Flow Injection Analysis of industrial water samples

by

Agata Dominika Makas, BSc (Hons)

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Abstract

An automated version of Flow Injection Analysis (FIA) instrumentation has been designed and evaluated for the quantitative determination of chemical oxygen demand (COD) for industrial samples. The distinct advantages over conventional COD assays are the significantly reduced analysis time (FIA complete in 10 min, compared with conventional COD assay times of 120 min); simplified sample handling; significantly reduced sample volume (less than 1 cm^3 for FIA); significantly reduced reagent consumption; and the avoidance of highly toxic reagents (such as Cr^{VI+}). The FIA system developed for this project included automation with a microcontroller, which enabled automatic sample introduction, which removed imprecision associated with the equivalent manual process.

Glucose was used as a calibration standard, and to assess the day to day reproducibility of the apparatus. A direct comparison between the FIA assay and a commercial system made on the same samples of aqueous paint waste washings (kindly donated by Crown Paints Ltd. Darwen, Lancashire) showed that the FIA system gave close equivalent quantitation, but with a consistent over prediction which is due to the systematic error associated with using glucose as a standard. A scaling factor would be required to ensure equivalent COD values. The FIA system generated bubbles and required systematic deposit removal in the coiled oxidation reactor; effects of air bubbles in the analysis has been studied and resolved in this project. The FIA-based COD assay had a linear range over 0.1 mM to 1.1 mM glucose concentration – equivalent to 18 mg/l to 198 mg/l; a limit of detection of 0.1 mM and a response time of 5 min, this was based on permanganate chemistry monitored at 525 nm using an Agilent Technologies Cary 60 UV-Vis Spectrophotometer fitted with a 1 cm path - length flow cell.

Declaration

No portion of the work referred to this dissertation has been submitted in support of an application for another degree qualification for this or any other university or institute of learning.

Agata D. Makas

Acknowledgements

At the beginning of the project I would like to thank Professor Peter Fielden who has supervised me through this study. He also inspired me to choose this particular subject.

In addition, I would like to thank Dr Sara Baldock who helped me with my project and Crown Paints Ltd. Company (Darwen, Lancashire) for access to their water samples.

Finally, I wish to thank my family for their support and encouragement throughout my studies.

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1 Introduction

The aim of the following study is to determine the potential of flow injection analysis (FIA) as an alternative technique for chemical oxygen demand (COD) measurements commonly used in industry.

The objectives of this study are to:

- review and adapt the COD method from the literature by Korenaga & Ikatsu (from 1981)[1],
- select the best operating conditions (i.e. temperature, time and reagents),
- to experimentally obtain and analyse of UV-vis spectrograms associated with the COD measurement process,
- investigate and solve problems that occurred during the performance of the experiment,
- develop a calibration procedure with construction and usage of calibration graphs over a useful range of sample concentrations and
- analyse of samples supplied by Crown Paints and compare of the results of the FIA procedure against those obtained from the supplier's COD analyser.

1.1 Flow Injection Analysis (FIA)

Flow Injection Analysis is a broadly applicable and well known technique used in analytical chemistry. It is based on a sample injection into a flowing carrier reagent stream where mixing occurs. Then the injected mixture of sample and carrier forms a peak profile that can then be detected[2], [3]. The FIA technique is an automated version of the wet - chemistry batch technique presented in Figure 1 where known volumes of sample and reagent are mixed in a beaker (or other volumetric container) and then put into an analytical measurement instrument[4].

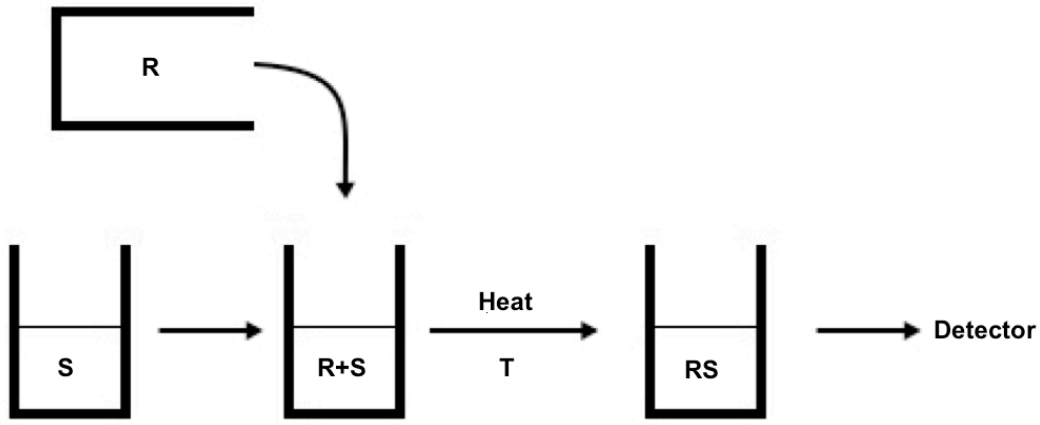


Figure 1: Batch process. *R* indicates analytical reagent(s), *S* is the sample, *RS* is the reaction product, *T* is time[4].

The physical foundations of FIA are related to dispersion and diffusion. Treatment of solute dispersion in a tube considers the relation between axial and radial dispersion on the shape of a sample plug as it moves downstream at a constant flow rate under laminar flow conditions as shown in Figure 2 and Figure 3.

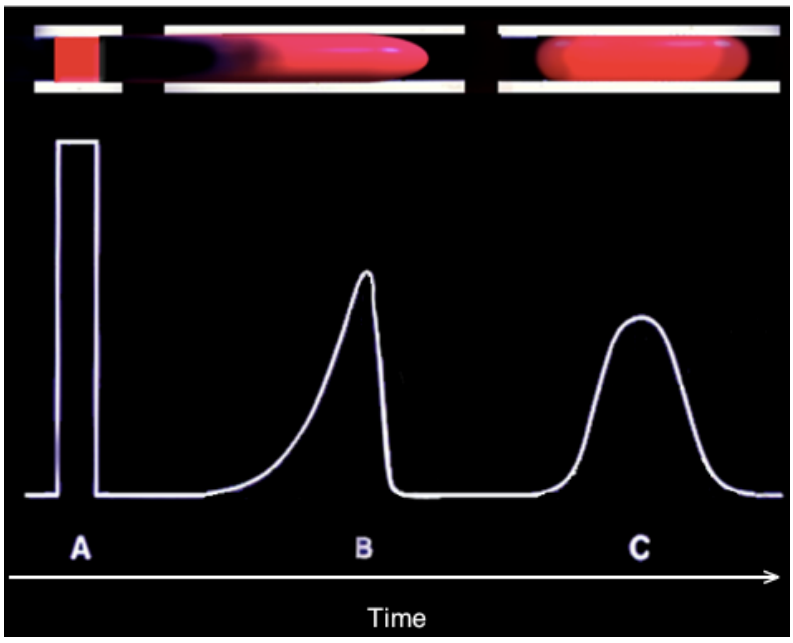


Figure 2: Sample dispersion and signal profiles in the tubing during FIA[5].

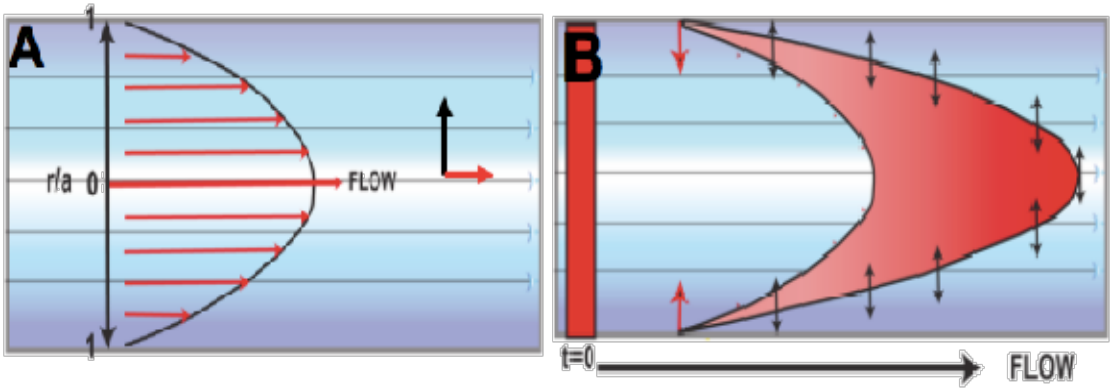


Figure 3: Laminar flow with parabolic shape (A) and bullet shaped, three-dimensional concentration gradient[5].

The flow velocity (u) may be calculated using equation:

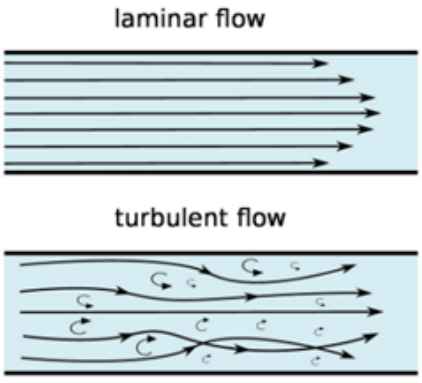
$u = 2\bar{u}\left(1 - \left(\frac{r}{a}\right)^2\right)\left[\frac{mm}{s}\right]$ where $\bar{u}$ is mean flow velocity $\left[\frac{\text{mm}}{\text{s}}\right]$, *r* is radial distance [mm], *a* is radius of the channel [mm].

Diffusion is the spreading of molecules or atoms due to a gradient in concentration. Diffusion length (*L*, distance travelled by reagents) in the tubing can be calculated using the following equation, which is based on Fick's Law.

$L^2 = 2Dt\ [mm^2]$ where *D* is diffusion coefficient (in aqueous solution it has a value between $6 \cdot 10^{-9}$ to $2 \cdot 10^{-9}$ $\frac{m^2}{s}$), *t* is time [s].

Movement of solvent may be descried by calculating the Reynolds Number. Mixing will change in different kind of flows.

The Reynolds number is the ratio of inertial forces to viscous forces and is a convenient parameter for predicting if a flow condition will be laminar or turbulent (Figure 4).



Re < 2000, particles move in straight lines, rare in water systems

Re > 4000, irregular movements of particle in the fluid, most common in water systems

Figure 4: Difference in laminar and turbulent flow in the tubing[6].

$Re = \frac{puL}{v}\left[\frac{\frac{g}{mm^3}\cdot\frac{mm}{s}\cdot mm}{\frac{g}{s\cdot mm}} = 1\right]$ where *Re* is Reynolds number, *u* is flow velocity $\left[\frac{\text{mm}}{\text{s}}\right]$, *p* is density of the fluid $\left[\frac{\text{g}}{\text{mm}^3}\right]$, v is kinematic viscosity of the fluid $\left[\frac{\text{g}}{\text{s}\cdot\text{mm}}\right]$ and *L* is characteristic linear dimension [mm].

FIA is simple, quick, versatile and gives quantitative results[7]. Analysis does not require large volumes and complex sample preparation. In every technique, the possibility of reconstruction and reproduction is the most important part. Flow injection analysis gives certainty that the sample volume does not significantly change between injections. Therefore, automation makes FIA a perfect technique for analysis. What is more automation helps in cost and hazard reduction, and the implementation of control and timing[8]. Furthermore, automation reduces the risk of contamination[9]. Since FIA is an automated technique, it significantly reduces human mistakes which leads to increased precision and improved reproducibility[4], [10].

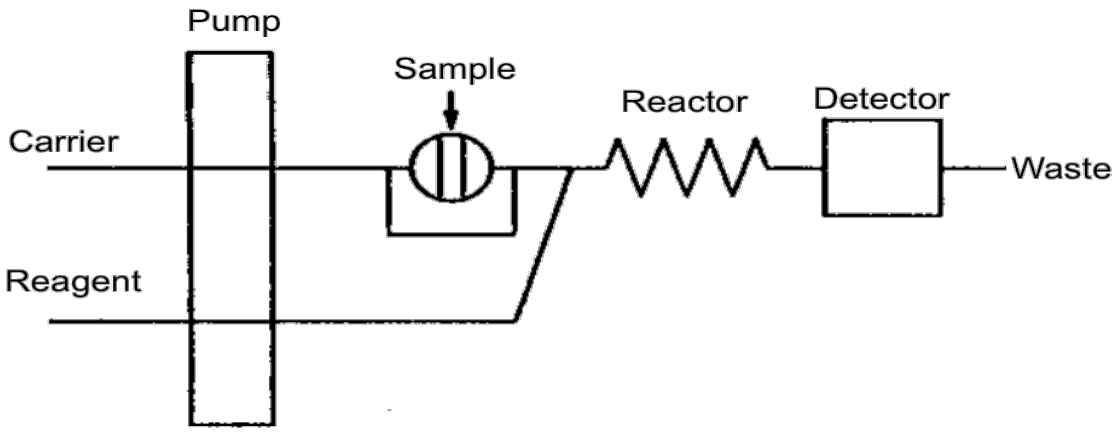


Figure 5: Simple flow injection manifold used in FIA[11].

Figure 5 shows a basic flow injection system which consists of a peristaltic pump which delivers sample and/or reagent into tubing, a sample injection valve, a detector described in section 1.2 and a data recorder[4], [8].

Components used to build the FIA system are relatively inexpensive. Moreover, the system can be easily modified to achieve optimised results or adapted to meet any type of task without complex changes[12]. Thus, the FIA technique is widely used in many areas. For example: chemical analysis, pharmaceutical, environmental and clinical chemistry, online monitoring of biotechnology, food and agriculture analysis[2], [7], [10]. Nowadays, monitoring waste and its treatment has become a big challenge, but even FIA may be used in that area[8].

1.2 Versatility of FIA coupled with various detectors

Various detectors may be coupled with a flow injection analysis system. The perfect detector should have a low noise level, a fast and linear response over a wide concentration range and high sensitivity[12].
Mostly used are detectors that measure: fluorescence, atomic absorption, pH or flame emission. What is more FIA system may be coupled with ion selective electrodes, a potentiostat or even high performance liquid chromatography (HPLC)[8], [12], [13].

A spectrophotometer coupled with a FIA system gives a setup which provides fast analysis with good precision. Moreover, it is easy to operate and relatively cheap[9]. Most of the analyses are done on – line which means the sample is analysed immediately once it reaches the detector in the flow cell. Another method is to collect a sample and put it in the container to be analysed as a ‘static’ sample[14].

Various flow cells may be used in FIA. They are made of different materials such as: Teflon, Plexiglas, polyetherimide (ULTEM) or Quartz[15]. The choice of the flow cell is based on the measured feature or wavelength range.

1.3 Detector used during the experiment – Spectrophotometer

An Agilent Technologies Cary 60 UV-Vis Spectrophotometer was used as a detector throughout the project. A xenon pulse lamp is the source of light and it provides both visible and UV light. The light beam (dimensions 1.5 x 1.0 mm) strikes the holographic diffraction grating (dimensions 27.5 x 35 mm with 1200 lines/mm), which separates the light into its component wavelengths (190 – 1100 nm). The grating is rotated so that only a specific wavelength of light reaches the exit slit. Then the light interacts with the sample. Then the detector measures the transmittance and absorbance of the sample. Detectors in the instrument used are dual silicon diodes (photodiode). A silicon diode detector enables the widest range of wavelengths to be measured without any loss of the sensitivity (Figure 7). Light transmitted through a material reduced exponentially as it travels through the sample. Beer-Lambert Law is the linear relationship between absorbance and concentration of an absorbing species. Absorbance is dimensionless and is described by the following equation:

$A = \varepsilon LC \left[M \cdot cm \cdot \frac{1}{M \cdot cm} = 1\right]$ where ε is wavelength dependent coefficient $\left[\frac{1}{\mathrm{M \cdot cm}}\right]$, *L* is a path length [cm] and *C* is concentration [M].

