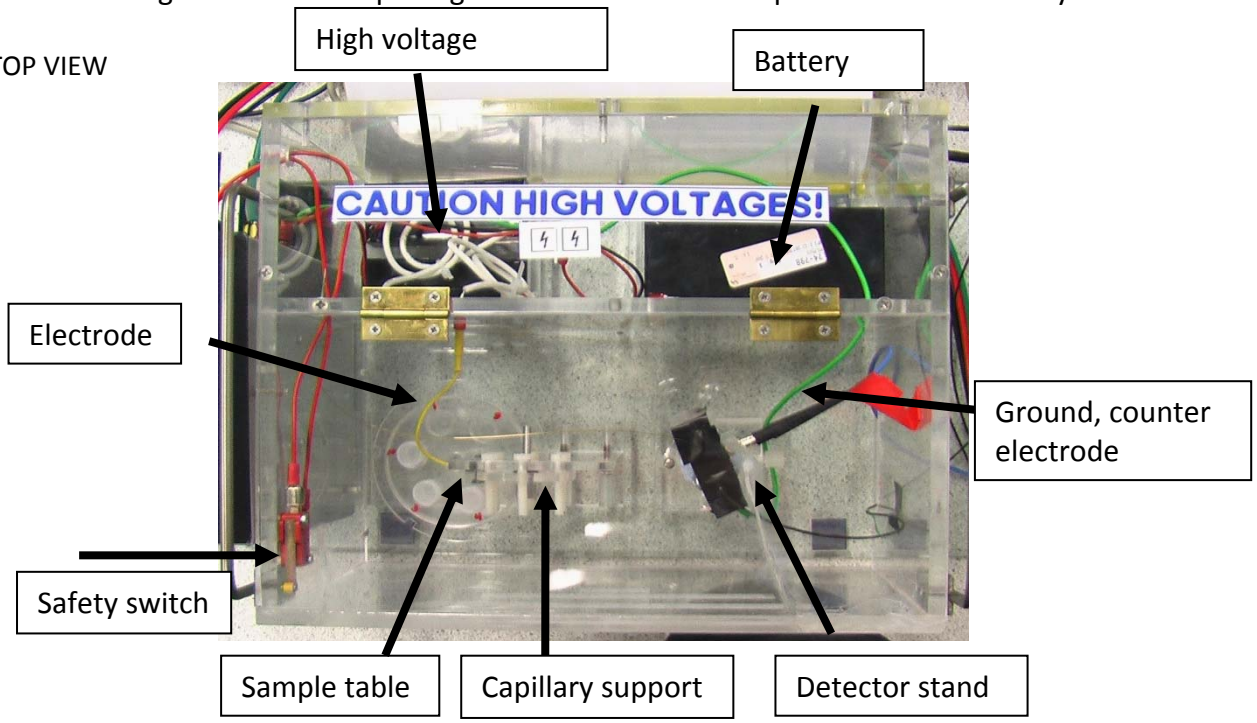
- To recharge the battery. (Disconnect the external power supply if this is connected). Turn switch to external and plug in the charger in the sockets for the battery. The charger itself is plugged in to the mains power.



SEPARATION BOX

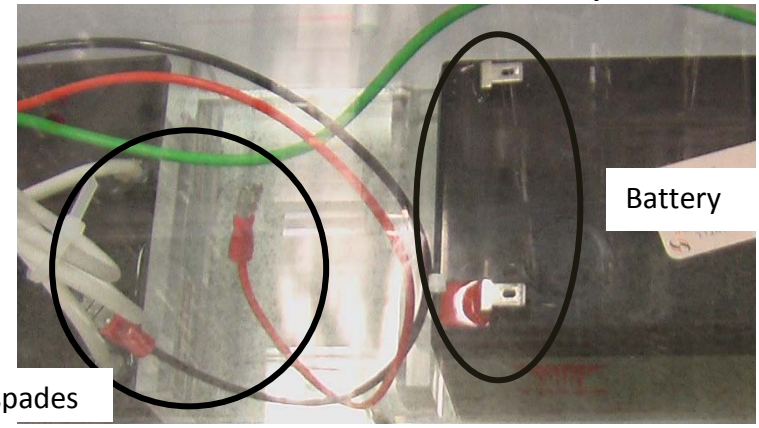
- The inside of the separation box is divided in two different sections separated by a Perspex wall.
  - The battery and the two High voltage modules are placed in the back.
  - The sample table, capillary support, electrodes and the detector stand is in the front. The right side has an opening for cables and at the top left corner is a safety switch.

TOP VIEW



- The battery and high voltage space can only be reached by unscrewing the back panel on the box.
- The back high voltage module gives negative voltage and the front gives positive voltage. They are connected to two output plugs in the separation wall. Black (negative) and red (positive).
- The battery is connected by fastening the cable spades to the outputs on the battery.

NOTE: Make sure it is RED cable to RED output and BLACK cable to BLACK output.



USING THE CE

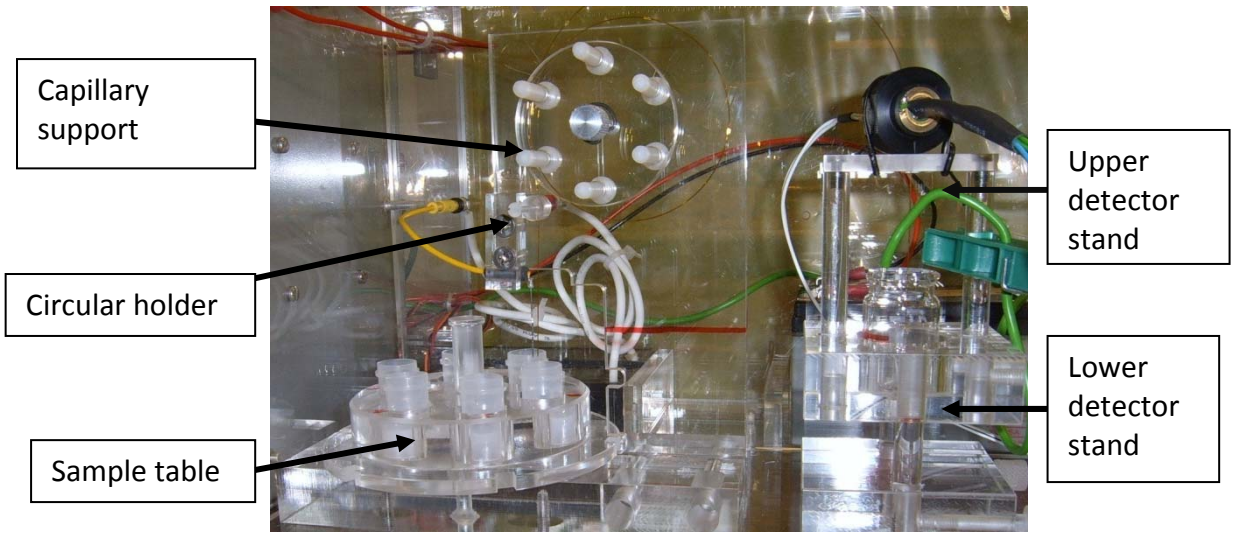
1. TURN ALL POWER OFF.

   1.1. The box is equipped with a safety switch which will interrupt the power to the system if the lid is opened. This is just a safety precaution and when installing detector etc. it is easy to accidently push the switch down and turn the electricity on so be careful when doing this.

2. Choose capillary and detector.
3. Install the capillary in the detector. Depending on which detector is used, this can be done afterwards, but it is usually easier to do it before.
4. Put the empty waste vial in the detector stand. This can be done after the detector has been fastened.
5. Fixate the detector to the detector holder by using cable ties, blue tack, electrical tape or similar smart things. The hole in the middle is best used for the capillary and the two small on the side can be used to fasten the detector. To protect the capillary a piece of PEEK tubing can be used.

   NOTE: Do not use metal to fasten the detector.

6. Fasten the capillary by pushing it through the small hole in the circular holder by the electrode and fasten the plastic screw. The end of the capillary should be on the same level as the electrode.
7. Wind the capillary around the wheel. The number of turns depends on the length of the capillary, make sure it does not touch the roof when closing the lid.
8. Put the sample table in run position (up) and adjust the lower part of the detector stand by one of the plastic screws, to make sure it is linear. (The red lines can be used as guidelines.) This is to prevent unintentional hydrodynamic injection. (also see 14)



9. Choose Polarity.

   9.1. Negative: Move the polarity switch to negative and put the electrode cable (yellow) in the black output.

   9.2. Positive: Move the polarity switch to positive and put the electrode cable (yellow) in the red output.

10. Place the ground cable in the waste vial and fasten it to the detector stand with a clothes-peg or similar.

11. Make sure the ON/OFF switch is turned to OFF.

12. Connect the CE to the desired power supply. If external power supply is used the most common voltage is 12 V.

    NOTE: Make sure the external power supply is set to min voltage before connecting it and then turn up the voltage, to prevent voltages over 18 V.

13. Put the vials with BGE and samples on the sample table. The volume in the vials should be the same.

14. Fill the waste vial with BGE. Adjust the level to the same as the samples by placing the BGE vial in run position and add/remove BGE from the waste vial. See 8.

15. When in the same position as in 14, make sure the capillary is under the surface in both vials. The height of the capillary in the waste vial can be changed by moving the upper part of the detector stand.

16. Switch software for the recording unit on.

17. Put BGE in run position. Close lid. Turn up to max voltage on the number turn. Switch the CE on and start the detectors recording. Since it's just BGE the baseline should be flat.

18. Injecting the sample. This can be done in two ways electrokinetically or hydrodynamically. Cleaning of the electrode and outside of the capillary should be done between changing vials. This can be done by having an extra vial with water and just dip the electrode and capillary in this vial.

    18.1. Electrokinetic injection.

        18.1.1. Turn the CE OFF.

        18.1.2. Open lid and put sample vial in run position, close lid.

        18.1.3. Use the number dial to set the injection voltage. 15 kV is good to start with and then optimize it.

        18.1.4. Turn the CE ON for the amount of time the injection should be done, then turn it off again. The optimum time is different for different samples. 5 s is good to start with and then optimize it.

        18.1.5. Turn the voltage back to max 15 kV if lower injection voltage is used and change back to BGE.

        18.1.6. Turn the CE and software recording ON at the same time.

18.2. Hydrodynamic injection.

18.2.1. Turn off the CE

18.2.2. Open lid and put the sample vial in run position.

18.2.3. Loosen the plastic screw on the lower part of the detector stand. Lower it (with the detector and the waste vial) to the lowest point possible and keep it there for the time needed for injection. Start with 1.5 min. (No voltage needed.) Then lift it up to the same level as before. Fasten the screw.

18.2.4. Change to BGE vial.

18.2.5. Close the lid and turn the CE and software recording ON at the same time.

19. Capillary flushing is best carried out manually with a syringe and connectors.

Appendix 4

Absorbance measurements for LED-AP detector

Figs. A-1 to A-5 show the absorbance measurements when main filter is not used. The x-scale is in [min:sec]. In Figs. A-1, A-4 and A-5 the pre-cleaning with deionised water was included in such a way the recording giving the graph with 11 steps.