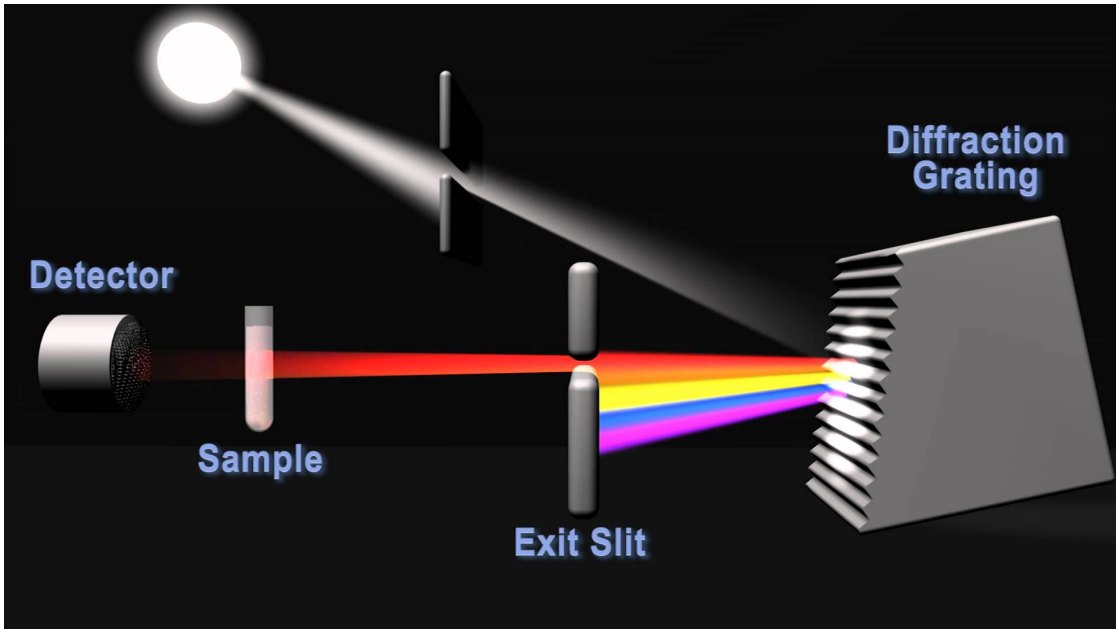


Figure 6: Depiction of the working principles of a spectrophotometer[16].

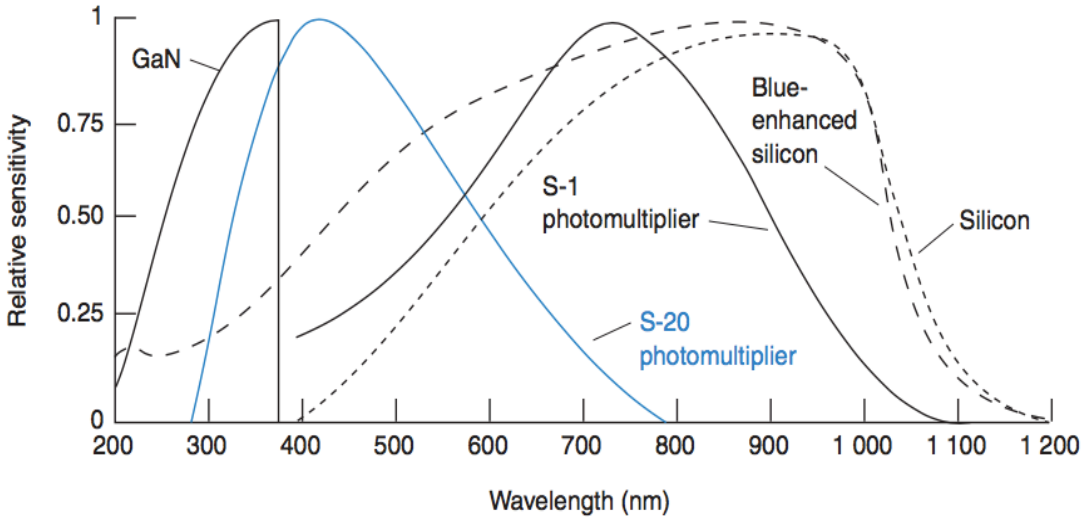


Figure 7: Detector response depends on the wavelength of the incident photons[17].

1.4 FIA vs Conventional Industrial Method for COD analysis

The most popular industrial method for COD analysis is based on the method that employs dichromate chemistry for the oxidation[18]. That includes usage of hazardous, toxic and expensive chemicals such as potassium dichromate ($K_2Cr_2O_7$), mercury sulfate ($HgSO_4$), silver sulfate (Ag_2SO_4) or ferroin indicator $[Fe(o\text{-}phen)_3]SO_4$. A silver compound is added as a catalyst to promote the oxidation of certain classes of organic compounds. A mercuric compound may be added to reduce the interference from oxidation of chloride ions. After the oxidation is completed, the amount of dichromate consumed is determined titrimetrically, titration is done using ferrous ammonium sulphate[19]. Either, the amount of reduced chromium or the amount of unreacted dichromate can be measured.

The process takes more than 2 hours (as a manual analysis)[20]–[22]. All steps required to be done before the analysis are shown in Figure 9. In the comparison of FIA and the Industrial Method for COD measurements is presented.

$$C_6H_{12}O_6 + 4\ K_2Cr_2O_7 + 16\ H_2SO_4 \rightarrow 6\ CO_2 + 4\ Cr_2(SO_4)_3 + 4\ K_2SO_4 + 22\ H_2O$$

Equation 1: Reaction of Industrial Method for COD.

All organic matter in the sample gets oxidized by a known excess of potassium dichromate in presence of sulfuric acid. Temperature in COD digester allows reaction to happen, then remaining, unreduced dichromate is determined by titration against ferrous ammonium sulphate, using ferroin as an indicator. The dichromate consumed by the samples is equivalent to the amount of oxygen required to oxidize organic pollutions in the water sample[23], [24].

Table 1: Comparison of FIA and Industrial Method for COD analysis.

	Flow Injection Analysis	**Industrial Method (potassium dichromate)**[23], [25]
Time of analysis	Less than 10 min	About 3 hours
Chemicals	Fairly safe at concentrations and quantities used	Significantly hazardous reagents and waste (including mercury)
Analysis	Automated	Requires titration and adding chemicals using pipettes
Typical sample volume	20 µl	2.5 ml
Detection range	5 - 198 mg/l (range used during this experiment)	3 - 900 mg/l
Equipment	Mostly available in every laboratory	Mostly available in every laboratory but also need COD digester
Sample preparation	None or just dilution with distilled water to match concentration range	Complex, including adding $K_2Cr_2O_7$ and H_2SO_4 as well as digestion
Calculation of COD concentration	Equation obtained from calibration graph eg. $COD = \frac{Abs - 0.9674}{-119.78}$	More complicated equation $COD = \frac{[(A - B) * N * 8 * 1000]}{V}$ A – volume of ferrous ammonium sulfate for blank B – volume of ferrous ammonium sulfate for sample N – concentration of ferrous ammonium sulfate V – volume of sample

From comparison of the two COD methods it is possible to see that FIA has many more advantages compared with the conventional Industrial Method. Flow injection analysis is much faster (including analysis and sample preparation) and safer for an operator (does not require use of any hazardous chemical); it does not even require an operator. It needs much smaller sample volumes, while the detection limit is still comparable. Furthermore, in the FIA calculation of the COD concentration of the sample it requires only the absorbance value of the peak height while an industrial method requires more complex calculations and more data to be known.

Nowadays, more laboratories start to use safer and faster techniques which excludes titrations using hazardous and toxic chemicals (Figure 8 ii), Crown Paints Ltd. is one of them. However, this technique still takes more than 2 hours and mercury – containing reagents are used. The method requires sample preparation and specially ordered COD Reagent vials (according to VWR website the cost of the vials vary from £1.50 to £1.90 each[26]).

One should know that COD vials are delivered from the USA, that will make costs higher. What is more, one must have an idea what the COD concentration may be before the analysis, because vials have various ranges (from 0 – 150 mg/l and 0 – 15 000 mg/l)[27], [28].

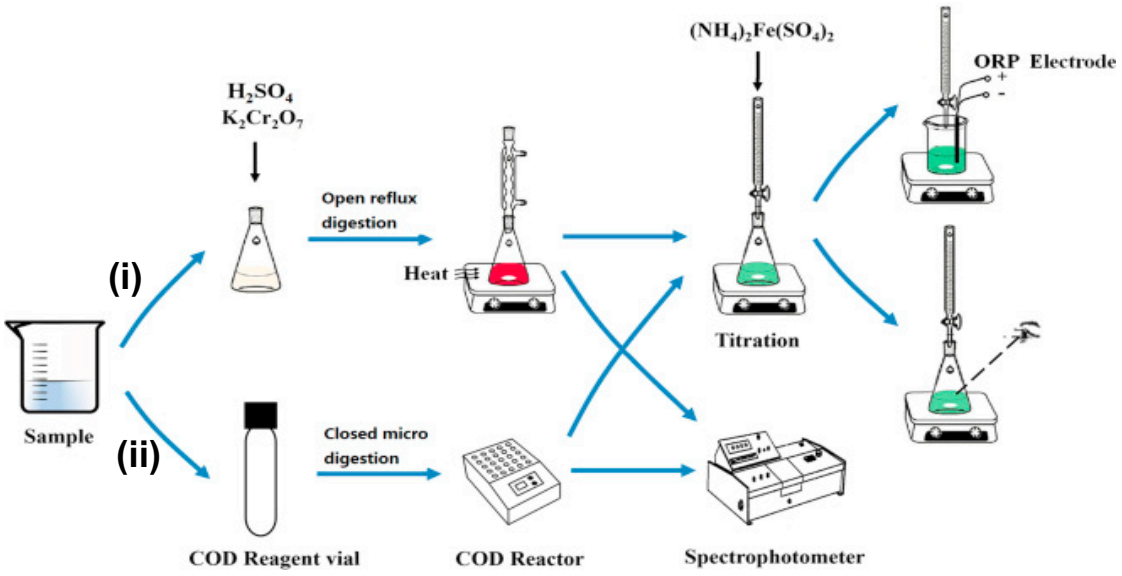


Figure 8: Two variations for the conventional Industrial Method for COD determination[29]. (i) represents the method that requires titration using hazardous reagents (details presented in Figure 9) and expensive chemicals and (ii) shows the safer method used by Crown Paints Ltd.

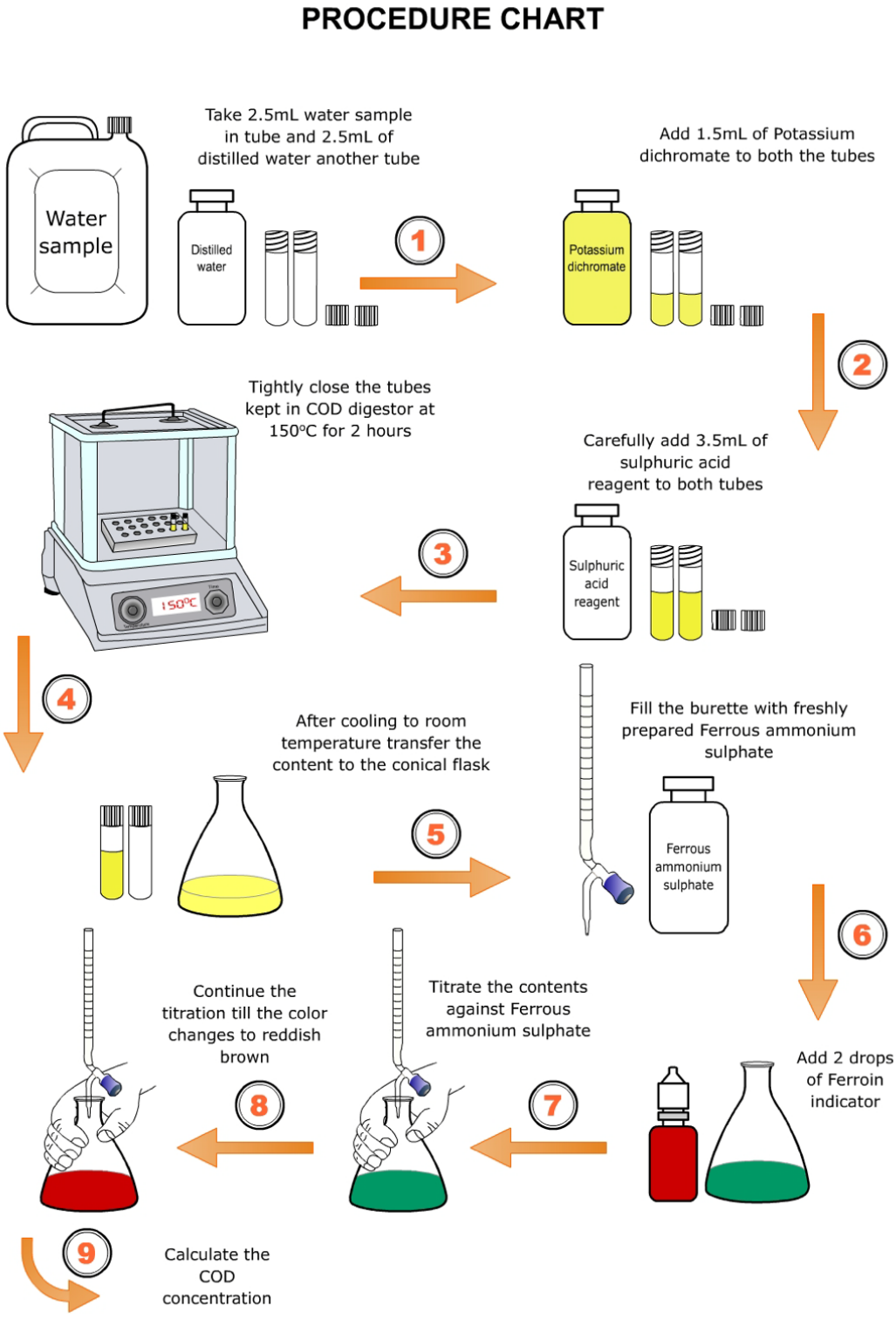


Figure 9: Procedure of the potassium dichromate method for COD, used in industry[23].

2 Project Part I

Acid/Base Reaction

2.1 Flow Injection Analysis system used for preliminary analysis

The project was started by setting up a Flow Injection Analysis system and running a simple experiment in order to understand the principles of the technique. Figure 10 shows the picture of how the FIA system was set up. It was made up from a two-way, six-port rotary valve, a set of tubing, a peristaltic pump, a flow cell QS 0.300 and an UV-Vis spectrophotometer. The pump used throughout the experiment was a Gilson® Minipuls 3 and an Agilent Technologies Cary 60 UV-Vis Spectrophotometer was used as the detector.

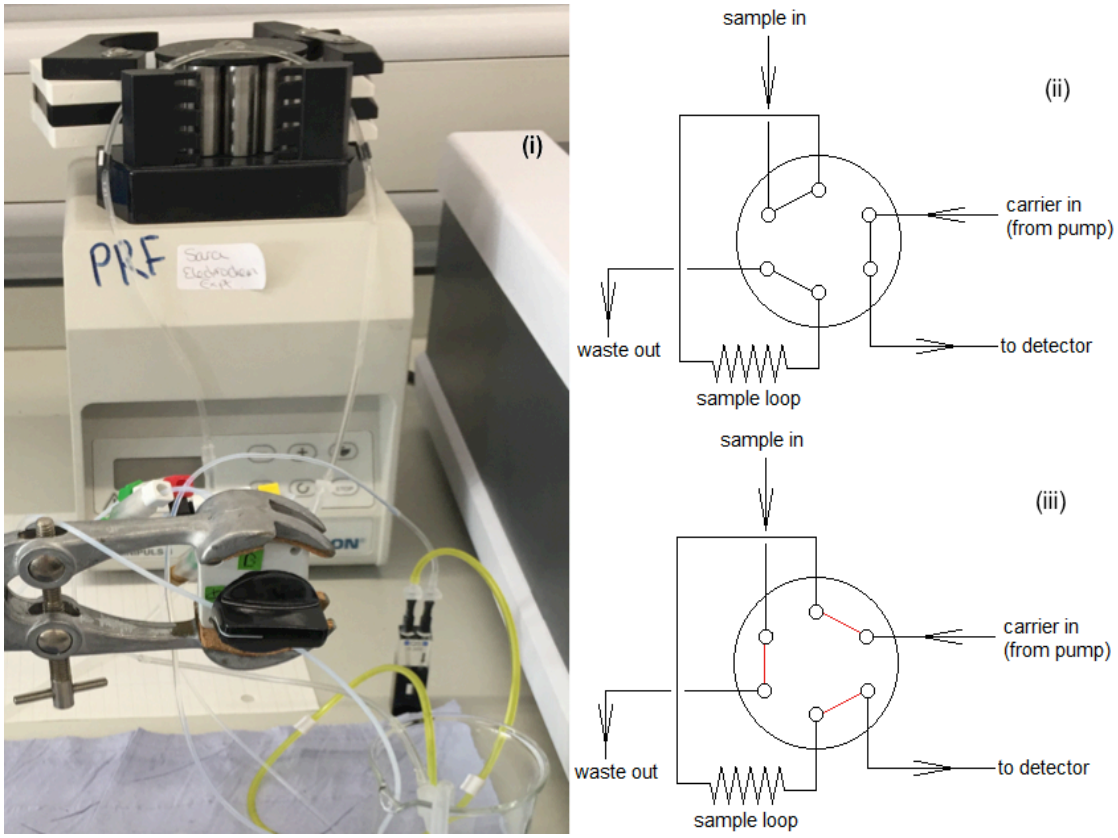


Figure 10: (i) Picture of the primary FIA system used for preliminary analysis. (ii) Presents the position of the valve while filling the loop and (iii) shows the position when running the sample.