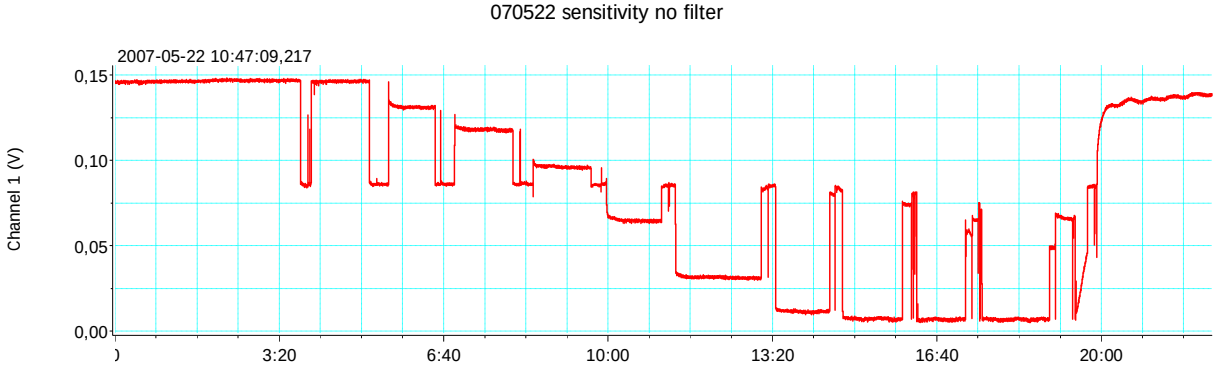


Figure A- 1 (Data in Table A-1, Appendix 5)

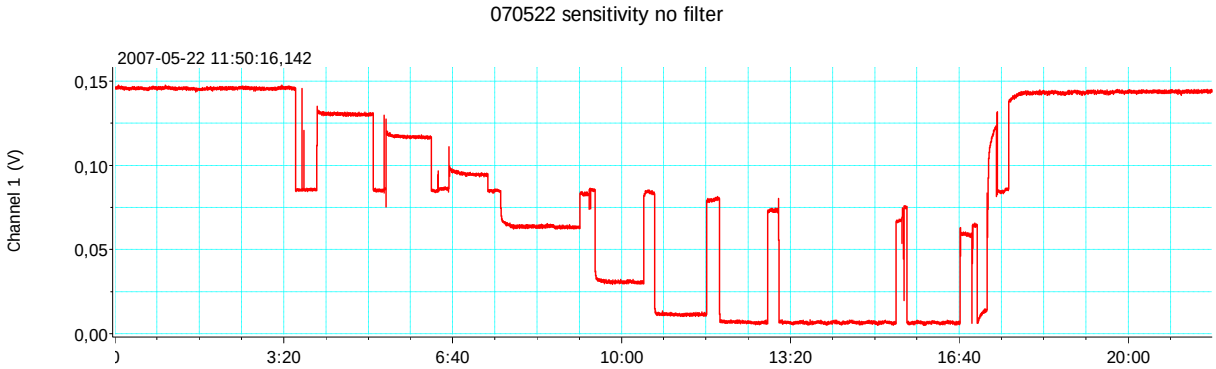


Figure A- 2 (Data in Table A-2, Appendix 5)

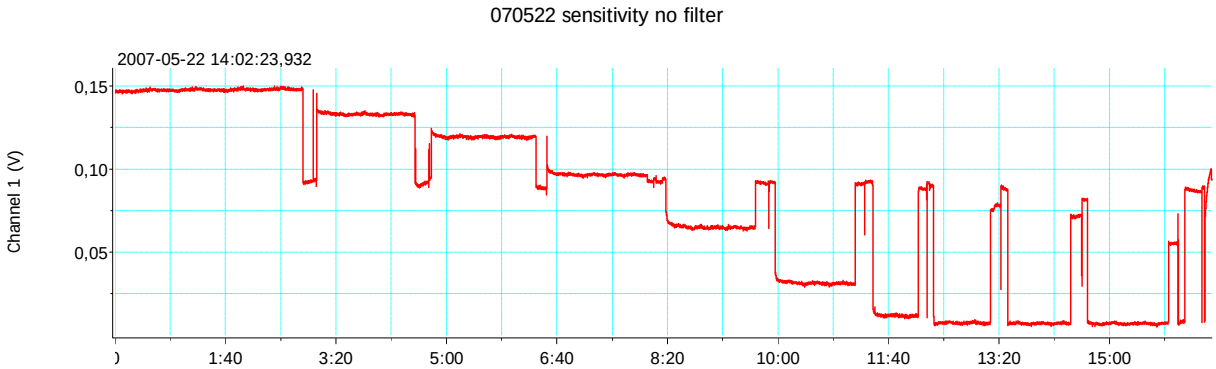


Figure A- 3 (Data in Table A-3, Appendix 5)

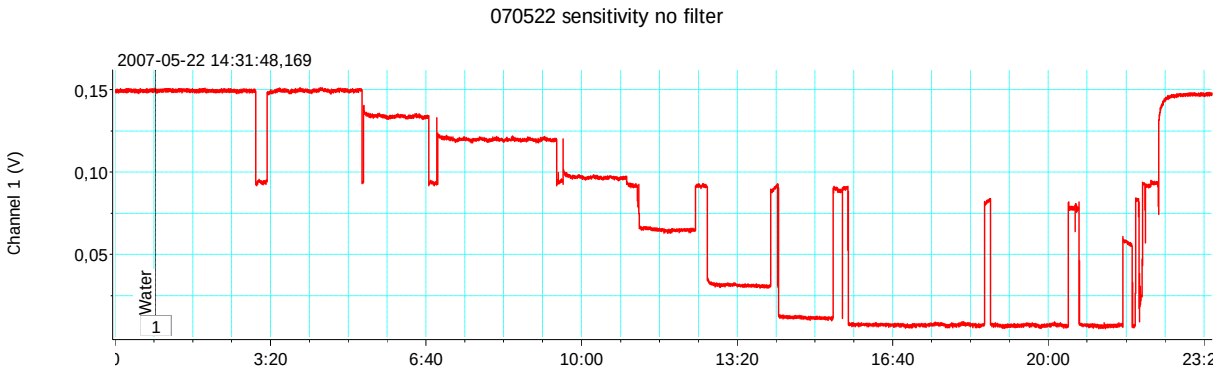


Figure A- 4 (Data in Table A-4, Appendix 5)

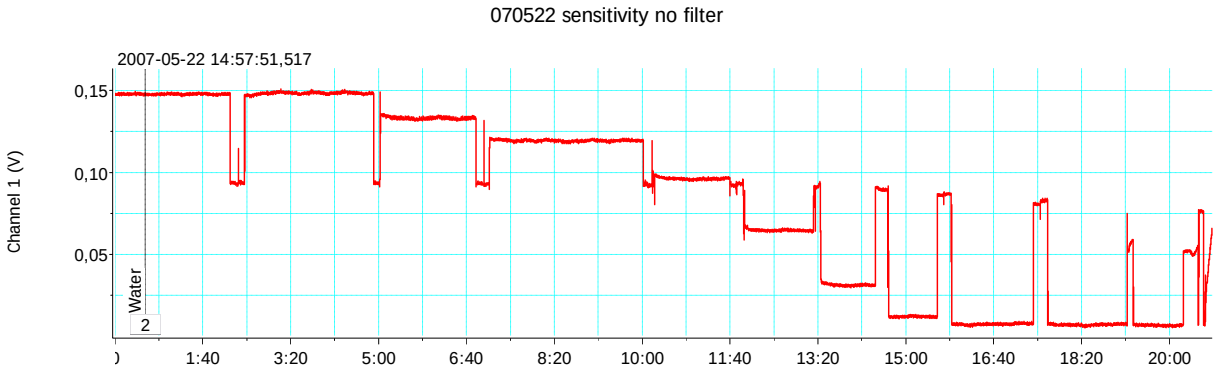


Figure A- 5 (Data in Table A-5, Appendix 5)

Figs. A-6 to A-10 show the absorbance measurements when the mains filter was used. The x-scale is in [min:sec]. In all figures the last part of the cleaning with deionised water was included in the recording.

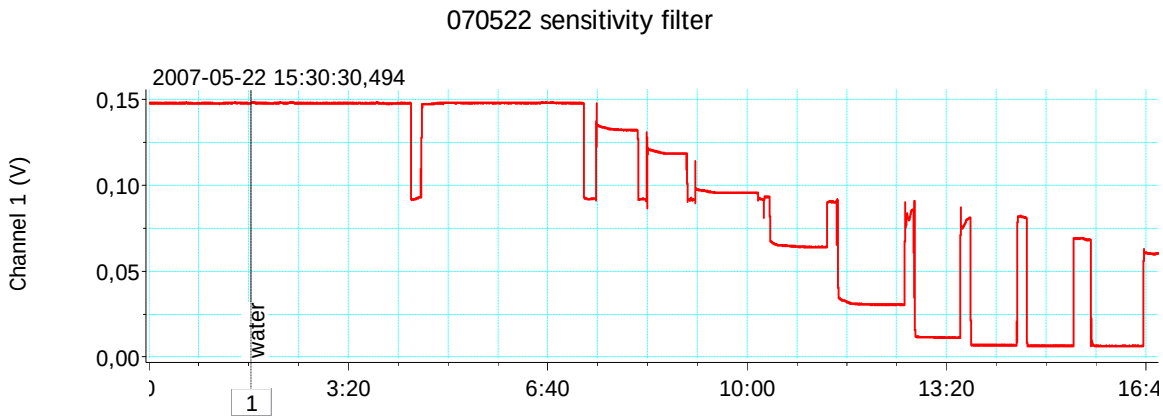


Figure A- 6 (Data in Table A-6, Appendix 5)

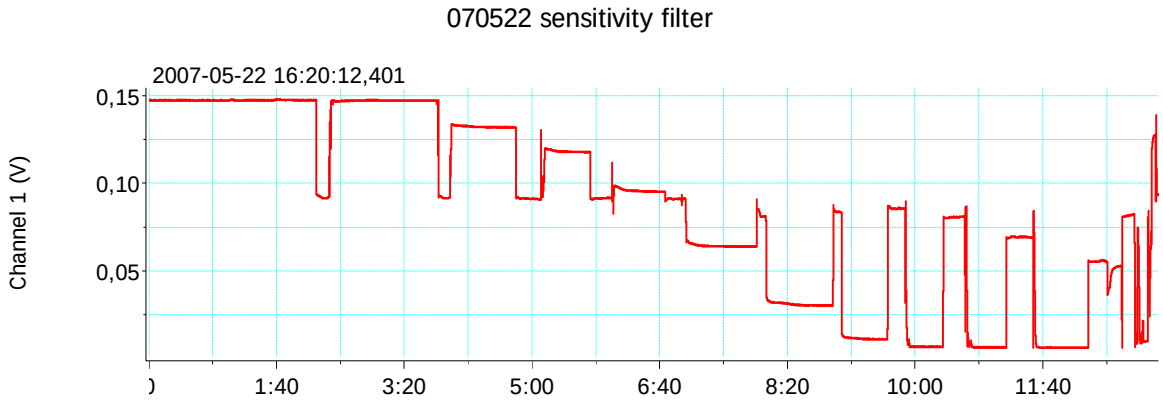


Figure A- 7 (Data in Table A-7, Appendix 5)