2.2 Investigation of FIA system using an acid/base reaction

The acid/base reaction between hydrochloric acid (HCl) and sodium hydroxide (NaOH) in the presence of phenolphthalein was introduced to run samples through the FIA system using a reaction that resulted in a noticeable colour change as shown in Figure 11. The experiment began with preparing 1 litre of 0.1 M of each analyte from stock solutions. Available reagents had to be diluted to obtain the required concentrations. Dilution was done as follows: 50 ml of 2 M NaOH to get a 1 litre of 0.1 M and 100 ml of 1 M HCl to get a 1 litre of 0.1 M, pH indicator was already available at the university and used as given. Phenolphthalein was prepared using 50% ethanol to give 0.1% m/V solvent.

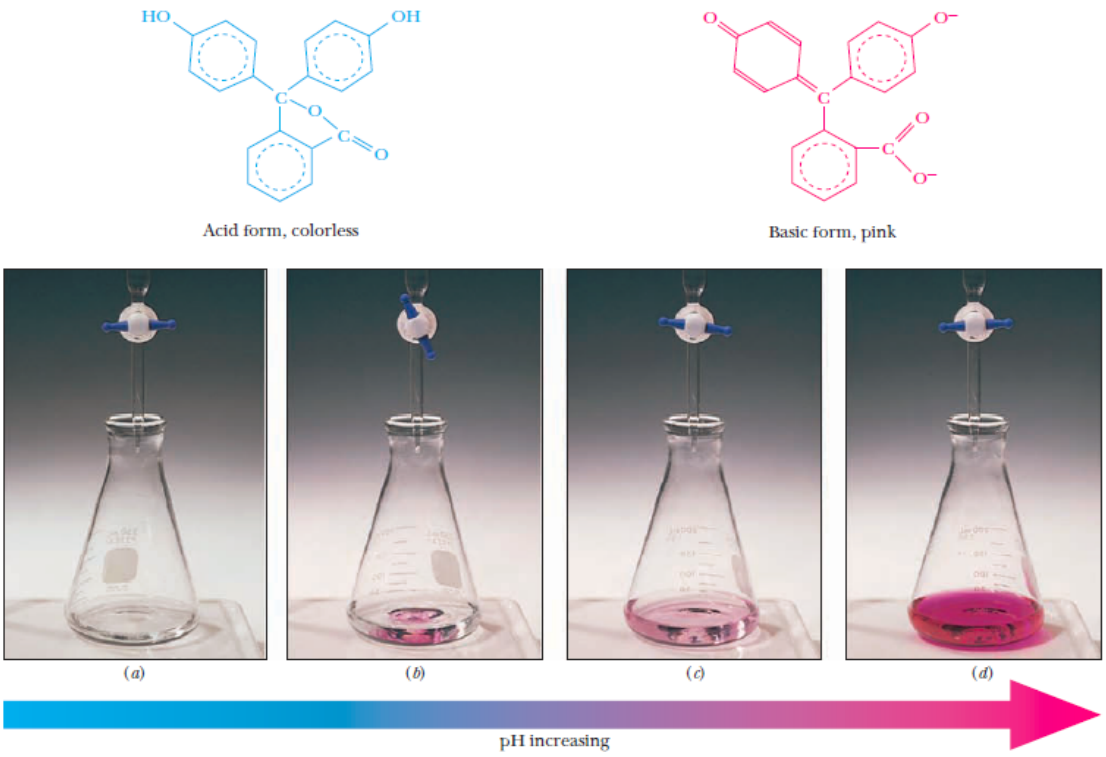


Figure 11: Show the acid and base forms of phenolphthalein, an indicator commonly used in the titration of strong acid with strong base. (a) The acidic solution is initially clear. (b) Adding base makes solution pink but the colour disappearing after swirling. (c) The first permanent pink indicates the endpoint of titration. (d) If the solvent becomes vividly coloured, it means base is in excess[30].

Indicators change colours in different environments. Phenolphthalein becomes pink in basic environment. Colour changing occurs where there is the same amount of both forms present in the mixture, the equilibrium lies exactly in the middle: $HIn \rightleftharpoons H^+ + In^-$

$pKa = \frac{[H^+][In^-]}{[HIn]}$ when equilibrium is in the centre then: $[In^-] = [HIn]$ so $pKa = H^+$ which is $pKa = pH$

pKa = 9.7 [30]

Based on that information, one knows that the indicator will change colour when the pH is the same value as its pKa value.

Firstly, the wavelength of the maximum absorbance had to be specified. To do so, 5 ml of HCl with 0.5 ml of phenolphthalein and 5 ml of NaOH were mixed and transferred into a cuvette. The spectrum was recorded between 400 - 800 nm and the highest absorbance was at 550 nm (Figure 12). Thus, it was the chosen wavelength to run all samples with hydrochloric acid, sodium hydroxide and phenolphthalein.

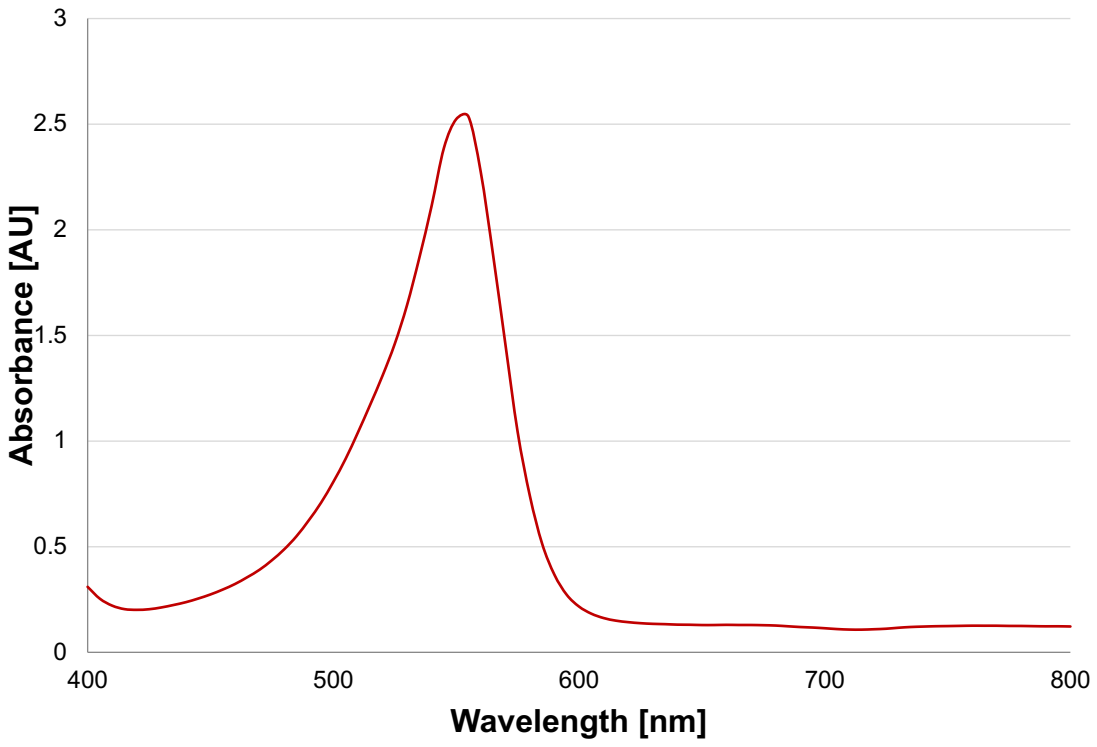


Figure 12: Spectrum of maximum absorbance at 550 nm for the acid and base analysis with phenolphthalein as the indicator.

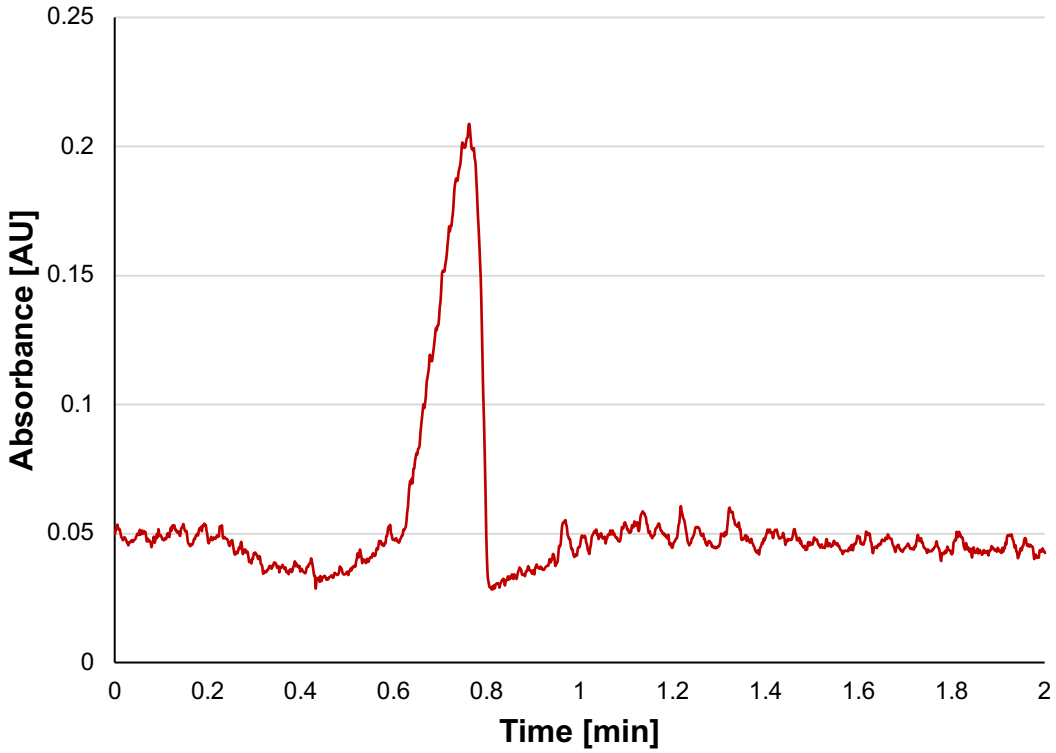


Figure 13: Spectrogram presenting acid/base titration at 550 nm.

A carrier solution of 100 ml of HCl and 1 ml of pH indicator were mixed and delivered from the flask by the peristaltic pump. The sample, NaOH in this case, was supplied by the syringe to fully fill the loop (74.6 μl). The carrier solution was colourless because phenolphthalein gives a pink colour only in basic solution (Figure 11). The valve position was changed (sample injected), and then the reaction began. It was possible to see a colour change which was measured by the spectrophotometer (Figure 13).

2.3 Pump calibration and optimisation of the pump speed

The peristaltic pump was calibrated by measuring the time needed to fill a 5 ml-measuring cylinder. The flow rate (*F*) was calculated using the following equation:

$F = \frac{V}{t}\ \left[\frac{\mu l}{s}\right]$ where *V* is volume and equals 5 ml = 5000 µl and *t* is time [s].

Table 2: Table showing data from the pump calibration used in the calibration graph from Figure 14.

Pump speed [RPM]	Time [s]	Flow rate [µl/s]
3.00	1766	2.8
5.00	1055	4.7
5.50	971	5.1
6.00	895	5.6
6.50	827	6.1
7.00	767	6.5
7.50	717	7.0
8.00	668	7.5
10.00	535	9.3

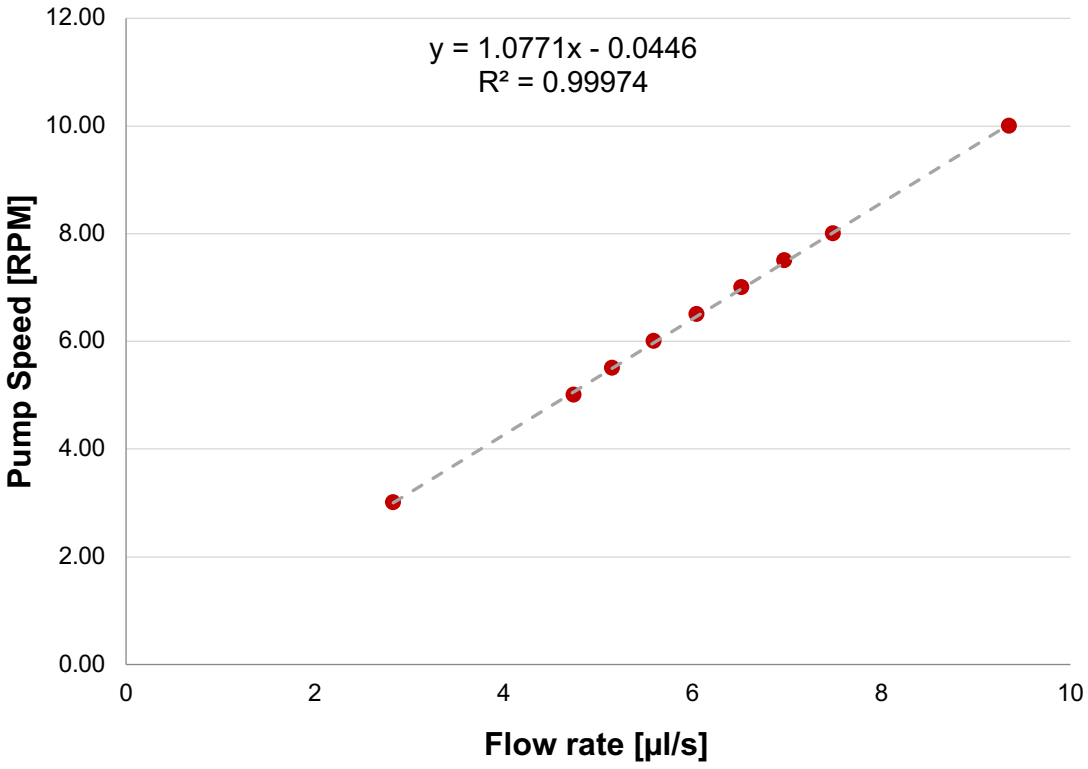


Figure 14: Pump calibration graph based on data from Table 2.

The main conclusion from calibration graph analysis is that the flow rate is predictable and linear over the range tested. In that analysis, it can be calculated based on the equation from it (*y = 1.0771x - 0.0446*). However, it may differ when using a different kind and length of tubing. Thus, new calibrations must be done when components of the pump system are changed.

The optimised pump speed has been selected based on the peak shape (Table 3). In Figure 15 the number of peaks in each analysis corresponds to the number of sample injections. In the two slowest analyses, only one sample has been analysed, in those that are faster two or three samples have been injected. One can see that the best peaks are in analyses (v) and (vi). Peaks are high, narrow and there is no fronting as in the first four analyses (sample peak with fronting has been highlighted by a blue circle in the first two spectrograms). Based on the optimisation results the best pump speed has been set as 11.0.

Table 3: Summary of spectrograms analysis from Figure 15.