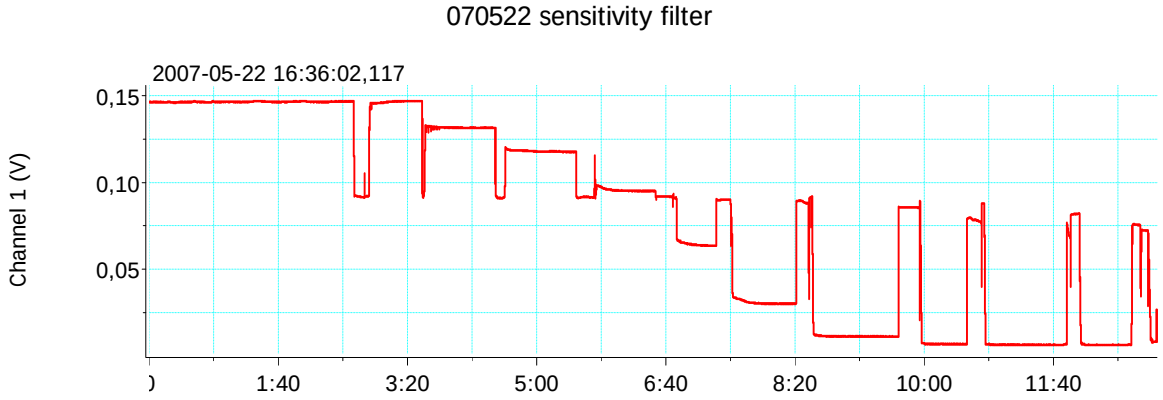


Figure A- 8 (Data in Table A-8, Appendix 5)

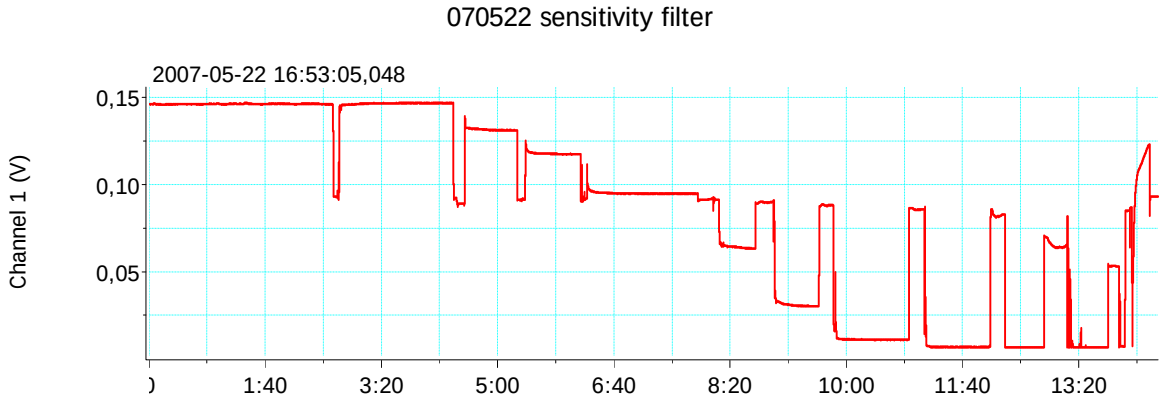


Figure A- 9 (Data in Table A-9, Appendix 5)

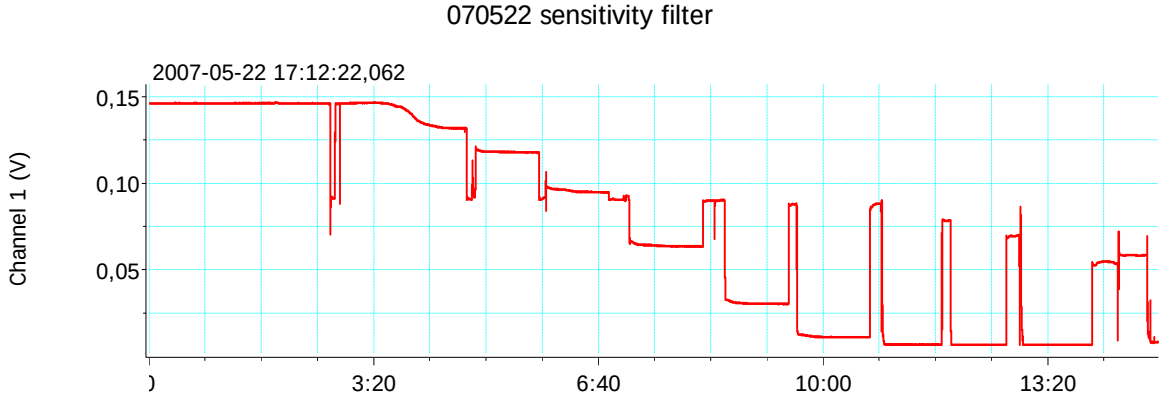


Figure A- 10 (Data in Table A-10, Appendix 5)

Appendix 5

Sensitivity calculations/graphs for LED-AP detector

Tables A-1 to A- 5 show the necessary measurements for sensitivity calculation when no mains filter was used. The values in Table A-1 are from measurement in Fig. A-1, Table A-2 from Fig. A-2 *etc.* Sensitivity is the ratio between the absorbance and the concentration.

Table A- 1 (Graph in Figure A-1, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(V_o/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,146	0	0
9	0,391	0,131	0,048	122,1
8	0,781	0,117	0,095	122,1
7	1,563	0,095	0,185	118,2
6	3,125	0,064	0,356	114,0
5	6,25	0,031	0,674	107,9
4	12,5	0,011	1,123	89,8
3	25	0,007	1,324	52,9
2	50	0,007	1,349	26,9
1	100	0,007	1,351	13,5

Table A- 2 (Graph in Figure A-2, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,146	0	0
9	0,391	0,130	0,048	123,5
8	0,781	0,117	0,097	123,6
7	1,563	0,094	0,188	120,3
6	3,125	0,063	0,362	115,9
5	6,25	0,031	0,676	108,2
4	12,5	0,011	1,110	88,8
3	25	0,007	1,336	53,4
2	50	0,007	1,348	27,0
1	100	0,006	1,350	13,5

Table A- 3 (Graph in Figure A-3, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,148	0	0
9	0,391	0,133	0,046	119,0
8	0,781	0,119	0,094	120,0
7	1,563	0,096	0,186	119,2
6	3,125	0,065	0,359	115,0
5	6,25	0,031	0,679	108,6
4	12,5	0,012	1,109	88,7
3	25	0,007	1,314	52,6
2	50	0,007	1,335	26,7
1	100	0,007	1,344	13,4

Table A- 4 (Graph in Figure A-4, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,149	0	0
9	0,391	0,134	0,048	122,7
8	0,781	0,120	0,096	123,3
7	1,563	0,096	0,190	121,5
6	3,125	0,064	0,365	116,7
5	6,25	0,031	0,686	109,7
4	12,5	0,011	1,119	89,5
3	25	0,007	1,322	52,9
2	50	0,007	1,341	26,8
1	100	0,007	1,360	13,6

Table A- 5 (Graph in Figure A-5, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,149	0	0
9	0,391	0,133	0,048	124,1
8	0,781	0,119	0,096	123,0
7	1,563	0,096	0,191	122,2
6	3,125	0,064	0,363	116,2
5	6,25	0,031	0,683	109,3
4	12,5	0,012	1,106	88,5
3	25	0,007	1,318	52,7
2	50	0,007	1,344	26,9
1	100	0,006	1,372	13,7

Tables A-6 to A- 10 show the sensitivity calculation measurements when the main filter was used. The values in Table A-6 are from measurement in Fig. A-6, Table A-7 from Fig. A-7 *etc.*

Table A- 6 (Graph in Figure A-6, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,148	0	0
9	0,391	0,132	0,049	124,9
8	0,781	0,118	0,097	123,8
7	1,563	0,095	0,191	121,9
6	3,125	0,064	0,365	116,6
5	6,25	0,030	0,689	110,2
4	12,5	0,011	1,126	90,1
3	25	0,007	1,343	53,7
2	50	0,006	1,365	27,3
1	100	0,006	1,373	13,7

Table A- 7 (Graph in Figure A-7, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,147	0	0
9	0,391	0,132	0,048	122,9
8	0,781	0,118	0,096	123,5
7	1,563	0,095	0,189	121,2
6	3,125	0,064	0,364	116,5
5	6,25	0,030	0,686	109,8
4	12,5	0,011	1,127	90,1
3	25	0,007	1,344	53,8
2	50	0,006	1,364	27,3
1	100	0,006	1,372	13,7

Table A- 8 (Graph in Figure A-8, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,147	0	0
9	0,391	0,131	0,048	122,5
8	0,781	0,118	0,096	123,0
7	1,563	0,095	0,189	120,9
6	3,125	0,064	0,363	116,1
5	6,25	0,030	0,691	110,5
4	12,5	0,011	1,125	90,0
3	25	0,007	1,342	53,7
2	50	0,006	1,367	27,3
1	100	0,006	1,370	13,7

Table A- 9 (Graph in Figure A-9, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,146	0	0
9	0,391	0,131	0,048	123,6
8	0,781	0,117	0,097	123,7
7	1,563	0,095	0,190	121,4
6	3,125	0,063	0,365	116,7
5	6,25	0,030	0,688	110,0
4	12,5	0,011	1,127	90,2
3	25	0,007	1,341	53,7
2	50	0,006	1,365	27,3
1	100	0,006	1,369	13,7

Table A- 10 (Graph in Figure A-10, Appendix 4)

Sample	Concentration [mM]	Voltage [V]	log(Vo/V) = Absorbance [AU]	Sensitivity [AU/M]
NaOH	0	(V_0) 0,147	0	0
9	0,391	0,132	0,047	120,9
8	0,781	0,118	0,096	123,0
7	1,563	0,095	0,190	121,3
6	3,125	0,063	0,364	116,6
5	6,25	0,030	0,689	110,2
4	12,5	0,011	1,127	90,2
3	25	0,007	1,343	53,7
2	50	0,006	1,365	27,3
1	100	0,006	1,370	13,7

Fig. A-11 show the sensitivity graphs from the five measurements when no filter was used.

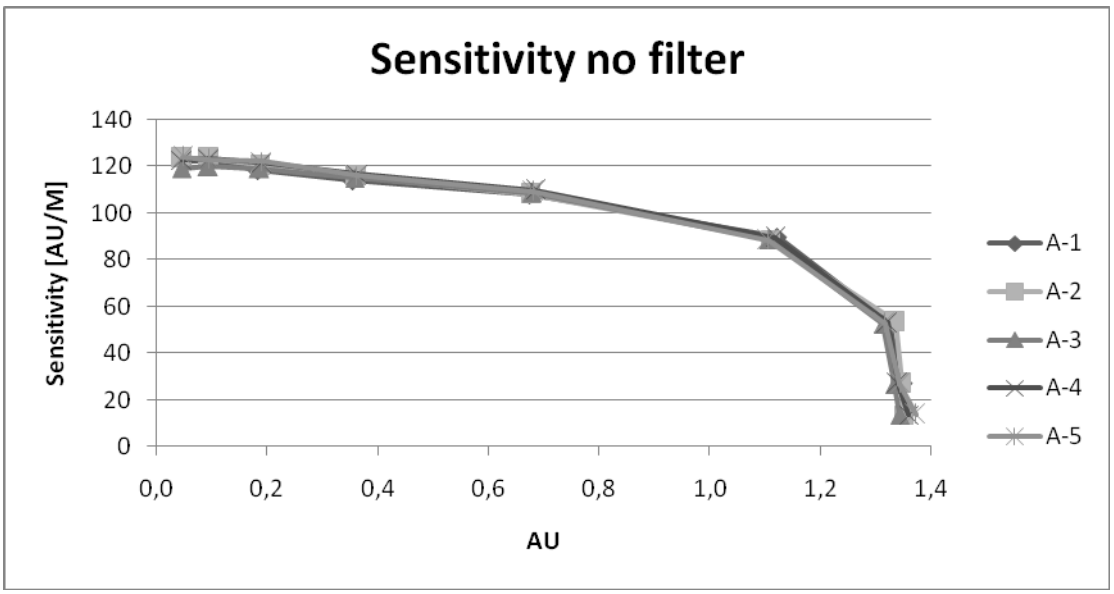


Figure A- 11: Sensitivity graphs when no filter was used.

In Table A-11 the average absorbance values from five measurements are listed, and these values are used for calculations of average sensitivity without main filter.

Table A- 11: Average absorbance and sensitivity without mains filter.

Sample	Concentration [mM]	Average Absorbance [AU]	Average Sensitivity [AU/M]
NaOH	0	0	0
9	0,391	0,048	122,3
8	0,781	0,096	122,4
7	1,563	0,188	120,3
6	3,125	0,361	115,6
5	6,25	0,680	108,7
4	12,5	1,113	89,0
3	25	1,323	52,9
2	50	1,343	26,9
1	100	1,356	13,6

Fig. A-12 shows the sensitivity graphs from five measurements when the main filter was used.

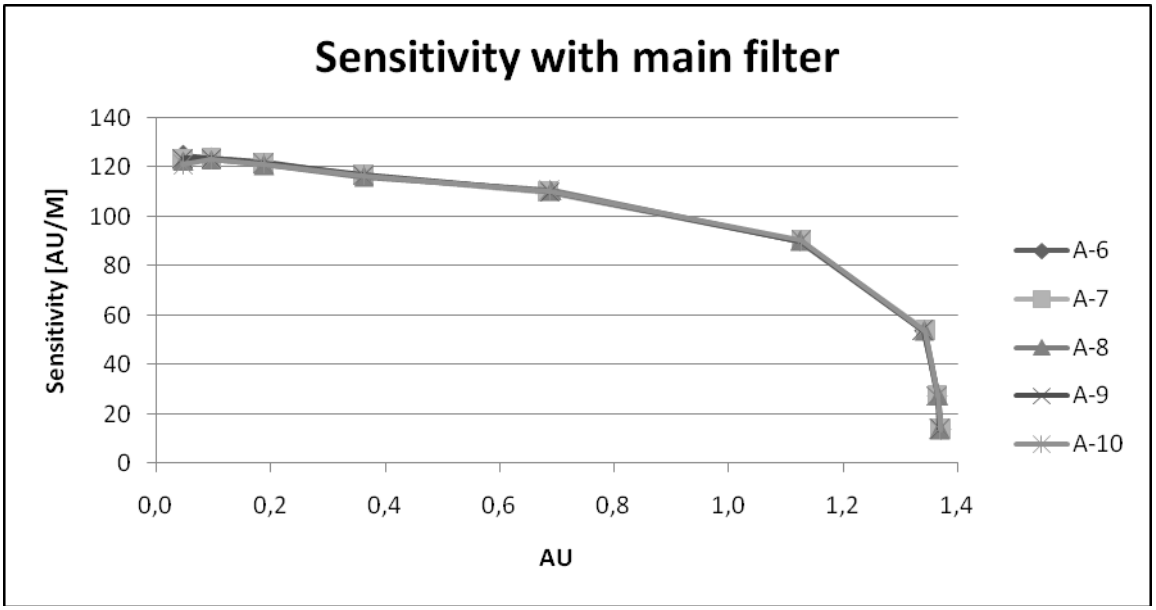


Figure A- 12: Sensitivity graph when the main filter was used.

In Table A-12 the average absorbance values from five measurements are listed, and these values are used for calculations of average sensitivity with main filter.

Table A- 12: Average absorbance and sensitivity with main filter.

Sample	Concentration [mM]	Average Absorbance [AU]	Average Sensitivity [AU/M]
NaOH	0	0	0
9	0,391	0,048	123,0
8	0,781	0,096	123,4
7	1,563	0,190	121,3
6	3,125	0,364	116,5
5	6,25	0,689	110,2
4	12,5	1,126	90,1
3	25	1,343	53,7
2	50	1,365	27,3
1	100	1,371	13,7

The values from Tables A-11 and A-12 are used to make an average sensitivity graph with and without mains filter (Fig. A-13). The horizontal dotted line show where the sensitivity graphs fall below 90 % of maximum sensitivity value [25]. The sensitivity is then recalculated to AU and mM using linear regression analysis.

Max linearity without the filter = 122.4 * 0.9 = 110.2 AU/M ≈ 0.61 AU ≈ 5.6 mM

Max linearity with the main filter =123.4 * 0.9 = 111.0 AU/M ≈ 0.64 AU ≈ 5.8 mM

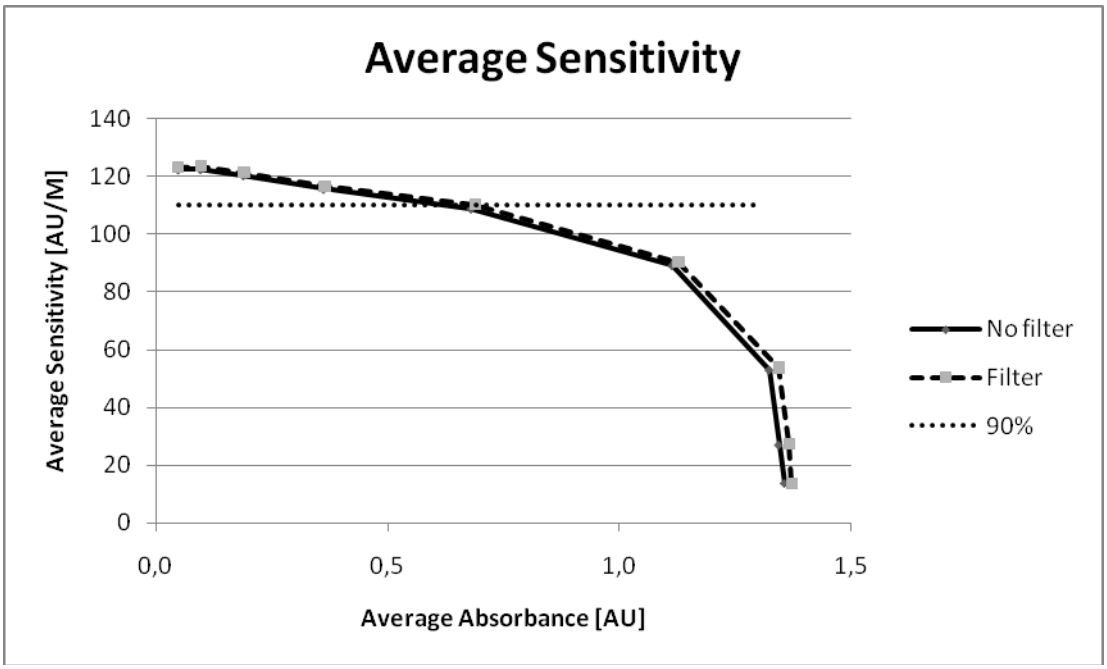


Figure A- 13: Average sensitivity graph.

From the average sensitivity the effective pathlength (ℓ_p) was calculated using Eq. 1 ($\ell_p = s/\varepsilon$). The sensitivity used was the average from the values at three lowest concentrations since the graph is flattest in this section. The diameter of the capillary is ≈75 µm. (ε = 16400 [21])

$$Without\ filter \rightarrow l_p = \frac{s}{\varepsilon} = \frac{(122.30 + 122.39 + 120.28)/3}{16400} = 0.00742\ [cm]$$

$$With\ main\ filter \rightarrow l_p = \frac{s}{\varepsilon} = \frac{(122.96 + 123.38 + 121.35)/3}{16400} = 0.00747\ [cm]$$

The Stray light was calculated using Eq. 2 ($I_o/I = 10^{-A}$), maximum absorbance was used.

$$Stray\ light\ without\ filter = \frac{I_o}{I} = 10^{-A} = 10^{-1.35} = 0.0447 \approx 4.5\ \%$$

$$Stray\ light\ with\ main\ filter = \frac{I_o}{I} = 10^{-A} = 10^{-1.37} = 0.0426 \approx 4.3\ \%$$

Appendix 6

LOD measurements for LED-IF detector, silicone oil

Figs. A-14 to A-18 show the measurements for LED-IF detector when the silicone oil was used. The line named 0.01 is a marker to indicate when fluorescein was injected. X-scale is in [min:sec].

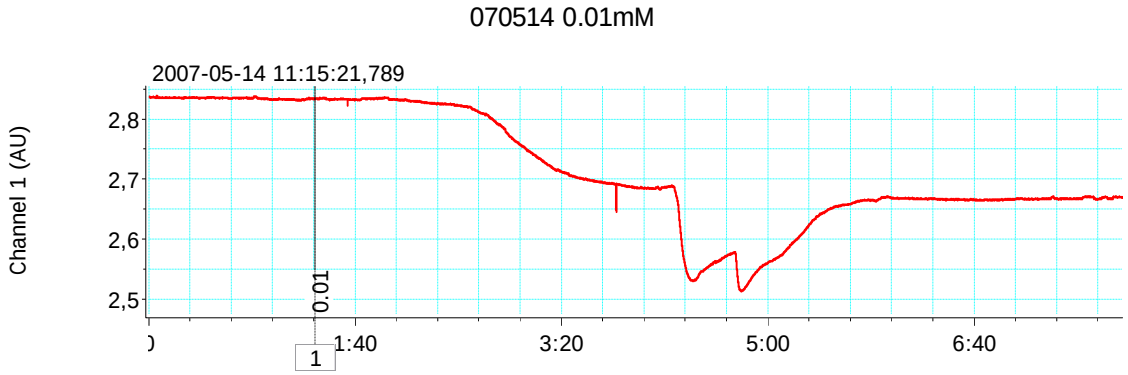


Figure A- 14: (Data in Table A-13, Appendix 8)

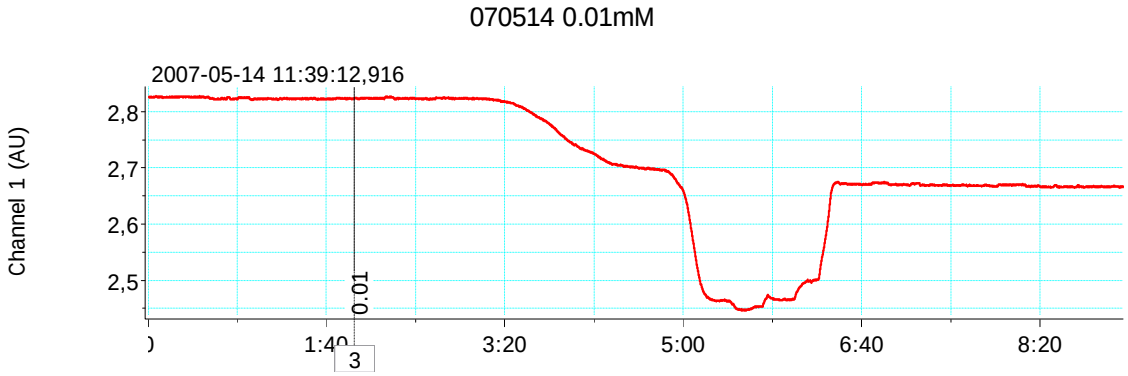


Figure A- 15: (Data in Table A-13, Appendix 8)