Analysis	Pump speed [RPM]	Description
i	5.0	Small and broad peaks, fronting present
ii	7.0	
iii	9.0	Peaks still small and some fronting present
iv	10.0	
v	11.0	High and narrow peaks
vi	11.5	

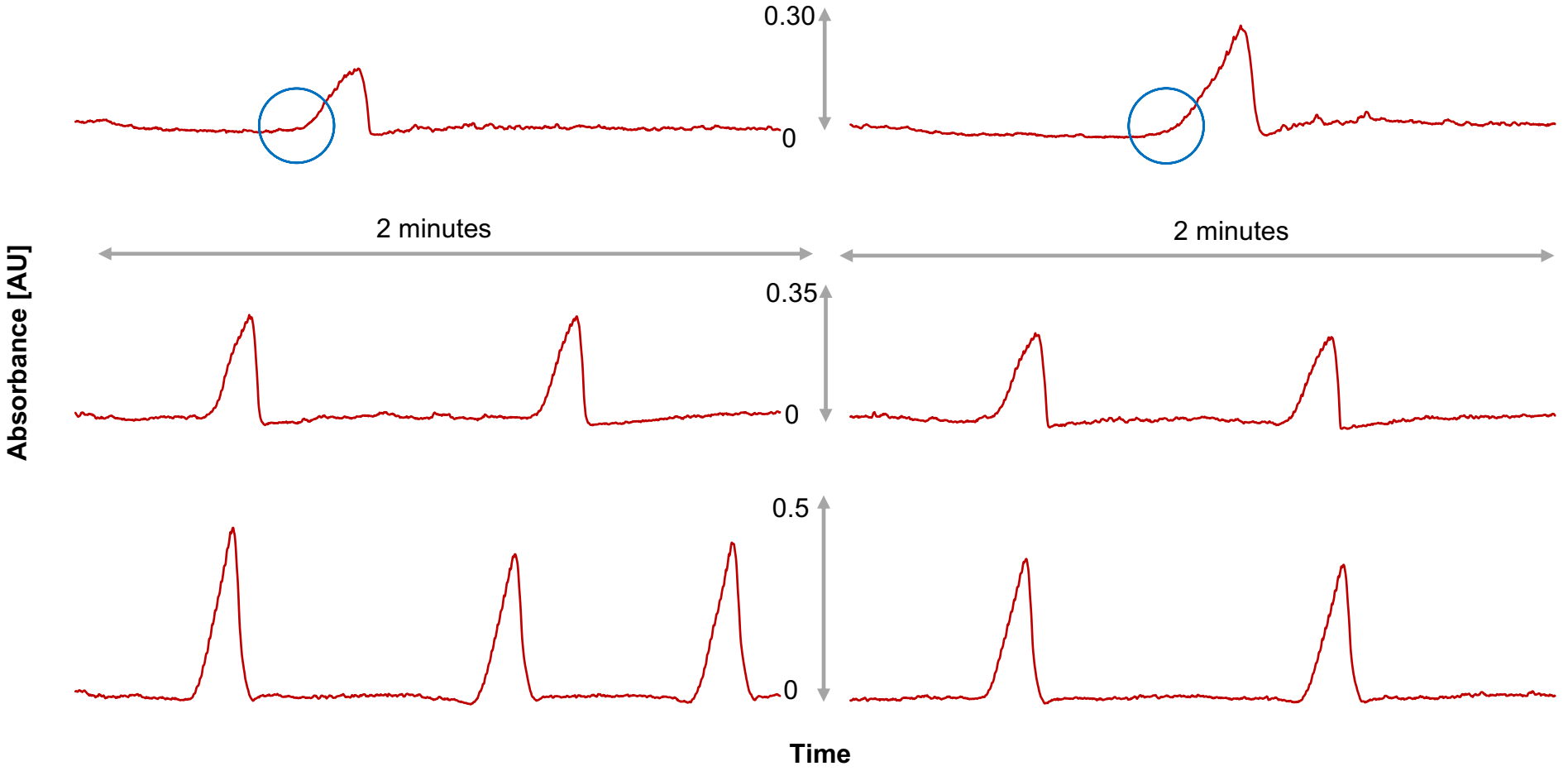


Figure 15: Spectrograms used to select the best pump speed. (i) indicates speed 5.0, (ii) speed 7.0, (iii) speed 9.0, (iv) speed 10.0, (v) speed 11.0 and (vi) speed 11.5. Additionally (i) and (ii) present fronting of peaks marked by a blue circle. Each analysis last 2 min.

2.4 Preliminary optimisation using food dye samples

To understand the principles of flow injection analysis, the first experiment was done only with distilled water and blue food dye (blue food colouring by Sainsbury's). A splash of dye (unknown volume) was diluted in the distilled water and used as a sample.

The first step of primary analysis required the appropriate wavelength to be established. The graph below shows maximum absorbance for the analysed sample at 620 nm. For analysis, a cuvette with 10 mm path length and 4.5 ml volume was used.

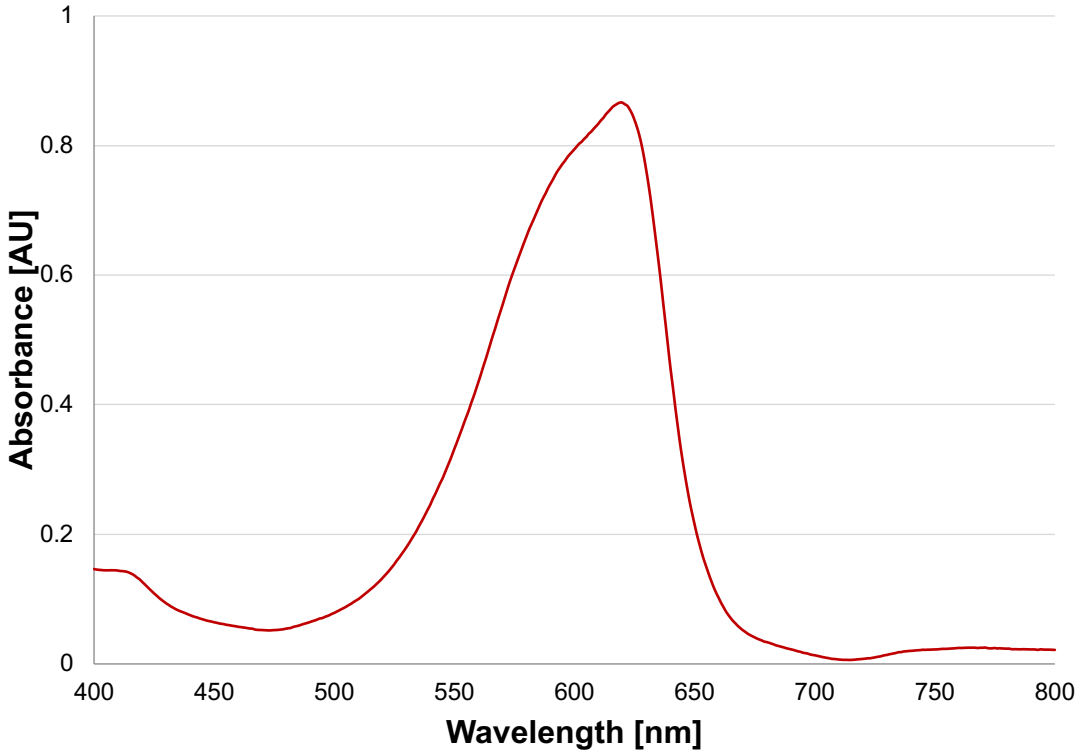


Figure 16: Spectrum of blue dye solvent shows maximum absorbance (at 620 nm) of sample used in primary analysis.

Based on primary analysis, the wavelength, which was used in the analysis of the food dye was 620 nm (Figure 16). In Figure 17, which presents analysis of blue dye samples, three peaks correspond to three sample injections are shown. The third peak is smaller than the other two probably due to insufficient loop filling.

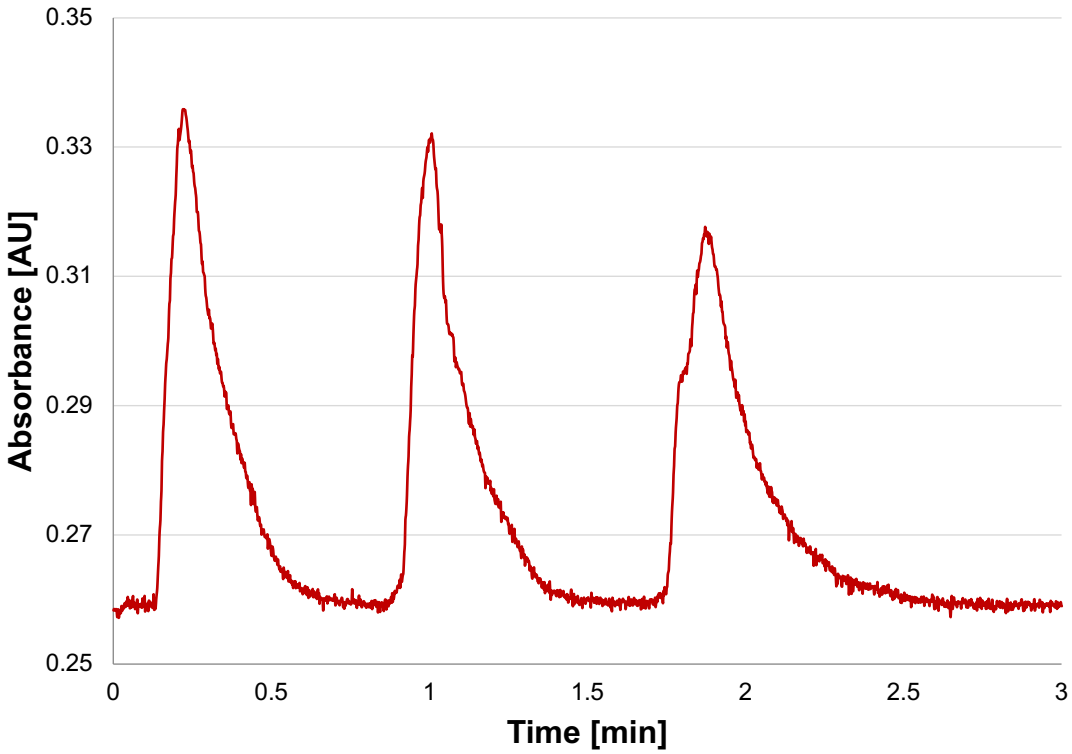


Figure 17: Spectrogram of flow injection analysis of blue food dye.

The loop length (*H*) was 95 mm with diameter (*r*) of 0.5 mm. That makes volume (*V*) of sample:

$V = \pi r^2 * H \left[mm^2 * mm = mm^3 = \mu l\right]$ where π equals 3.14.

$V = 3.14 * (0.5)^2 * 95 = 74.6\ \mu l$

During this research to wash and fill the loop, 4 ml of sample was used. That excess amount of sample was used to make sure the loop was filled properly. Only then do the peaks have consistent height.

3 Project Part II

Chemical Oxygen Demand Determination

3.1 Development of a FIA system to analyse of Chemical Oxygen Demand

Chemical oxygen demand (COD) is defined by the amount of oxygen that is required to oxidize organic materials in a measured solution[20]–[22],[31]. Commonly expressed in mass of oxygen consumed over volume of solution which in SI units is mg/l[32]. The test of COD is valuable for assessing organic pollution levels in environmental water samples and it is one of the major parameters measured in industrial water samples[1],[12],[33]. This is due to organic pollutants which must be controlled before getting rid of industrial waste water. It used to be measured by manual titration (Figure 9) but FIA allows quicker and safer analysis[34].

For COD determination, another FIA system with three valves has been designed as seen in Figure 18, Figure 19 and Figure 20.

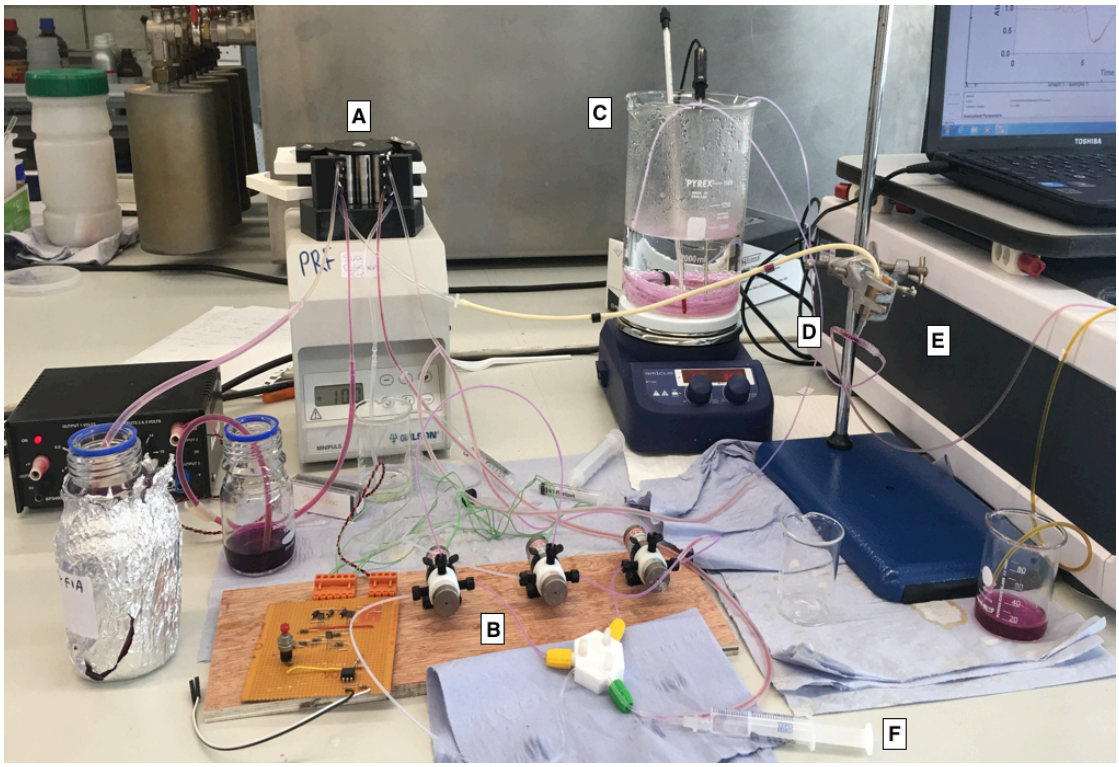


Figure 18: Set up of the FIA system in the laboratory at the university with the sample delivered into the sampling loop using a syringe. A: peristatic pump; B: automated valves; C: heated reaction coil; D: debubbler; E: UV-Vis Spectrophotometer; F: sample injection port.

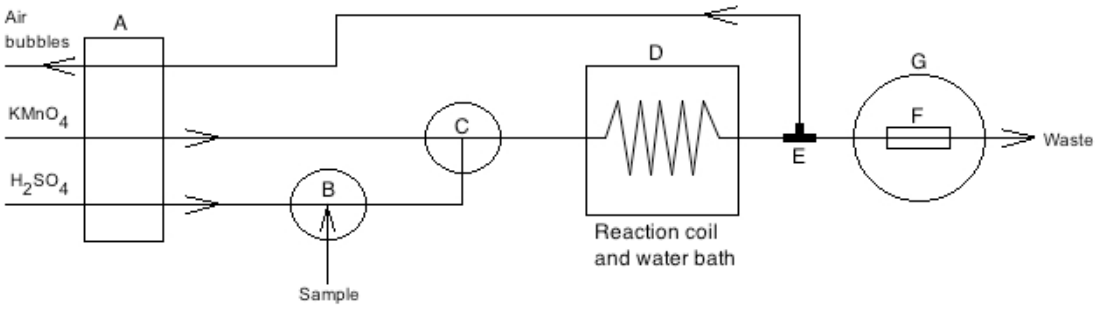


Figure 19: Diagram of the FIA system developed for COD analysis. A: peristatic pump Gilson® Minipuls 3; B: constant-volume sampling valve; C: mixing joint; D: reaction coil (15 m) and water bath; E: debubbler; F: flow cell QS 0.300; G: detector set at 525 nm (Agilent Technologies Cary 60 UV-Vis Spectrophotometer) and data recorder[12].

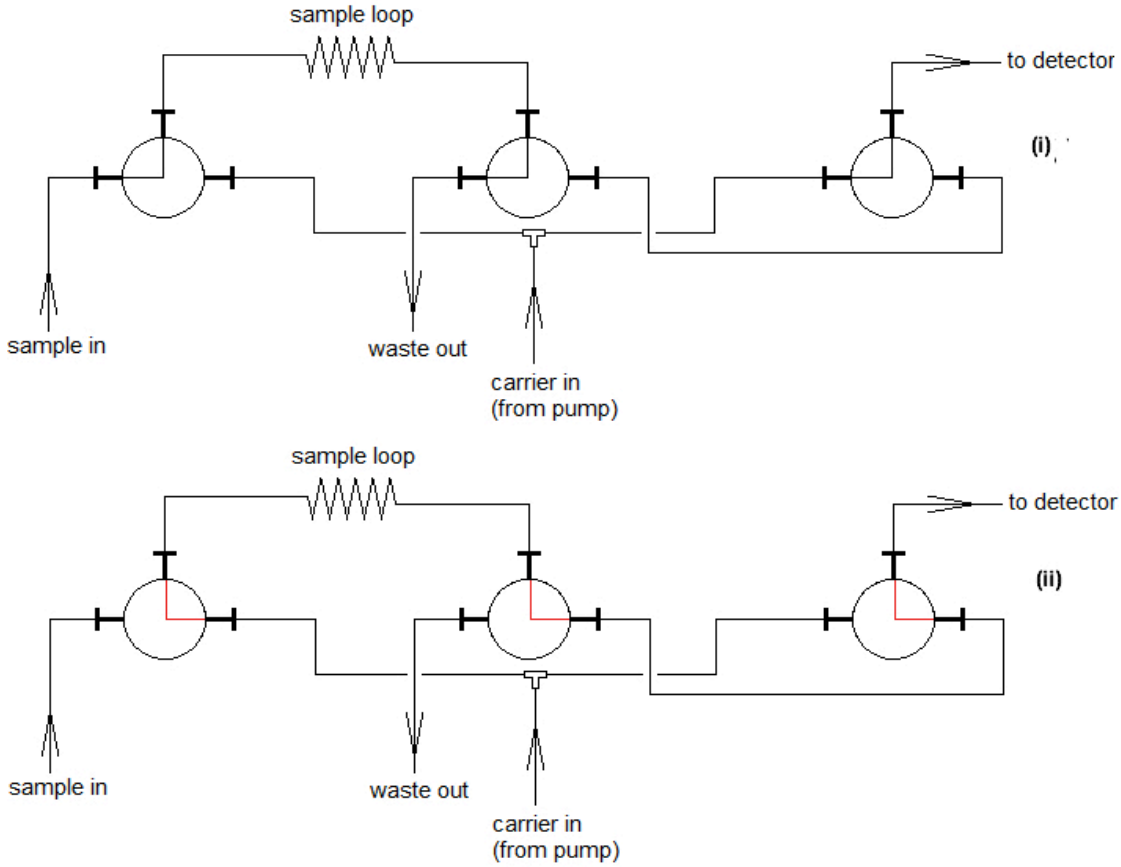


Figure 20: Schemes of the valve positions during loop filling (i) and during analysis (ii).