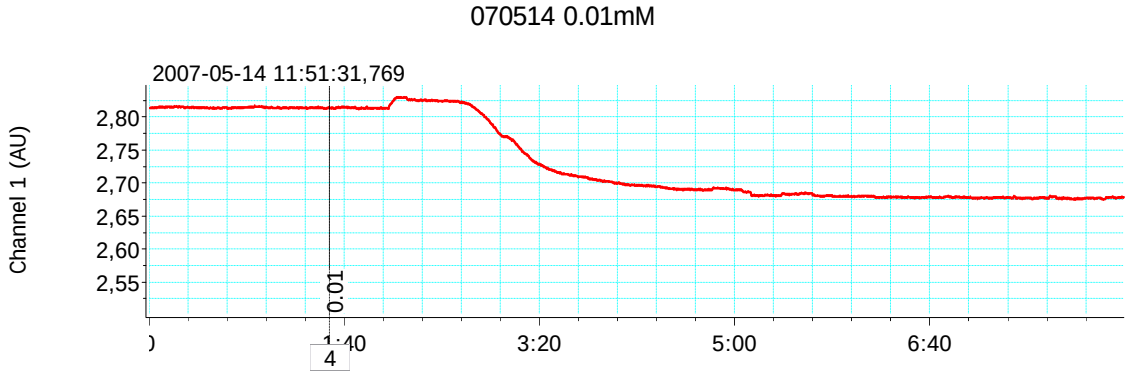


Figure A- 16 (Data in Table A-13, Appendix 8)

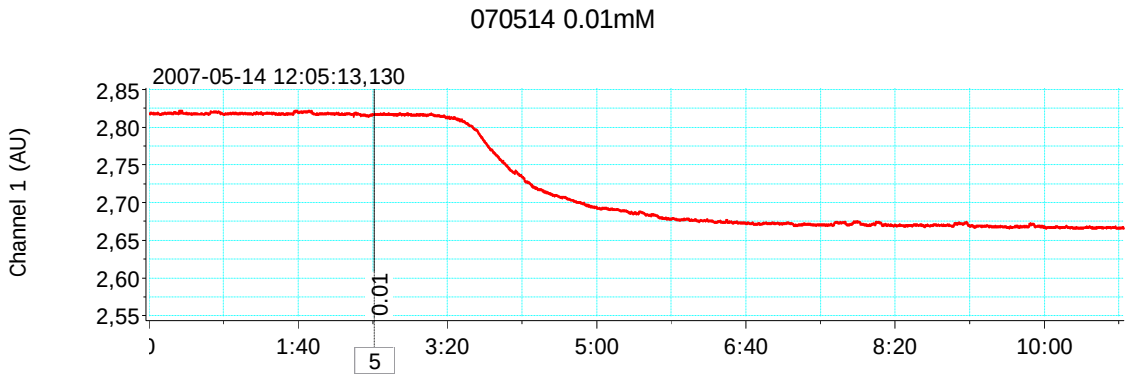


Figure A- 17: (Data in Table A-13, Appendix 8)

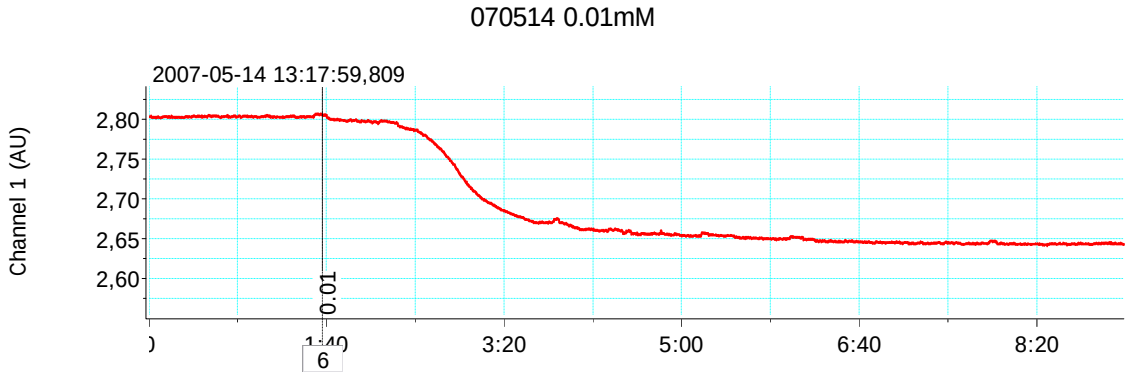


Figure A- 18: (Data in Table A-13, Appendix 8)

Appendix 7

LOD measurements for LED-IF detector, immersion oil

Figs. A-19 to A-23 show the measurements for LED-IF detector when the immersion oil was used. The line named 0.01 is just a marker to indicate when fluorescein was injected. X-scale is in [min:sec].

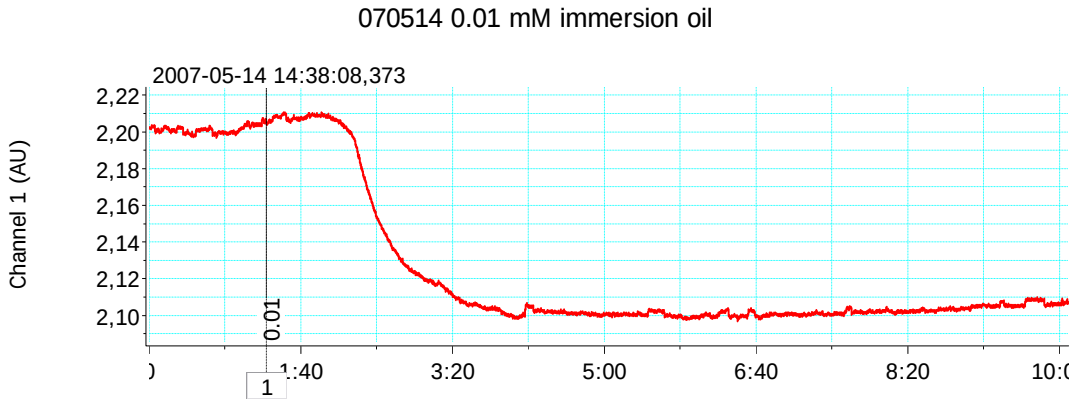


Figure A- 19 (Data in Table A-14, Appendix 9)

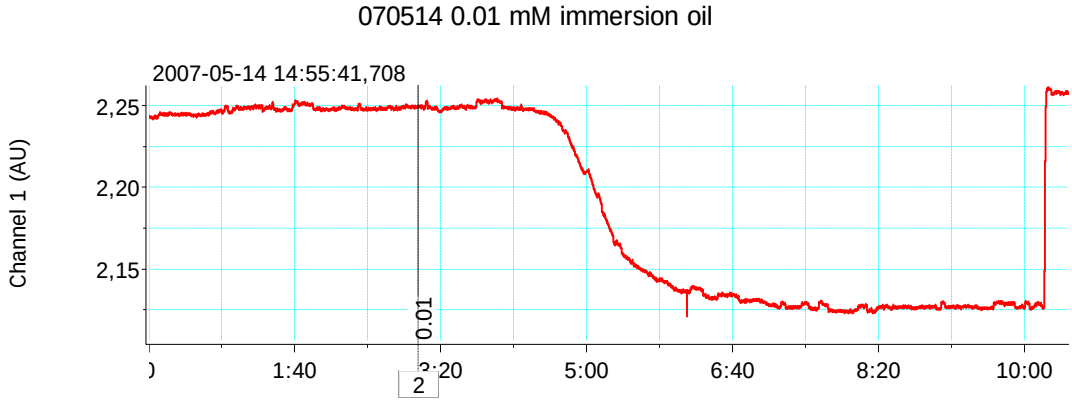


Figure A- 20 (Data in Table A-14, Appendix 9)

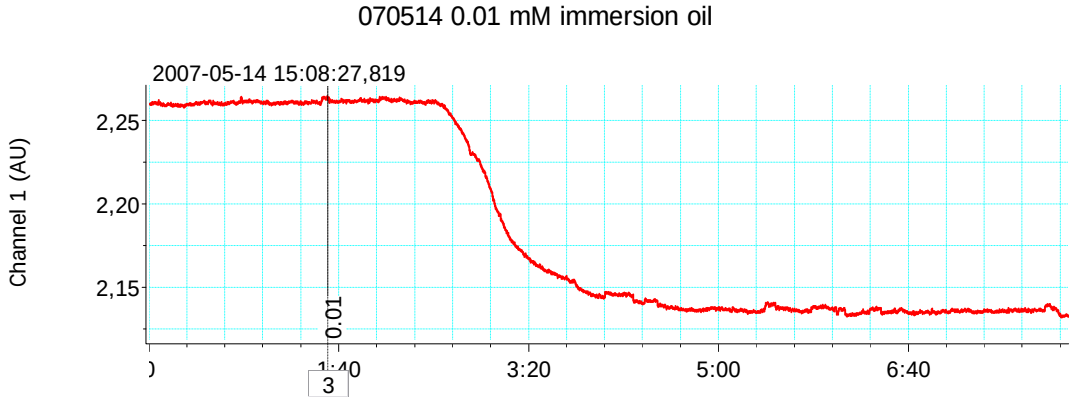


Figure A- 21 (Data in Table A-14, Appendix 9)

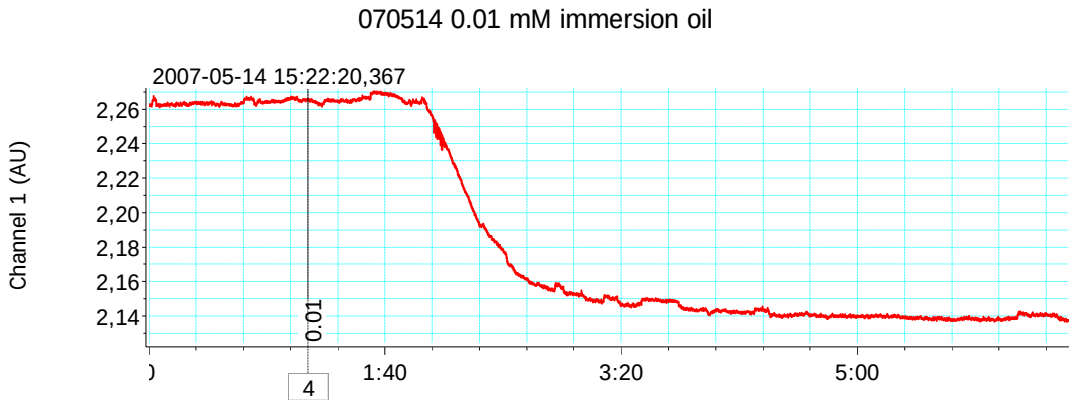


Figure A- 22 (Data in Table A-14, Appendix 9)

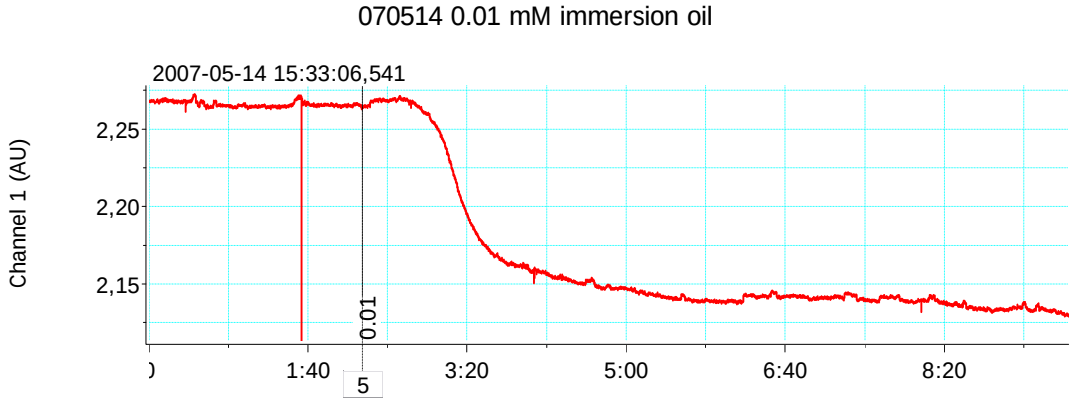


Figure A- 23 (Data in Table A-14, Appendix 9)

Appendix 8

LOD calculations for LED-IF detector, silicone oil

Table A-13 shows the limit of detection (LOD) calculations when silicone oil was used.