Three-way solenoid - actuated valves (round structures in Figure 20) were introduced to allow automated operation of the FIA system.

3.2 Microcontroller programming used to control the sample injection

To automate the process, a microprocessor, the Arduino UNO (Figure 21) has been used to automate the sample injection process. Automation of the analysis allows measurement of several samples without supervision and also eliminates human error, and so improves sample injection precision. A LCD display was added to the microcontroller to get information about the progress of the analysis. It requires programming using a simplified 'C-type language'. In Appendix I the code is available.

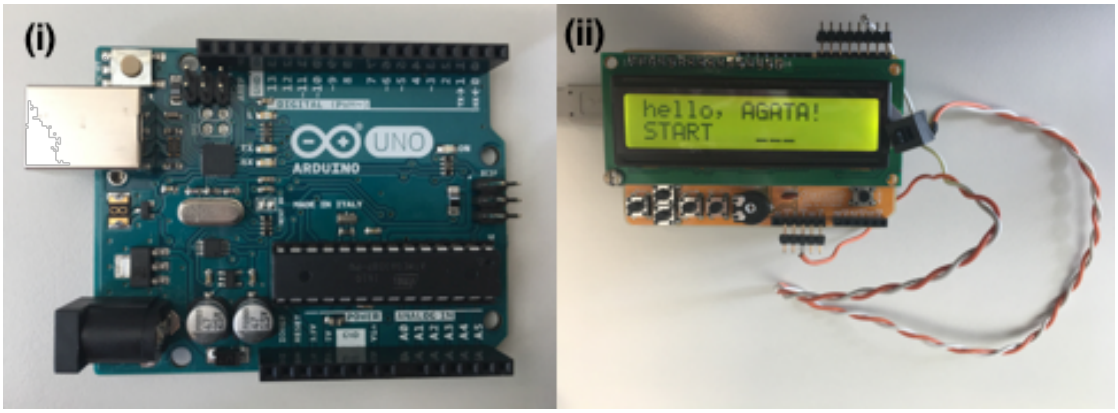


Figure 21: (i) Arduino UNO microcontroller, (ii) microcontroller with screen.

3.3 The chemistry used in the FIA method for COD analysis[1]

3.3.1 Potassium permanganate ($KMnO_4$) solution preparation

Potassium permanganate was ACS reagent, ≥99.0% by Fluka and its solution was prepared as follows:

1. 0. 8 g of potassium permanganate has been diluted in 1100 ml of distilled water (Figure 22 i) as stated in the journal method[1].
2. Solution was boiled and left to stand overnight in the dark (Figure 22 ii).
3. Next day (after ~ 12 h) the solution has been filtered using a Hydrophilic PTFE 0.2 μm filter (Fisher brand) fitted on a 20 ml syringe then storage in amber glass bottle in the fridge for future use.

4. Before use the solution had to be diluted using distilled water to obtain a ratio of 14 : 1. (14 H_2O : 1 $KMnO_4$). The dilution factor was worked out by checking different ratios of $KMnO_4$ and H_2O. This diluted solution should give absorbance of 0.95 ± 0.05 at 525 nm.

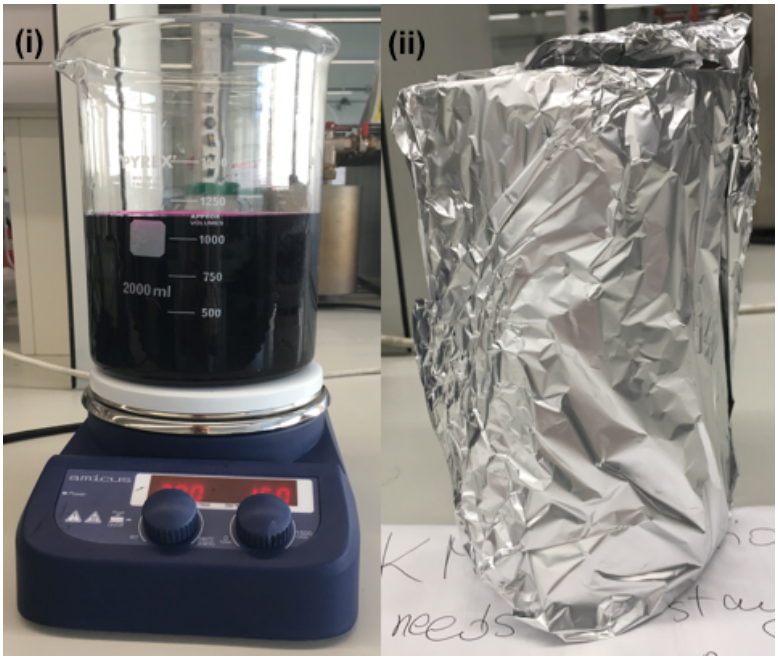


Figure 22: Preparation of potassium permanganate solution while boiling (i) and standing overnight in dark (ii).

3.3.2 Sulfuric acid (H_2SO_4) solution preparation

Sulfuric acid was ACS reagent, 95.0 - 98.0% by Fluka. Half way through the project that acid has been replaced by: Sulfuric acid ACS reagent, 95.0% solution in water by Acros Organics. Solutions were prepared as follows:

1. 98% H_2SO_4 has been diluted to give 15% solution (91.8 ml or 169 g in 1000 ml with 95.0 - 98.0% acid; respectively 94.7 ml or 173 g of 95.0% acid).

   Amount needed to prepare 15% H_2SO_4 from 95.0-98.0% acid has been calculated using dependency:

   $C_1 \cdot V_1 = C_2 \cdot V_2$ where C_1 is molar concentration of stock solution, V_1 is volume of stock solution, C_2 is moles concentration of final solution and V_2 is volume of final solution.

The transformation of the formula for use with percentage concentrations:

$C_n = \frac{n}{V} \left[\frac{mol}{dm^3}\right]$ where C_n is molar concentration, n is number of moles and V is volume.

$n = \frac{m_s}{M_s} \left[\frac{g}{g/mol} = mol\right]$ where m_s is mass of the substance and M_s is molar mass of the substance.

$C_p = \frac{m_s}{m_r} \cdot 100\% \Longrightarrow m_s = \frac{C_p \cdot m_r}{100\%}$ where C_p is percentage concentration, m_s is mass of the substance, m_r is total mass of the solution.

$d = \frac{m}{V} \Longrightarrow m = d \cdot V \left[\frac{g \cdot ml}{ml} = g\right]$ where d is density, V is volume and m_r is total mass of the solution.

Based on these dependencies it is possible to transform the formula for molar concentration as follows:

$C_n = \frac{C_p \cdot d \cdot V}{M_s \cdot V} = \frac{C_p \cdot d}{M_s}$

$\frac{C_{p98\%} \cdot d_{98\%} \cdot V_{98\%}}{M_S} = \frac{C_{p2} \cdot d_2 \cdot V_2}{M_S}$ because the substance remains the same, molar mass stays the same and the equation gets shorted to:

$C_{p98\%} \cdot d_{98\%} \cdot V_{98\%} = C_{p2} \cdot d_2 \cdot V_2$ where $C_{p98\%}$ = 0.98; $d_{98\%}$ = 1.84 g/cm^3; $V_{98\%}$ = 91.8 cm^3; V_2 = 1000 cm^3.

The unknowns are: C_{p2} and d_2.

However, it is possible to obtain the values from standard tables for sulfuric acid[35] which can be seen in Table 4.

Table 4: Standard tables for sulfuric acid[35].

C [%]	*d* [g/cm3]	*C · d*
1	1.004	0.0100
2	1.011	0.0202
3	1.017	0.0305
4	1.024	0.0410
5	1.030	0.0515
6	1.037	0.0622
7	1.044	0.0731
8	1.051	0.0841
9	1.058	0.0952
10	1.065	0.1065
11	1.072	0.1179
12	1.079	0.1295
13	1.066	0.1386
14	1.093	0.1530
15	**1.100**	**0.1650**
16	1.107	0.1771
95	1.830	1.7385
98	1.840	1.8032

$0.98 \cdot 1.84 \cdot 91.8 = C_{p2} \cdot d_2 \cdot 1000 \implies \frac{0.98 \cdot 1.84 \cdot 91.8}{1000} = C_{p2} \cdot d_2$

$C_{p2} \cdot d_2 = 0.1655$ from Table 4 one can see that 0.1655 is closest to the value for a concentration of 15%.

Acid has been measured on the scale then mass of acid needed is:

$m = 1.84 \cdot 91.8 \approx 169\ g$

The same dependency has been used in calculation of amount needed to prepare solution from 95.0% sulfuric acid:

$C_{p95\%} \cdot d_{95\%} \cdot V_{95\%} = C_{p2} \cdot d_2 \cdot V_2$ where $C_{p95\%} = 0.95$; $d_{95\%} = 1.774$ g/cm^3; $V_{95\%} = 94.7$ cm^3; $V_2 = 1000$ cm^3.

The unknowns are: C_{p2} and d_2.

$$0.95 \cdot 1.84 \cdot 94.7 = C_{p2} \cdot d_2 * 1000 \Longrightarrow$$

$$\frac{0.95 \cdot 1.83 \cdot 94.7}{1000} = C_{p2} \cdot d_2$$

$C_{p2} \cdot d_2 = 0.1646$ from Table 4 one can see that 0.1655 is closest to value for concentration of 15% (marked in red).

Acid has been measured on the scale then mass of acid needed is:

$m = 1.83 \cdot 94.7 \approx 173\ g$

2. A stock acid containing 0.1 M $(NH_4)_2SO_4$ has been prepared by adding 6.607 g of ammonium sulfate to 500 ml of 15% sulfuric acid.
3. Then 0.5% m/V $KMnO_4$ solution containing 0.1 M ammonium sulfate is added in the ratio 2 : 1 to 15% acid (0.5 g of solid $KMnO_4$ in 100 ml of water $\rightarrow$ filtered plus 200 ml of 15% H_2SO_4 with added 3.96 g of $(NH_4)_2SO_4$ to generate solution containing 0.1 M).
4. To get an acid solution ready for analysis, 200 ml of acid containing 0.1 M $(NH_4)_2SO_4$ has been added to 80 ml of 0.5% m/V KMnO4 solution containing 0.1 M ammonium sulfate (ratio of 5 : 2).

3.3.3 Preparation of glucose ($C_6H_{12}O_6$) standards

The glucose used: D - (+) - Glucose, ACS reagent by Sigma-Aldrich. Solutions were prepared as follows:

1. 1 M glucose stock solution was prepared and then diluted to 0.01 M.
2. From 0.01 M solution, glucose standards were prepared at six concentrations: 0.1 mM, 0.3 mM, 0.5 mM, 0.7 mM, 0.9 mM and 1.1 mM (Figure 23).

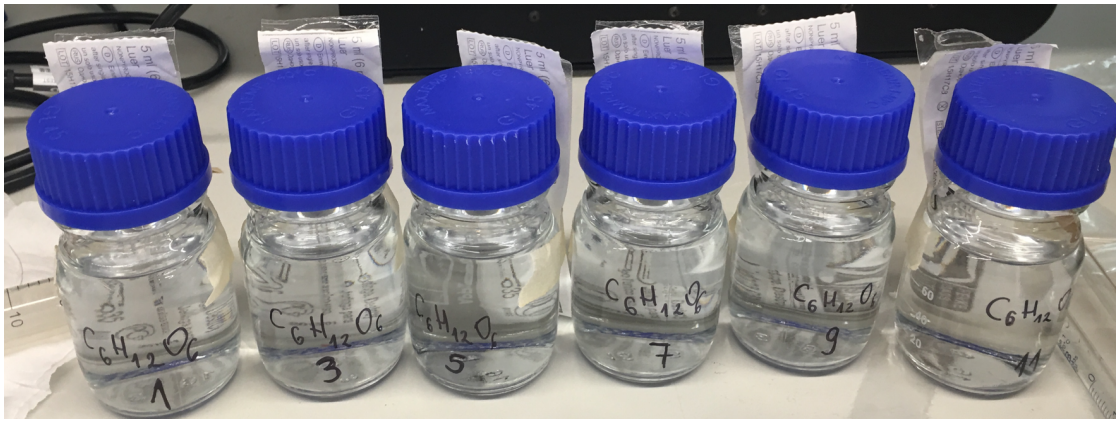


Figure 23: Glucose standards used in the experiment.

There are a number of chemicals which may be used as a standard for that particular experiment. Previous studies used a mixture of L-glutamic acid and lactose (in 5 : 1 ratio), sodium salicylate, sodium acetate or sodium oxalate as a calibration standard.

In this study glucose was used because it matched the protocol from the article that was used for the FIA method[1]. Moreover, glucose is cheap, stable in water solution and safe to use. Also, calculation of COD concentration is straight forward.

3.3.4 Iron (II) sulfate ($FeSO_4$) residue cleaning reagent preparation

Iron (II) sulfate heptahydrate was: ACS reagent, ≥99.0% by Fluka. Solution was prepared as follows:

1. 0.5 M solution has been prepared.
2. For cleaning the system, it has been mixed with 15% H_2SO_4 solution in ratio 1 : 1.

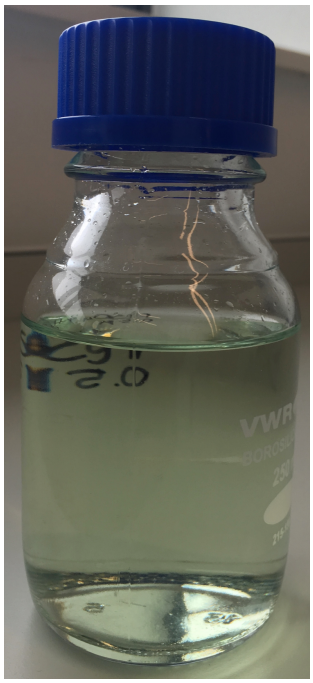


Figure 24: Iron (II) sulfate ($FeSO_4$) in H_2SO_4 use as a cleaning solvent.

3.4 Wavelength selection for COD experiment

This particular experiment was based on the article by Korenaga and Ikatsu[1]. However, some of the conditions such as: the solutions used in the experiment (their concentrations), and operating conditions, especially the wavelength have been re-evaluated. To do so, some of the mixed solutions, taken from the waste have also been examined (Figure 25). In the analysis, it has been confirmed that 525 nm is the appropriate wavelength and therefore it has been used throughout the experiment.

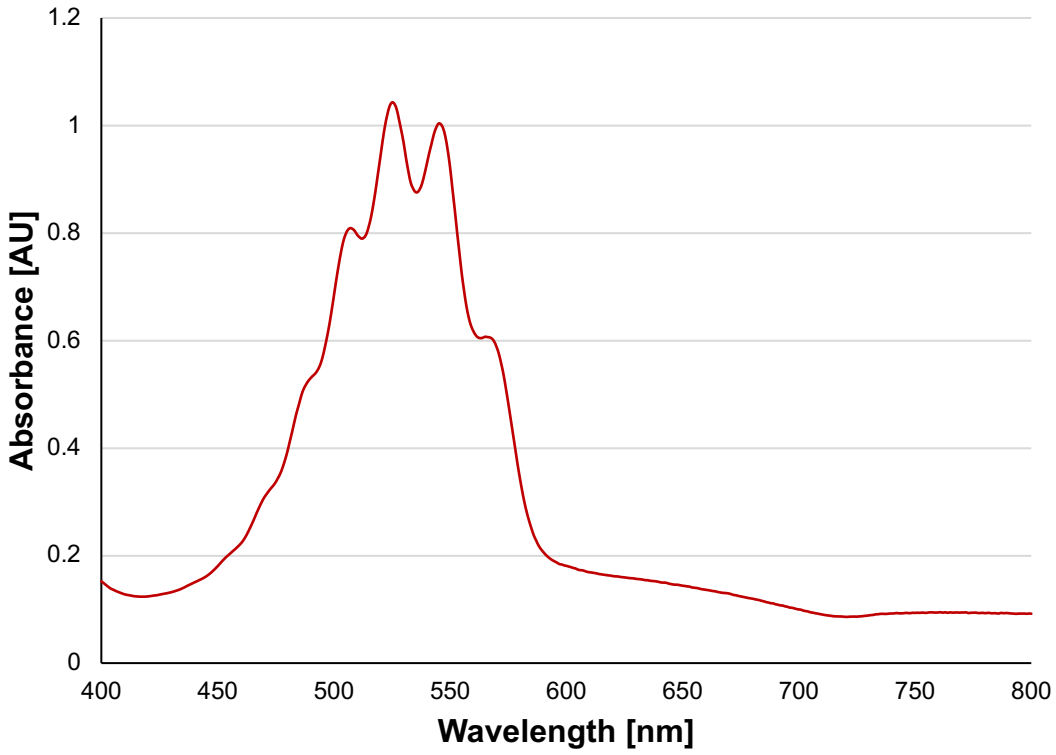


Figure 25: Selection of the wavelength of maximum absorbance for solvents used during COD analysis ($KMnO_4$ + H_2SO_4).