Details:
The Graph nr refers to Figure number in Appendix 6.
Concentration (C) refers to the concentration of fluorescein.
Baseline is the absorbance value before the injection.
Signal is the absorbance value after injection.
The noise was determined from the baseline measurement before the injection was done.
Change is the difference between the baseline and the signal.
S/N is the ratio between the change and the noise.
LOD is the calculated concentration (C_{LOD}) when $S/N_{LOD} = 3$:

$C/C_{LOD} = (S/N)/(S/N_{LOD})$

$C_{LOD} = 0.01*3/(S/N)$

Average is the average value of LOD.
STDAV is standard deviation for LOD. This was calculated by using the STDAV function in Excel (Swedish version).

Table A- 13

Silicone oil

Graph Nr	Concentration [mM]	Baseline	Signal	Noise N [AU]	Change S	S/N	LOD [mM]
A- 14	0.01	2.833	2.666	0.003	0.167	55.67	0.00054
A- 15	0.01	2.823	2.666	0.003	0.157	52.33	0.00057
A- 16	0.01	2.813	2.667	0.003	0.146	48.67	0.00062
A- 17	0.01	2.817	2.667	0.003	0.15	50.00	0.00060
A- 18	0.01	2.803	2.643	0.003	0.16	53.33	0.00056
						Average	0.00058
						STDAV	0.00002

Appendix 9

LOD calculations for LED-IF detector, immersion oil

Table A-14 shows the limit of detection (LOD) calculations when immersion oil was used.

Details:
The Graph nr refers to Figure number in Appendix 7.
Concentration (C) refers to the concentration of fluorescein.
Baseline is the absorbance value before the injection.
Signal is the absorbance value after injection.
The noise was determined from the baseline measurement before the injection was done.
Change is the difference between the baseline and the signal.
S/N is the ratio between the change and the noise.
LOD is the calculated concentration (C_{LOD}) when $S/N_{LOD} = 3$:

$C/C_{LOD} = (S/N)/(S/N_{LOD})$

$C_{LOD} = 0.01*3/(S/N)$

Average is the average value of LOD.
STDAV is standard deviation for LOD. This was calculated by using the STDAV function in Excel (Swedish version).
The first measurement was excluded when the average and STDAV was calculated as it is an outlier according to both Q-test and Grubbs test.

Table A- 14

Immersion oil

Graph Nr	Concentration [mM]	Baseline	Signal	Noise N	Change S	S/N	LOD [mM]
~~**A-19**~~	~~0,01~~	~~2,207~~	~~2,1~~	~~0,003~~	~~0,107~~	~~35,67~~	~~0,00084~~
A-20	0,01	2,248	2,126	0,003	0,122	40,67	0,00074
A-21	0,01	2,261	2,135	0,003	0,126	42,00	0,00071
A-22	0,01	2,265	2,138	0,003	0,127	42,33	0,00071
A-23	0,01	2,265	2,141	0,003	0,124	41,33	0,00073
						Average	0,00072
						STDAV	0,00001

Appendix 10

Linearity measurements and calculations for LED-IF detector

Figs. A-24 to A-28 show the linearity measurements for LED-IF. X-scale is in [min:sec].

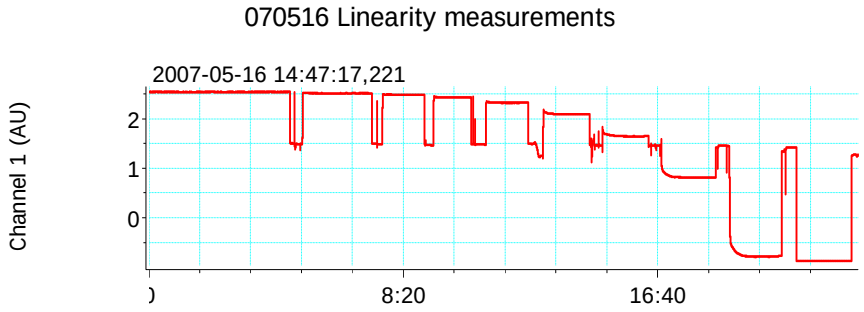


Figure A- 24 (Data in Table A-15)

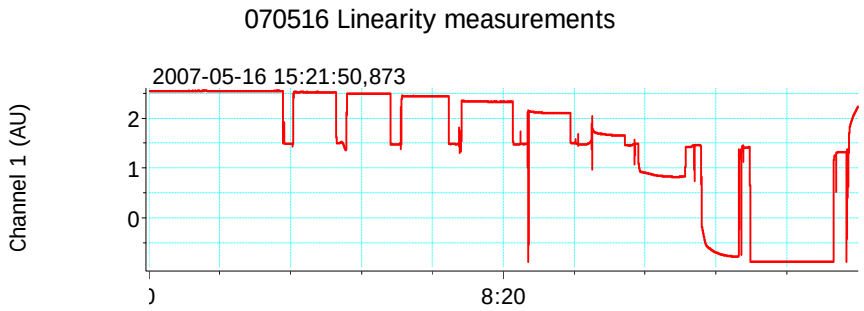


Figure A- 25 (Data in Table A-16)

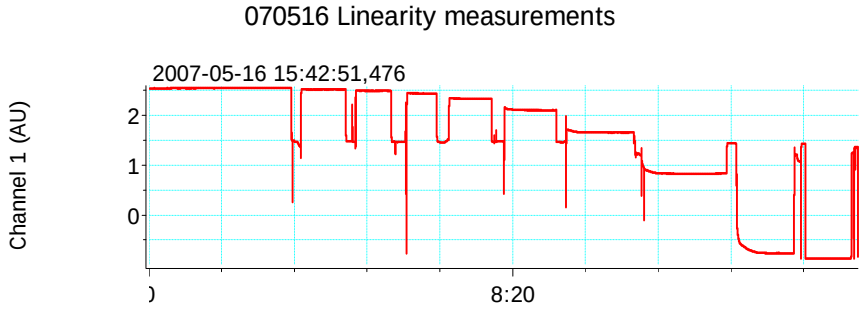


Figure A- 26 (Data in Table A-17)

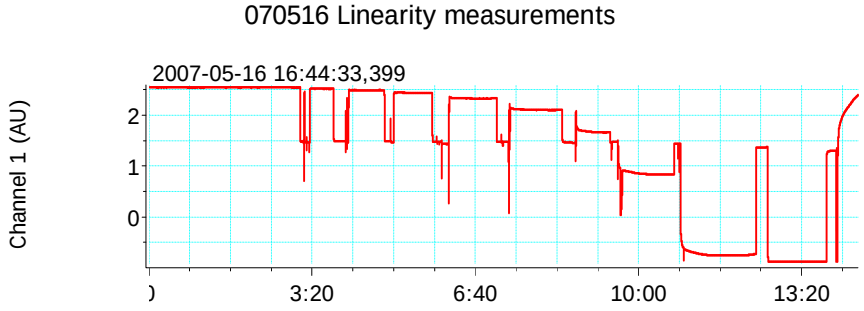


Figure A- 27 (Data in Table A-18)

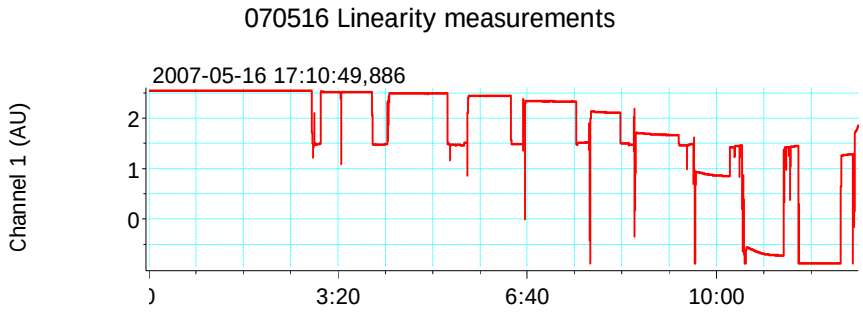


Figure A- 28 (Data in Table A-19)

Calculations and graphs.

Tables A-15 to A-19 show the measurements needed for the linearity calculation. The detector was designed to measure absorbance, but during the measurements intensity was recorded. That is why the higher the concentration of sample , the lower intensity (higher absorbance value) was observed.

Table A- 15 (Graph in figure A-24)

Concentration [mM]	Signal	Zeroed signal	Intensity [AU]
0,4	-0,886	-3,420	3,42
0,2	-0,796	-3,330	3,33
0,1	0,804	-1,730	1,73
0,05	1,638	-0,896	0,90
0,025	2,088	-0,446	0,45
0,0125	2,315	-0,219	0,22
0,00625	2,426	-0,108	0,11
0,003125	2,478	-0,056	0,06
0,0015625	2,507	-0,027	0,03
0	2,534	0,000	0,00

Table A-16 (Graph in figure A-25)

Concentration [mM]	Signal	Zeroed signal	Intensity [AU]
0,4	-0,886	-3,425	3,43
0,2	-0,774	-3,313	3,31
0,1	0,817	-1,722	1,72
0,05	1,649	-0,890	0,89
0,025	2,096	-0,443	0,44
0,0125	2,323	-0,216	0,22
0,00625	2,434	-0,105	0,11
0,003125	2,485	-0,054	0,05
0,0015625	2,513	-0,026	0,03
0	2,539	0,000	0,00

Table A- 17 (Graph in figure A-26)

Concentration [mM]	Signal	Zeroed signal	Intensity [AU]
0,4	-0,886	-3,422	3,42
0,2	-0,775	-3,311	3,31
0,1	0,820	-1,716	1,72
0,05	1,648	-0,888	0,89
0,025	2,095	-0,441	0,44
0,0125	2,322	-0,214	0,21
0,00625	2,429	-0,107	0,11
0,003125	2,481	-0,055	0,06
0,0015625	2,508	-0,028	0,03
0	2,536	0,000	0,00

Table A- 18 (Graph in figure A-27)

Concentration [mM]	Signal	Zeroed signal	Intensity [AU]
0,4	-0,886	-3,424	3,43
0,2	-0,761	-3,299	3,30
0,1	0,824	-1,714	1,71
0,05	1,653	-0,885	0,89
0,025	2,095	-0,443	0,44
0,0125	2,319	-0,219	0,22
0,00625	2,428	-0,110	0,11
0,003125	2,481	-0,057	0,06
0,0015625	2,510	-0,028	0,03
0	2,538	0,000	0,00

Table A- 19 (Graph in figure A-28)

Concentration [mM]	Signal	Zeroed signal	Intensity [AU]
0,4	-0,886	-3,421	3,42
0,2	-0,728	-3,263	3,26
0,1	0,850	-1,686	1,67
0,05	1,663	-0,872	0,87
0,025	2,107	-0,428	0,43
0,0125	2,324	-0,211	0,21
0,00625	2,431	-0,104	0,11
0,003125	2,480	-0,055	0,06
0,0015625	2,509	-0,026	0,03
0	2,535	0,000	0,00

The intensity values from Tables A-15 to A-19 (corresponding to the recorded values from Figs. A-24 to A-28) were plotted over the concentration range giving the linearity graph in Fig. A-29.