3.5 Calibration for the COD analysis experiment

To optimise operating conditions, prepared glucose standards have been analysed using the water bath set at different temperatures, a low temperature is considered better operationally with the added advantage of safer working conditions for the researcher. Moreover, Korenaga and Ikatsu[1], found that a temperature around 100^{o}C promoted air bubble formation. Therefore, all reagents had to pass through the glass de-gasser before analysis. Thus, the author of this research project analysed four temperatures: 50^{o}C, 60^{o}C, 70^{o}C and 80^{o}C.

At every temperature, at least two sets of data have been collected to confirm the results.

3.5.2 Analysis at 50°C

Table 5: Data used for constructing the calibration graph from Figure 26, n = 3.

Concentration [mM]	Absorbance [AU]	Standard deviation
0.1	1.044	0.035
0.3	1.039	0.030
0.5	1.046	0.028
0.7	1.050	0.043
0.9	1.053	0.045
1.1	1.036	0.051

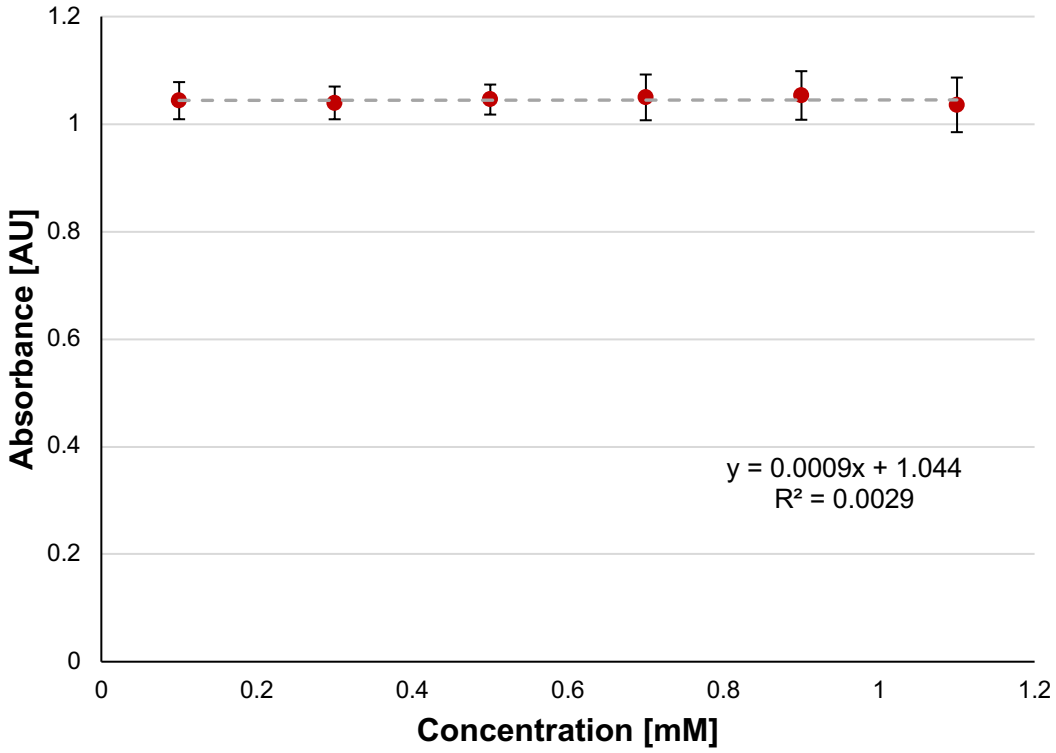


Figure 26: Calibration graph for glucose standards at 50°C.

From calibration graph in Figure 26 it is possible to notice that absorbance of the standards is not linearly dependent on concentration. The coefficient of determination (R^2) value, 0.0029, is far too low to be considered as reliable ($R^2 > 0.9$). In every analysis at least one value was out from the expected results. In conclusion, a temperature of 50°C seems to be too low to get valuable results.

3.5.3 Analysis at 60°C

Table 6: Data used for constructing the calibration graph from, n = 4.

Concentration [mM]	Absorbance [AU]	Standard deviation
0.1	0.920	0.112
0.3	0.919	0.125
0.5	0.906	0.122
0.7	0.906	0.124
0.9	0.901	0.125
1.1	0.886	0.126

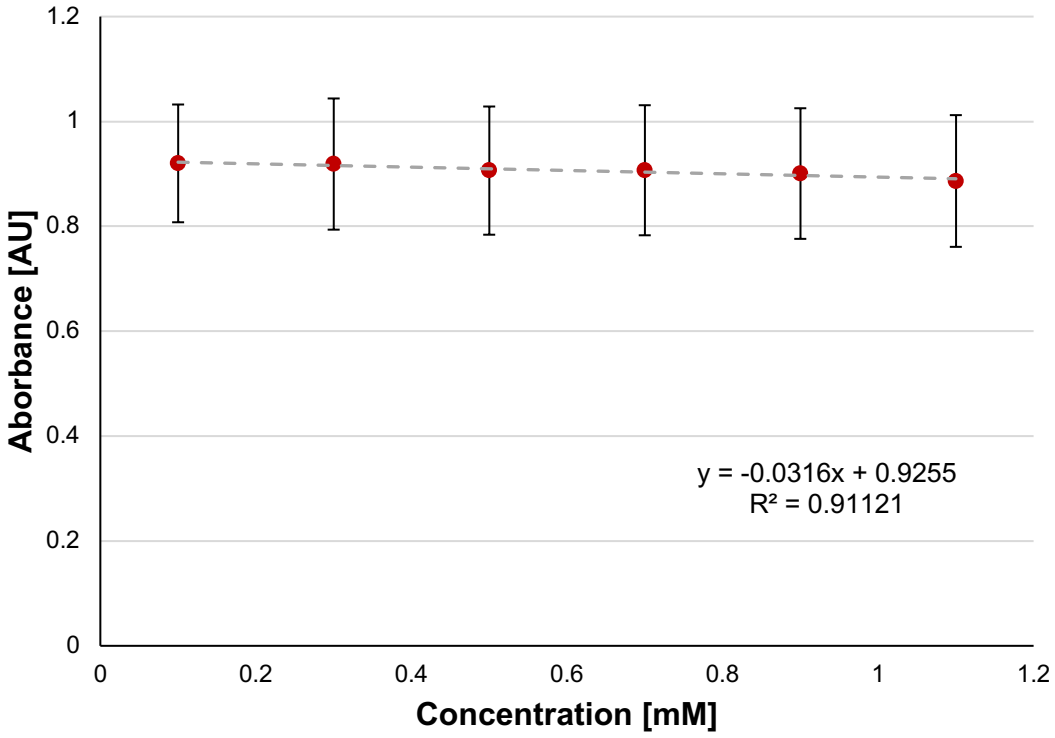


Figure 27: Calibration graph for glucose standards at 60°C based on data from Table 6.

Results obtained for the glucose standard at 60°C allows construction of a valid calibration graph. What has to be emphasised, is that in some analyses the glucose standard at a concentration of 0.1 mM was lower than expected which may be due to the due to a lower spectrometer sensitivity at 60°C for the lowest concentrations. Nethertheless when compared with results in section 3.5.2, 3.5.4 and 3.5.5 in this study, 60°C is the most suitable for operation during the planned experiment. The coefficient of determination R^2 value is 0.911 which makes it reliable.

3.5.4 Analysis at 70°C

Table 7: Data used for constructing the calibration graph from Figure 28, n = 2.

Concentration [mM]	Absorbance [AU]	Standard deviation
0.1	0.956	0.015
0.3	0.919	0.005
0.5	0.909	0.015
0.7	0.901	0.024
0.9	0.895	0.040
1.1	0.861	0.037

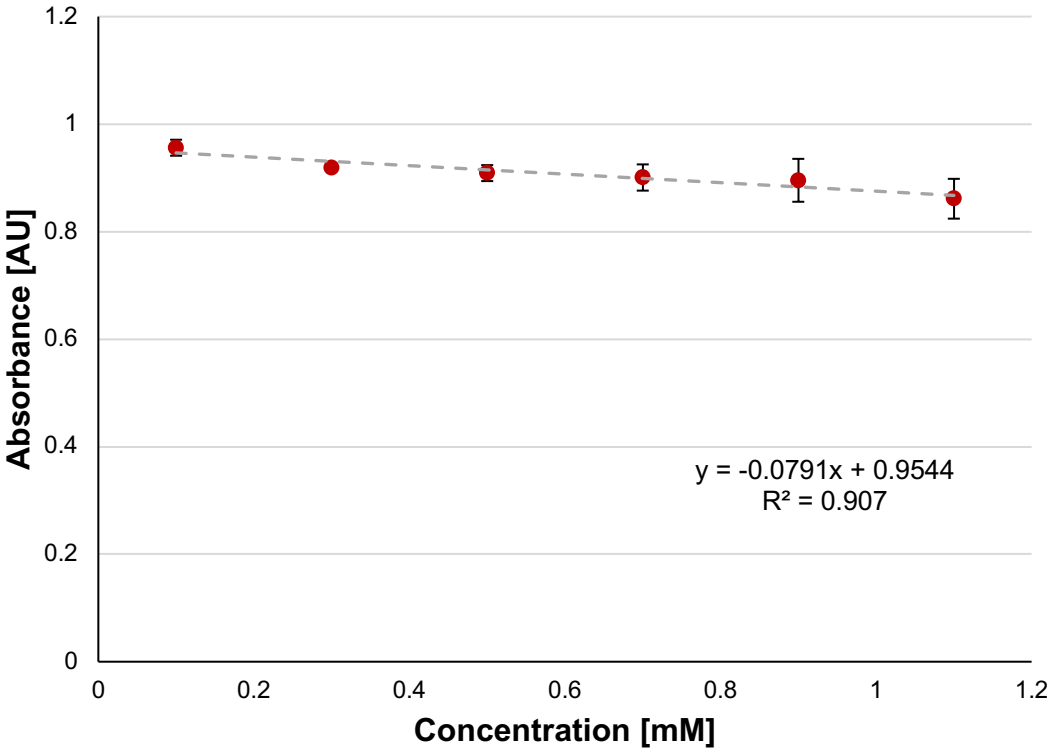


Figure 28: Calibration graph for glucose standards at 70°C based on data from Table 7.

Figure 28 presents the calibration graph at 70°C where the absorbance is linearly dependent on concentration. Although the sensitivity is higher, the coefficient of determination value is lower than for the previous temperature of 60°C. That makes the lower temperature (60°C) preferable. Moreover, temperatures above 70°C are difficult to maintain during the analysis, when using a water bath for temperature control.

3.5.5 Analysis at 80°C

Table 8: Data used for constructing the calibration graph from Figure 29, n = 3.

Concentration [mM]	Absorbance [AU]	Standard deviation
0.1	0.813	0.145
0.3	0.779	0.142
0.5	0.747	0.144
0.7	0.713	0.157
0.9	0.695	0.159
1.1	0.662	0.152

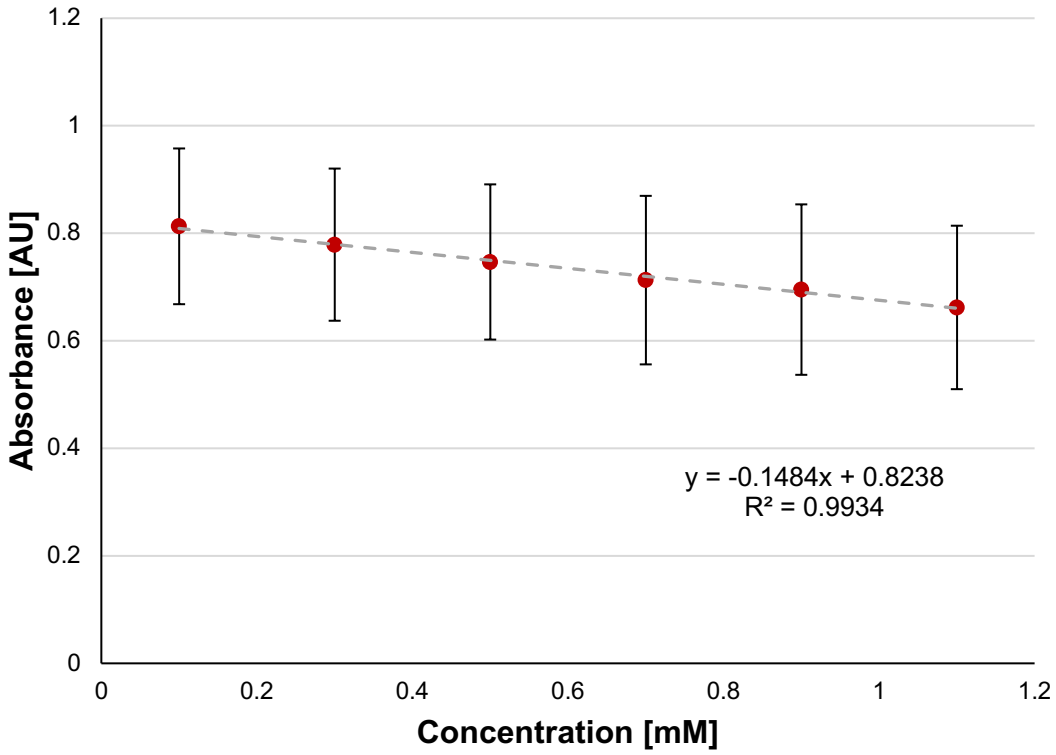


Figure 29: Calibration graph for glucose at 80°C based on data from Table 8.

In Figure 29, one can notice that differences in absorption between the glucose standards are very small that may result in imprecise outcomes. What is more, at 70°C and 80°C, keeping temperature stable throughout the whole experiment was a challenge. At 80°C, the water bath had to be covered by aluminium foil to prevent heat loss. That made these two temperatures not practically suitable for the analysis of COD in water samples.

4 Results

4.1 Analysis of industrial samples

During the project, industrial water samples from Crown Paints Ltd (Darwen, Lancashire, UK) were provided. The samples were collected on 12th June and 24th July 2017 and analysed the next day in the industry laboratory and within two days at the University. Samples were delivered in screw tight bottles (volume of 100 ml) and were stored in the fridge at a temperature of 6°C (Figure 30). At the University samples were analysed within two days. Samples were analysed in two ways using:

1) diluted samples with distilled water in the ratio 1:10,

2) sample straight from the storage bottle without dilution or any posttreatment.

Between the samples calibration standards were analysed.

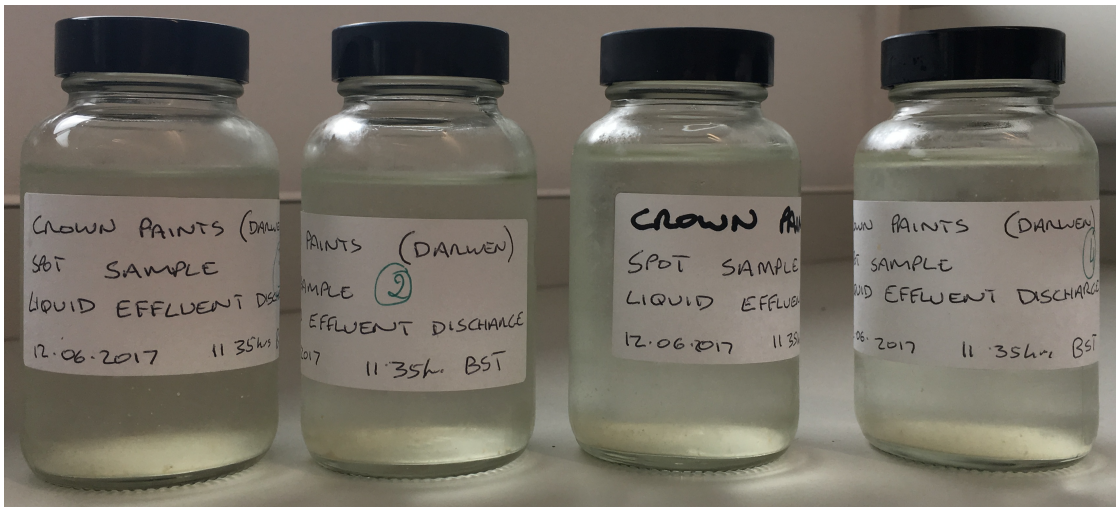


Figure 30: Industrial samples of water from Crown Paints company.

Table 9 presents obtained results from FIA and from Crown Paints'. FIA concentration values are the average taken from two days' analysis. Below the table, there is the conversion method of concentration from millimolar to mg/l.

Table 9: Comparison of COD values obtained using FIA vs the Industrial Method used in Figure 31.

Sample	Concentration [mM] by FIA	Concentration [mg/l] by FIA	Concentration [mg/l] by Industrial Method
CP 1	8.87	1598	1035
CP 1_1	15.6	2814	1320
CP 2_1	14.5	2613	1118

Converting COD concentration from mM to mg/l:

$M_{C6H12O6}$ = 180.156 g/mol = 180 156 mg/mol

1 mol = 1000 mM

180 156 mg $\rightarrow$ 1000 mM

x $\rightarrow$ 8.869 mM

$$x = \frac{180\,156 * 8.869}{1000} \approx 1598 \left[\frac{mg * mM}{mM} = mg\right]$$

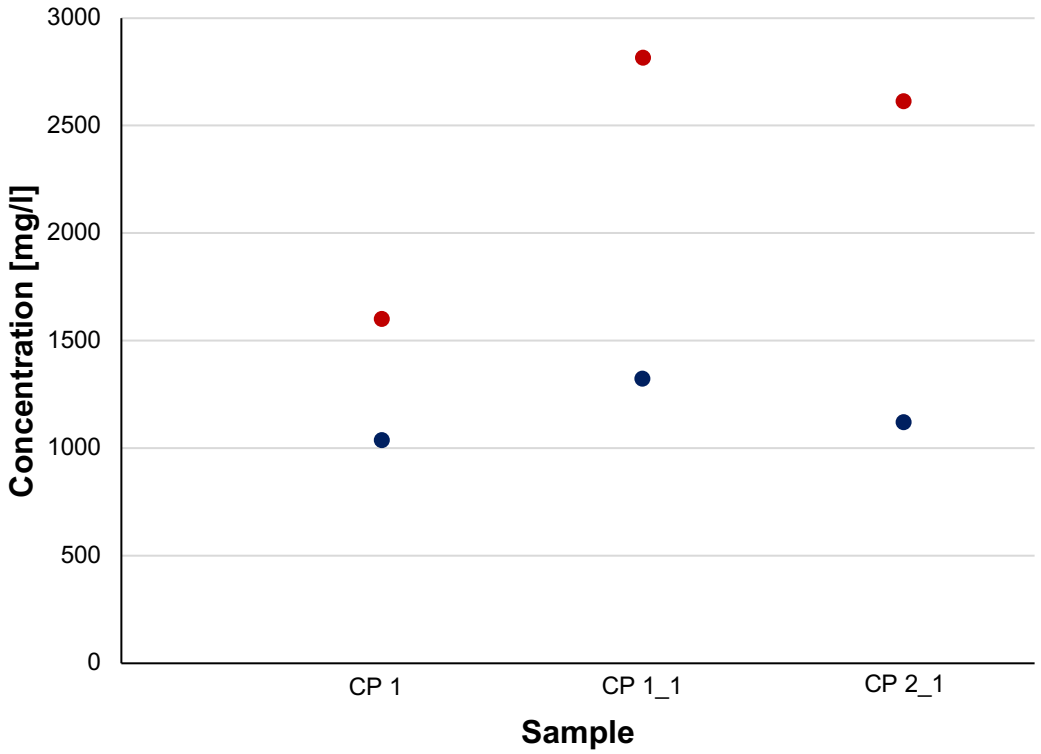


Figure 31: Graph with comparison of COD concentration values obtained from analysis using FIA • vs Industrial Method • based on data from Table 9. CP 1 is sample from 12th June and CP 1_1 and CP 2_1 are two samples from 24th July.

From analysing Figure 31 it is possible to observe that all concentration values obtained from FIA are much higher than those from the Industrial Method. However, one is able to observe a correlation between both values. The FIA values are around two times higher than those obtained from the Industrial Method. More samples should be analysed to confirm that. Samples from Crown Paints have been analysed at the University within two days after delivery and in the supplier's laboratory only once on the day the samples were bottled for analysis. It may be advisable to analyse all samples in both laboratories at the same time. That would allow a more robust comparison of the results obtained.

4.2 Recognition of the peak before the main sample peak

In almost every analysis the peak before the main negative sample peak may be seen, as shown in Figure 32. The peaks slightly vary in size but appear in most of the results. The question then comes to mind: why does the absorbance increase before sample reaches the detector?

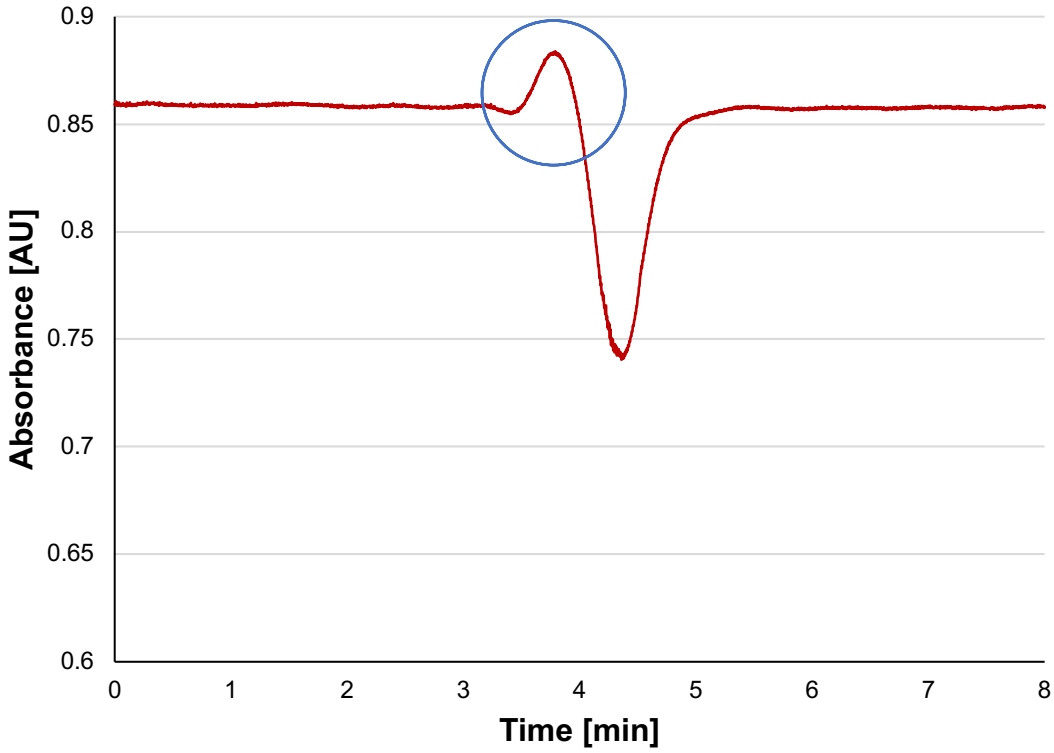


Figure 32: Example of spectrogram with a peak before the main sample peak.

The author did not find any explanation in the literature. However, the most possible reason is scattering. Appendix II presents analysis of the pre-peak. The possible impact on the height of the peak has on the following parameters: temperature, concentration and stability of used standards, sequence of samples (first sample or last one) were all studied. The effect of particles and air bubbles on the analyses were under investigation as well.

The stability of the standards was investigated. The glucose standard diluted with distilled water used in the experiment is stable[36]. There is no significant change in the undetermined peak height between the 0.1 mM and 1.1 mM glucose standards. One can assume that there is no correlation between the standard concentration and the pre-peak peak height.

Temperature does not have great influence on the undetermined peak height. Three different temperatures have been examined (50^{o}C, 60^{o}C and 70^{o}C). What is important, is that at 60^{o}C the peak heights seem to be lower (average peak absorbance is 0.023) than at 70^{o}C (average peak absorbance is 0.102) and at 50^{o}C (average peak absorbance is 0.054).

Standards were analysed in both orders, from the lowest to highest and from the highest to lowest. There is no correlation of the undetermined pre-peak peak height and the sample order. Nevertheless, the sample analysed after cleaning the system always has a lower peak than samples analysed before the cleaning. This researcher noticed that undetermined peak heights get bigger from analysis to analysis until cleaning.

Particles become a problem after the system has been running for longer than an hour. Although accumulation of the particles has not been detected during this project, it was possible to observe that particles' absorbance peaks heights are very low and there are many of them as seen in Figure 33.

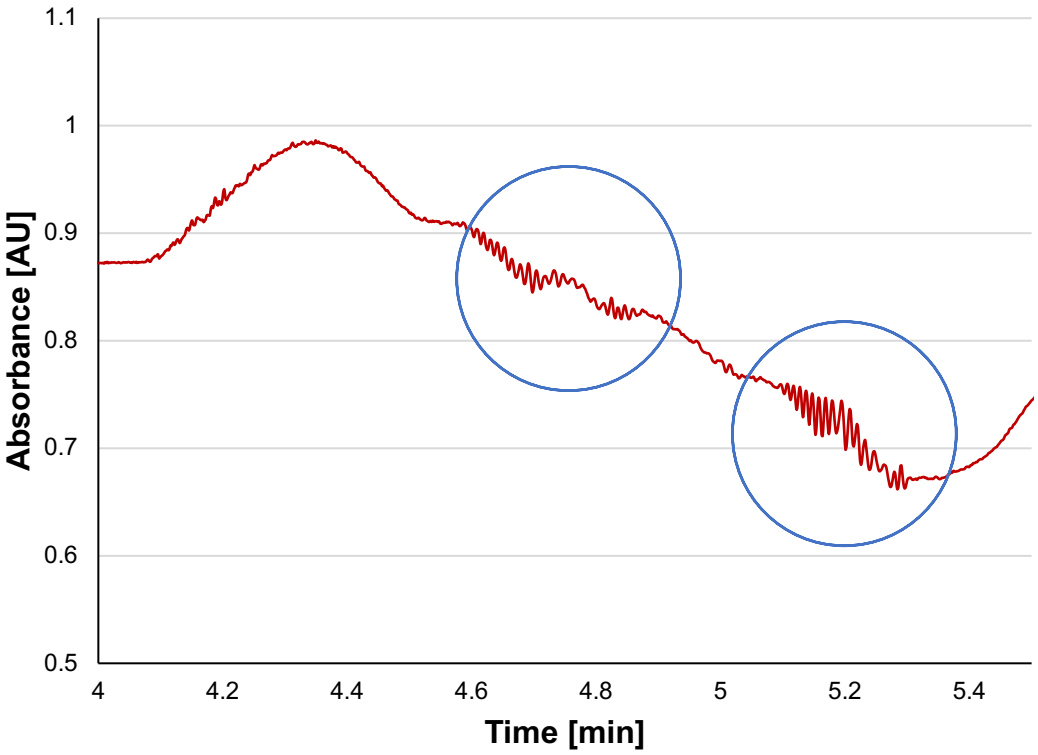


Figure 33: Fragment of the spectrogram (from 4 – 5.5 minutes) showing the influence of particles on the spectrometer response. Particles peaks are marked by blue circles.

Air bubbles may also have the result of increasing absorbance. However, all the air bubbles identified during the experiment resulted in very sharp and narrow peaks. These peaks may be observed in figures presented in the Discussion section. One can see, that it is not possible to confuse air bubble peaks with any other phenomena as they are distinctly rapid and short - fired.

5 Discussion - trouble shooting during the experiment

5.1 Gas bubbles

During the experiment, gas bubbles disrupted the analysis. There were so many of them that accurate analysis of results was impossible (Figure 34). After investigation two possible sources of the gas bubbles have been found. One is through the gas injection into tubing with the sample or reagents. A second possibility is that gas is the product of the reaction that occurs in the reaction coil and the gas is carbon dioxide:

$$5\ C_6H_{12}O_6 + 24\ KMnO_4 + 36\ H_2SO_4 \rightarrow \mathbf{30\ CO_2\uparrow} + 24\ MnSO_4 + 12\ K_2SO_4 + 66\ H_2O$$

or

$$C_6H_{12}O_6 + 8\ KMnO_4 + 4\ H_2SO_4 \rightarrow \mathbf{6\ CO_2\uparrow} + 8\ MnO_2\downarrow + 4\ K_2SO_4 + 10\ H_2O$$

Equation 2: Reactions that occur in the reaction coil.

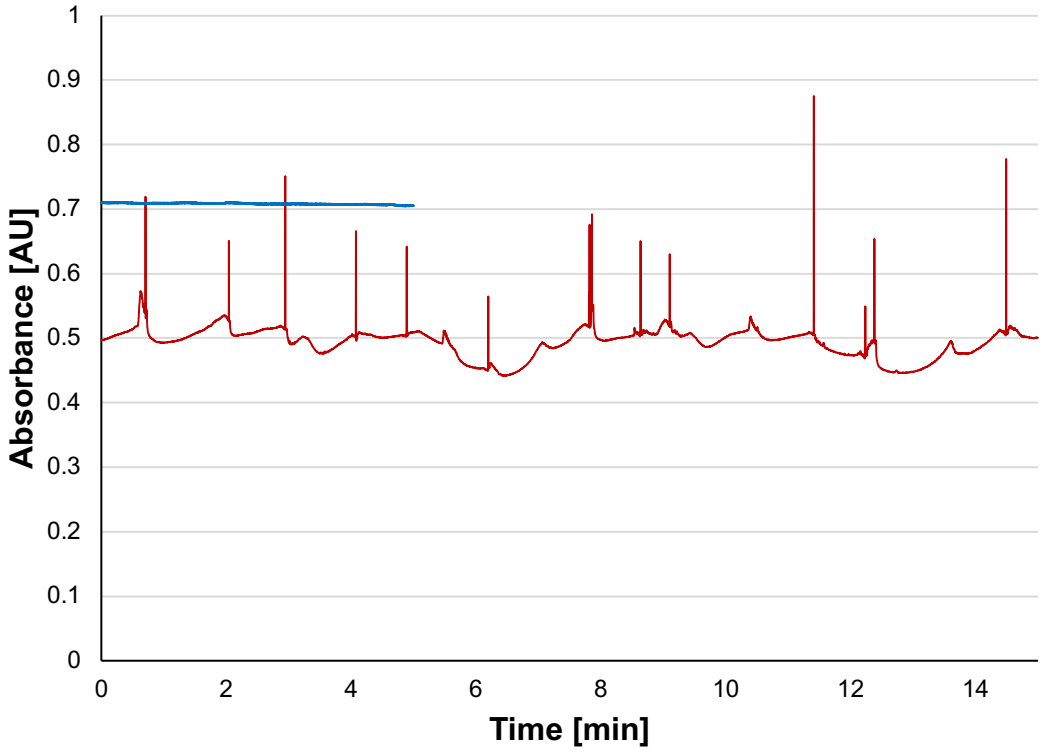


Figure 34: Spectrogram of blank sample with gas bubbles coming out of the system where a debubbler was not used (red graph, *t = 15 min*). All the peaks one can see in this trace are gas bubble peaks. The blue graph (*t = 5 min*) shows the blank sample with a debubbler fitted and is included to show the high degree of effectiveness.

A debubbler has been introduced to the flow system to prevent gas bubble problems in the future experiments (Figure 35). It is a glass T-shaped tube connected to the pump (which pumps out the bubbles) and to the other tubing. The debubbler diameter is 1 mm, it is 30 mm long, the glass part coming off the tube is 13 mm long. Tubing connected to the debubbler was made from three different tubes (first is 42 mm long and 2.3 mm diameter; second measured 46 mm with 1.65 mm diameter; last is 13 mm and 0.8 mm diameter).
The only disadvantage of using that debubbler is that a small amount of the solution is lost with the gas bubbles, however it is not a significant volume, so it has no influence on the obtained results.