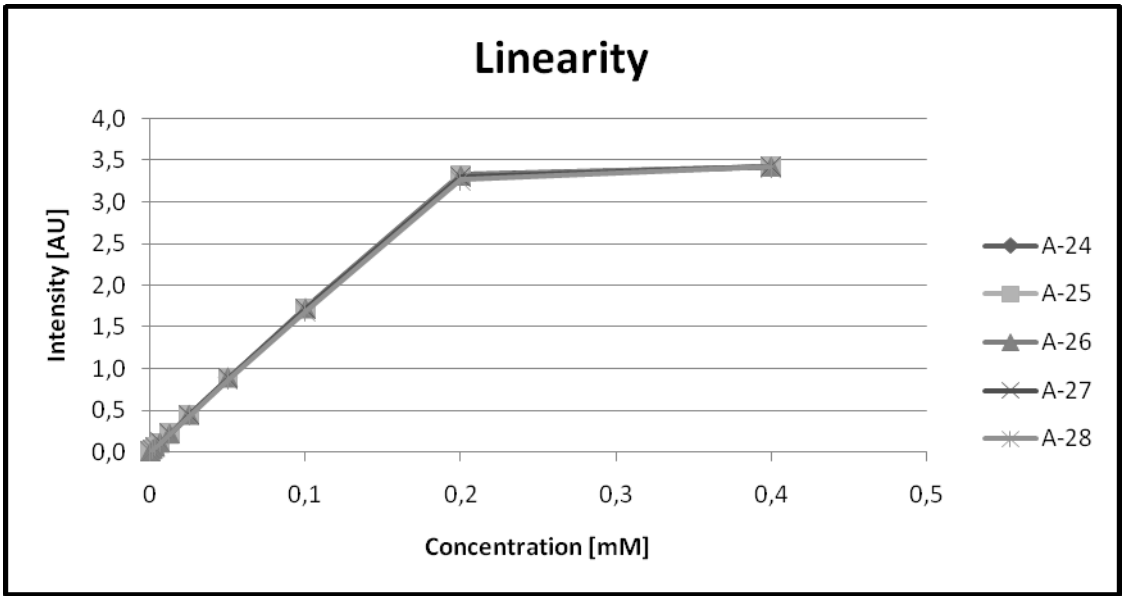


Figure A- 29: Linearity graph for fluorescein using the LED-IF detector.

Table A-20 is showing the calculated average intensity from the 5 measurements. The average intensity values over concentration are plotted in the average linearity graph in Fig. A-30.

Table A- 20: Calculated average intensity.

Concentration [mM]	Intensity [AU]
0,4	3,42
0,2	3,30
0,1	1,71
0,05	0,89
0,025	0,44
0,0125	0,22
0,00625	0,11
0,003125	0,06
0,0015625	0,03
0	0,00

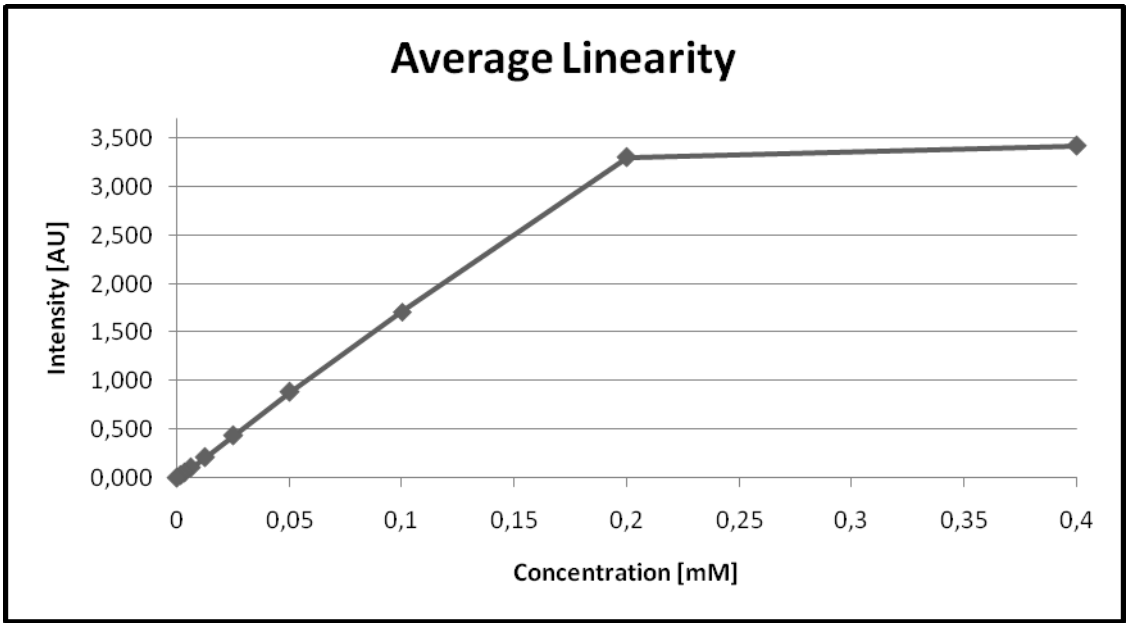


Figure A- 30: Average linearity graph for fluorescein using the LED-IF detector.

The upper linearity limit was determined from the graph at the point where line changes slope (at concentration of 0.2 mM). The regression coefficient value was calculated to 0.999 using Excel.

Appendix 11

Anion separations for LED-AP detector in CE

Figs. A-31 to A-38 show the separations for the LED-AP detector in the CE. The injection was done for 4 seconds at -15 kV. X-scale is in [min:sec]

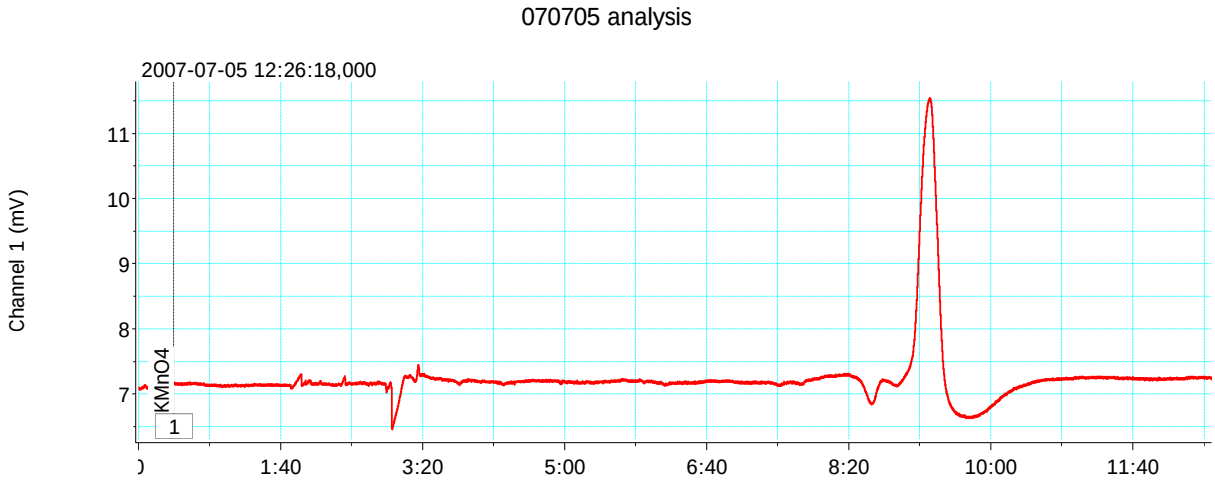


Figure A- 31: Anion MnO_4^-

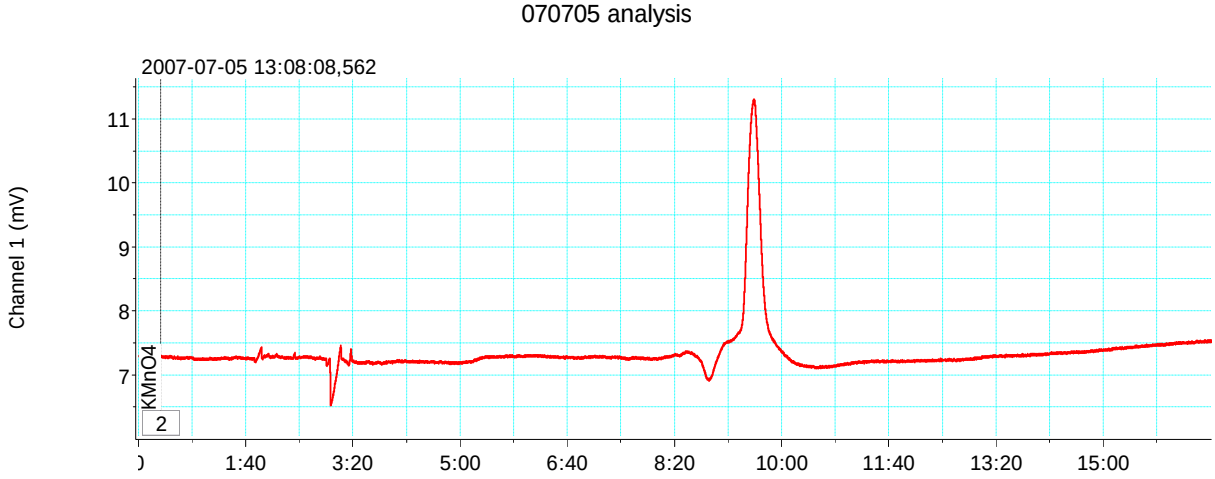


Figure A- 32: Anion MnO_4^-

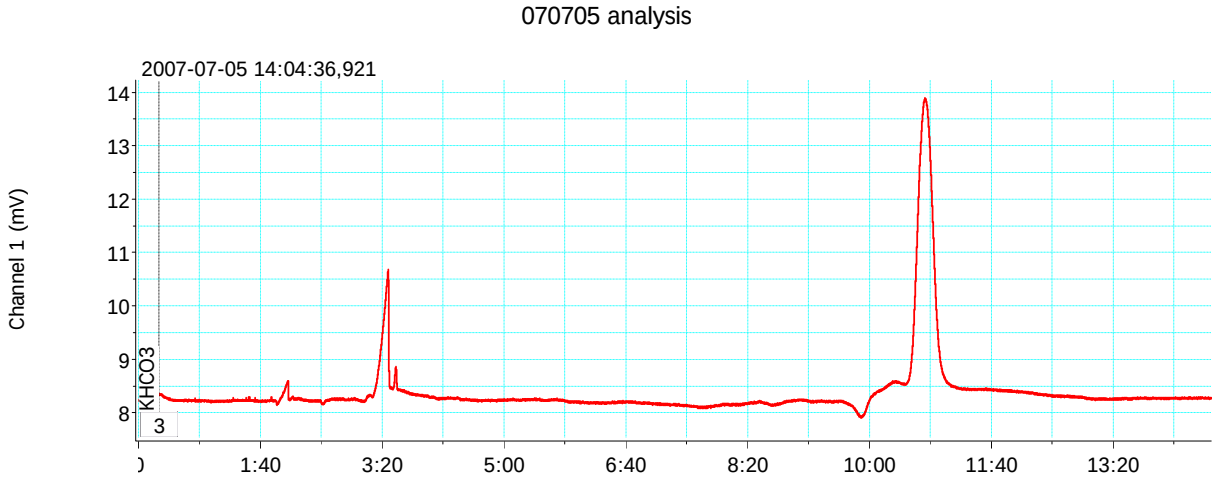


Figure A- 33: Anion HCO_3^-

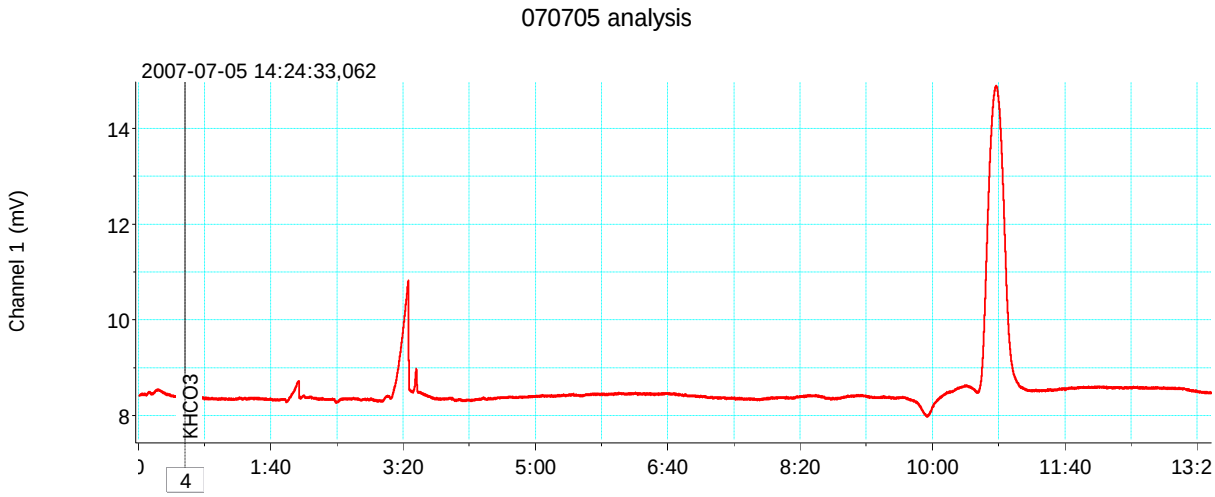


Figure A- 34: Anion HCO_3^-

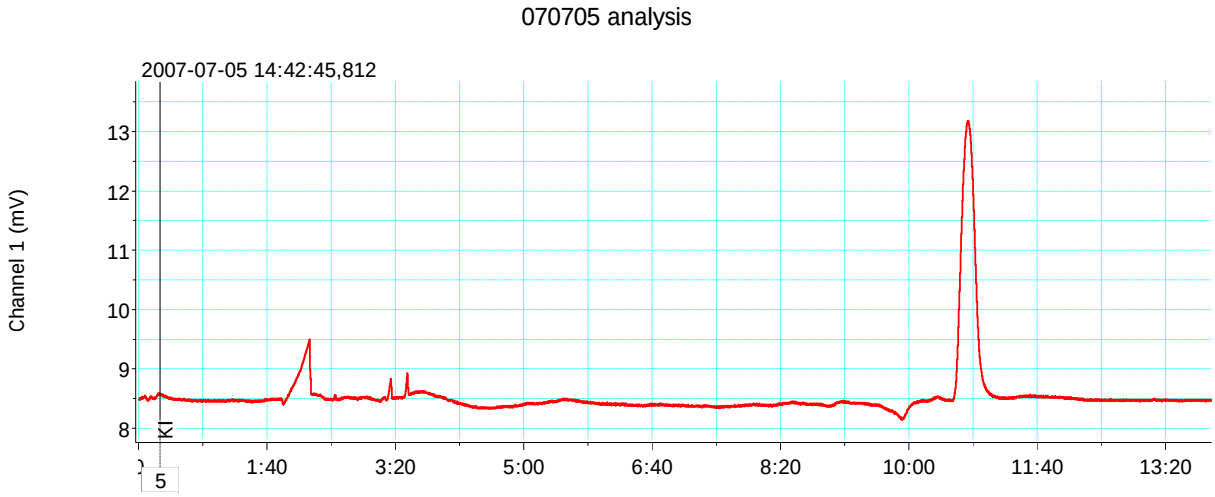


Figure A-35: Anion I^-

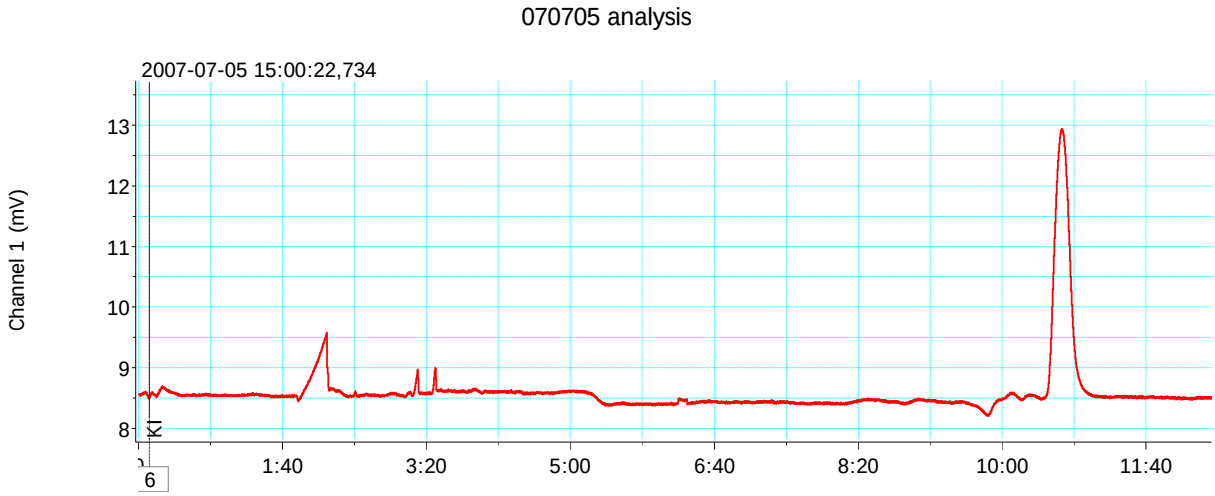


Figure A- 36: Anion I^-

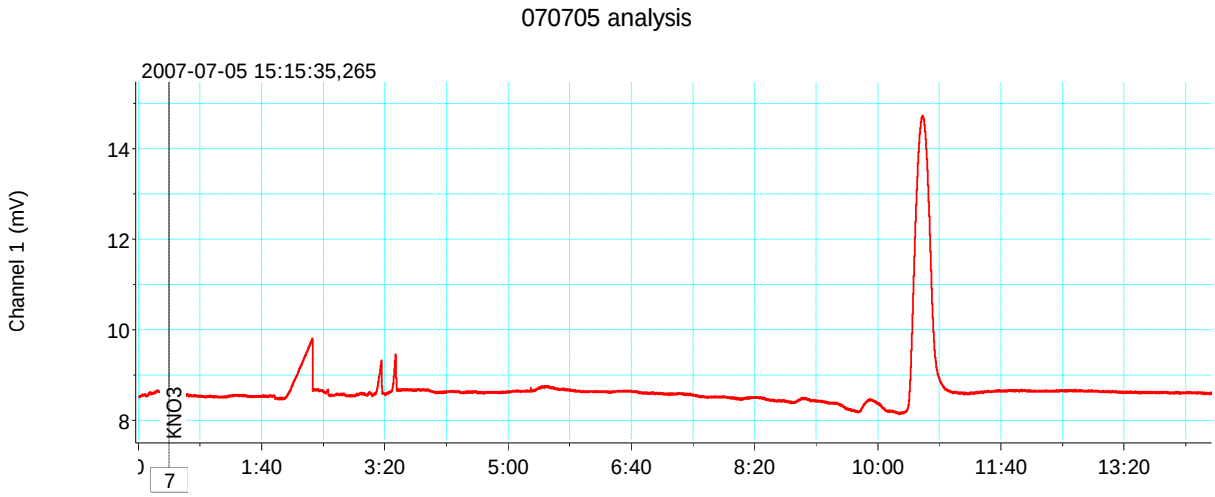


Figure A-37: Anion NO_3^-

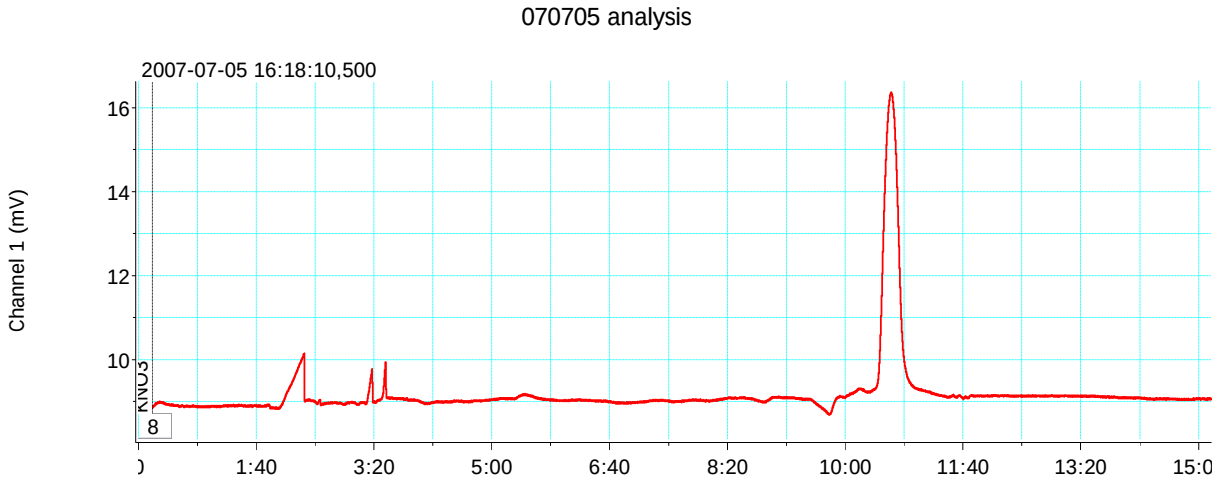


Figure A-38: Anion NO_3^-

Table A-21 shows the migration times for the anions. The separation number equals the figure number that can be found above.

Table A- 21: Peak times.

Separation Nr	Anion	Anion peak [min:sec]	EOF peak [min:sec]
A-31	MnO_4^-	2:58	9.17
A-32	MnO_4^-	2:59	9.33
A-33	HCO_3^-	3:25	10:45
A-34	HCO_3^-	3:23	10:47
A-35	I^-	2:13	10:45
A-36	I^-	2:10	10:40
A-37	NO_3^-	2:21	10:36
A-38	NO_3^-	2:20	10:38