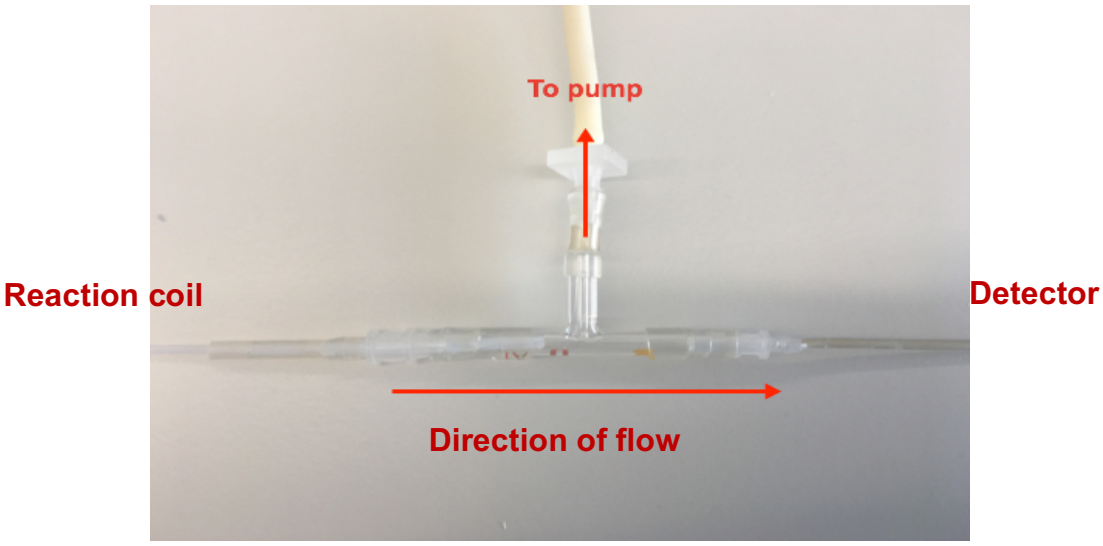


Figure 35: Debubbler with marked flow.

5.2 Cleaning the system

After about an hour of operation a black/brown sediment of manganese dioxide (MnO_2) starts to precipitate in the reaction coil (Equation 2 presents two possible reactions). The system can be easily cleaned by pumping a solution of 0.5 M iron (II) sulfate in sulfuric acid (1 : 1) through it (Figure 36).
Reaction of the cleaning process:

$$MnO_2\downarrow + 2\ FeSO_4 + 2\ H_2SO_4 \rightarrow MnSO_4 + Fe_2(SO_4)_3 + 2\ H_2O$$

Equation 3: Reaction of the cleaning process.

Figure 36: Pictures showing clearing of the system with solution of $FeSO_4$ and H_2SO_4. From left hand side: blocked reaction coil is cleaned until all sediment disappears (right hand side).

5.3 Position of the flow cell in the spectrophotometer

Another problem observed in the presented analysis was the position of the flow cell in the spectrophotometer. The light beam from the instrument hits the sample at 26 mm high. The flow cell was 45 mm high in total and its black surface was 26 mm high, so the beam reaches the black surface of the flow cell, when it was pushed all the way down to the bottom of the sample holder in the instrument (Figure 37 and Figure 38).

The flow cell used in this project has a window at 9 mm which ends at 20 mm from the bottom of the cell. It means that the flow cell had to have a higher window, so the light beam hits the sample, not the black surface of the cell. To achieve that, the flow cell was moved up in the sample holder and placed in line with the cell lifter (Figure 38).

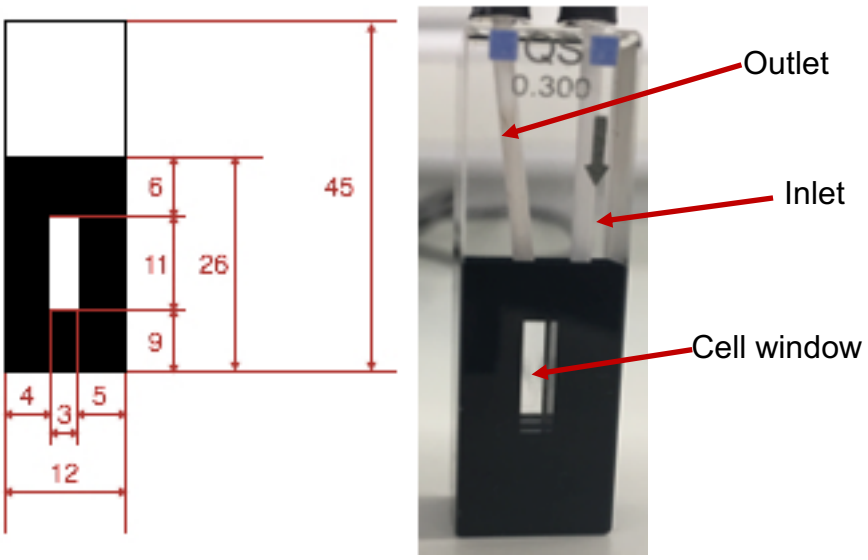


Figure 37: Scheme of the flow cell (1 cm path length) used in the experiment with all dimensions (in mm).

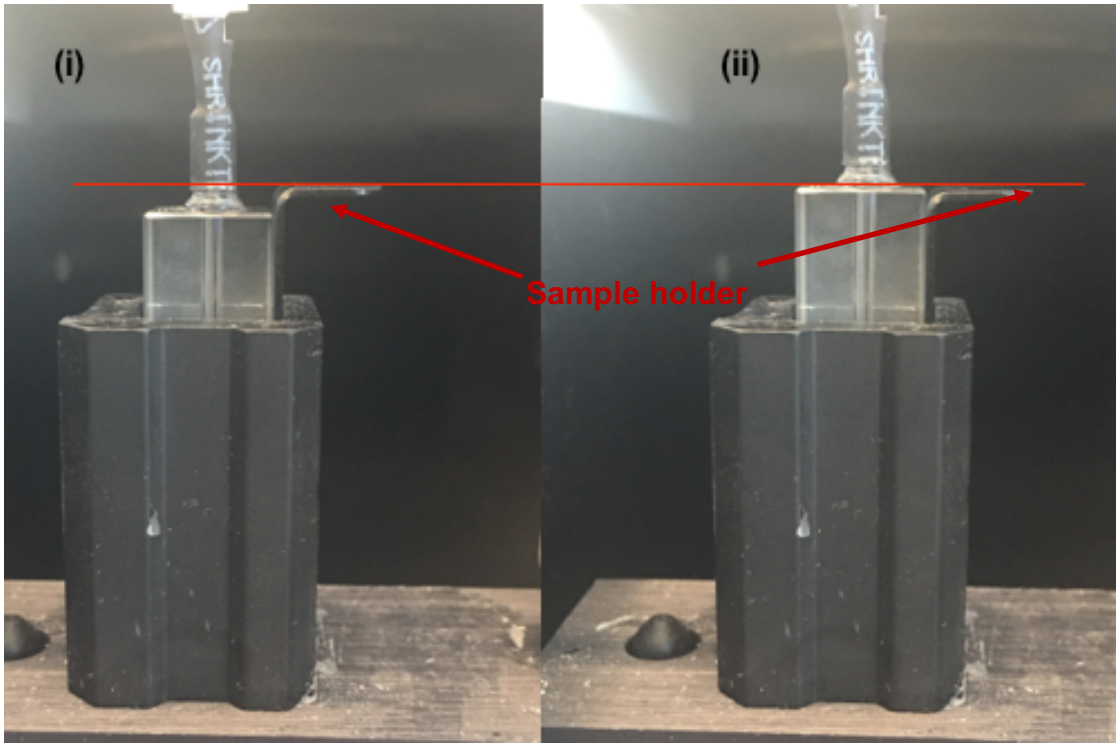


Figure 38: Picture showing position of the flow cell in the instrument with cell pushed all the way down (i) and how it was placed during the experiment (ii).

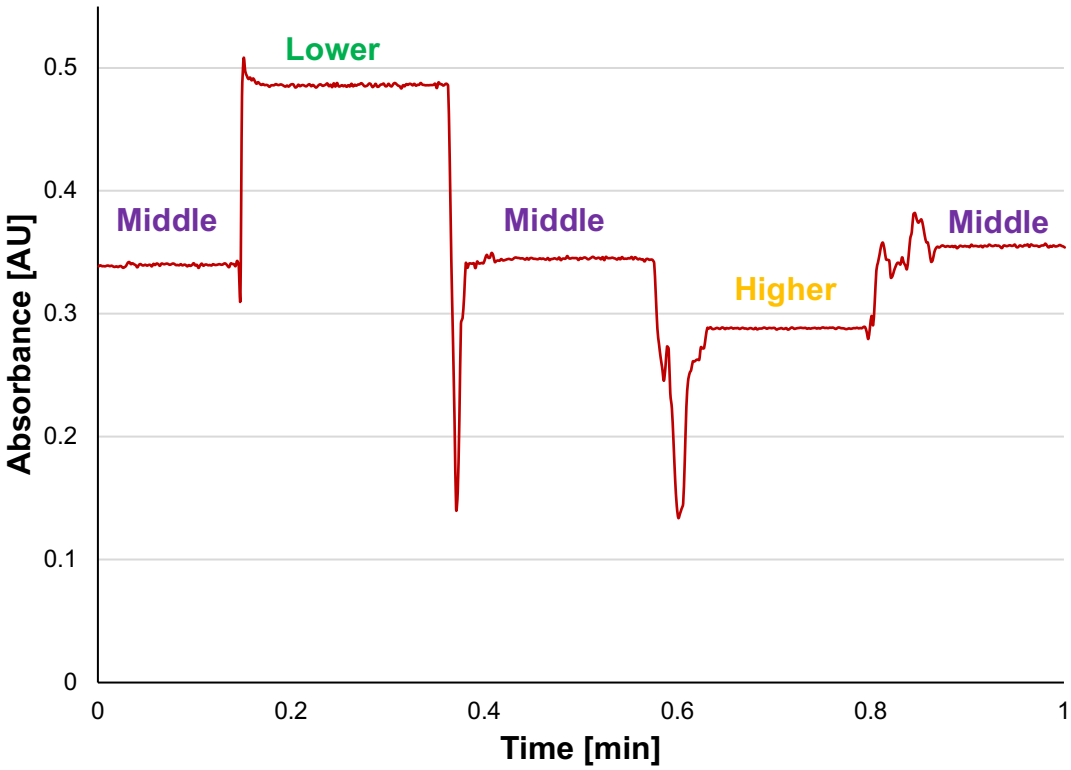


Figure 39: Graph showing how the movement of the flow cell affects the spectrogram.

In Figure 39 the influence of flow cell movement on absorbance is presented. At the beginning of the analysis, the flow cell has been held at the same distance from the bottom of the sample holder during each analysis. Then it had been deliberately pushed all the way down to the bottom and the absorbance increased. This is due to the light hitting the black surface rather than the transparent part of the cell. Accordingly, if the flow cell was taken up, peaks become smaller than the base line. This is because of the fact that distilled water has an absorbance higher than the transparent part of the flow cell.

5.4 Ageing of reagents

During the time of the experiment, this researcher has noticed that the base line absorbance significantly changed and whilst values of 1.2 were obtained in the first day, they were 0.75 a few days later. That problem has been thoroughly checked by analysing blank samples in a week time. Every day at least three blanks without any air bubbles peaks have been obtained. Then an average was calculated to obtain as accurate data as possible (Table 10).

Afterwards, all data were plotted on the graph to show how reagent ageing changes the base line absorption (Figure 40).

Table 10: Average absorbance from blank samples analysed which were used to construct the graph shown in Figure 40. Day 7 and 8 being a weekend.

Day	Average Absorbance [AU]
1	0.924
2	0.891
3	0.885
4	0.851
5	0.809
6	0.741
9	0.710

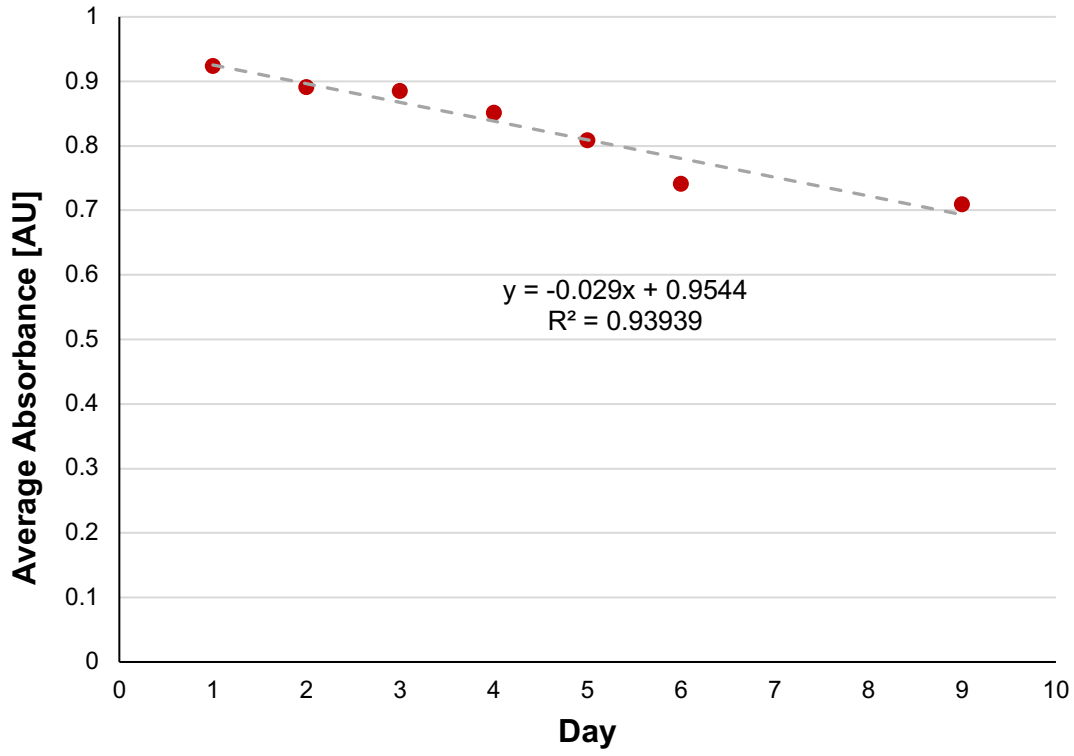


Figure 40: Average absorbance from analysis of the blank samples. Based on data from Table 10.

Analysis of Figure 40 allows one to conclude that it is not possible to compare results from different days without running standards alongside the samples. Only calibration graphs constructed during the same analysis makes the data reliable and allows for precise COD concentration calculations.

6 Conclusion

The simplicity of the FIA system makes the technique an alternative to the commercial COD instruments used in industry. During this project, this researcher compared results obtained from flow injection analysis and from the external company results (Crown Paints Ltd.).
The outcome of that comparison is that there is not enough evidence that FIA could be used as an alternative for the Industrial Method. However, data obtained so far look promising. If more experiments undertaken and if the data would confirm that the technique is reliable, the company may be able to reduce expenses and the time required for analysis. Unfortunately, time for this project did not allow conducting more comparative experiments with industrial waste samples.

Components used to make the FIA system are relatively inexpensive and it can be easily modified to achieve the best possible results or adapted to meet any type of task without complex changes[12]. That makes flow injection analysis a perfect technique for analysis of various samples.

A few problems which occurred during the project were relatively easy to solve. However, it needed some research and additional work and analysis. Everyone who uses FIA should be aware that gas bubbles may become a big problem during the analysis and may require a debubbler or degasser of the reagents. Another problem was sediment which appeared after around 1 hour of testing. Although pumping cleaning solvent throughout tubbing allows continuation of the analysis.

In the future, more experiments should be done using different standards. Then results may be compared with Industrial results and from results presented in this thesis. Korenaga[34] used a mixture of L-glutamic acid and lactose (in 5 : 1 ratio). Furthermore, other scientists used sodium salicylate, sodium acetate or sodium oxalate[25]. Comparison would allow an informed choice of the best standards for FIA-based COD determination.

7 Bibliography

[1] T. Korenaga and H. Ikatsu, “Continuous Flow Injection Analysis of Aqueous Environmental Samples for Chemical Oxygen Demand,” *Analyst*, vol. 106, pp. 653–662, 1981.

[2] M. Trojanowicz and K. Kołacińska, “Recent advances in flow injection analysis,” *Analyst*, vol. 141, no. 7, pp. 2085–2139, 2016.

[3] C. Ranger, “Flow Injection Analysis. Principles, techniques, applications, design.,” *Anal. Chem.*, vol. 53, no. 1, p. 20A–32A, 1981.

[4] B. Karlberg and G. Pacey, “Flow Injection Analysis a Practical Guide,” *Tech. Instrum. Anal. Chem.*, pp. 1–28, 1989.

[5] J. Ruzicka, E. Hansen, and A. Starzynski, “Flow Injection Analysis,” 2017.

[6] “Nuclear Power.” [Online]. Available: http://www.nuclear-power.net/nuclear-engineering/fluid-dynamics/reynolds-number/. [Accessed: 10-Feb-2017].

[7] F. R. Mansour and N. D. Danielson, “Reverse flow-injection analysis,” *Trends Anal. Chem.*, vol. 40, pp. 1–14, 2012.

[8] A. A. Kulkarni and I. S. Vaidya, “Flow injection analysis : an overview,” *J. Crit. Rev.*, vol. 2, no. 4, pp. 19–24, 2015.

[9] T. Zhou, S. Feng, Y. Huang, D. Yuan, J. Ma, and Y. Zhu, “Determination of Aluminum in Natural Waters by Flow Injection Analysis with Spectrophotometric Detection,” *Anal. Lett.*, vol. 49, no. 11, pp. 1669–1680, 2016.

[10] J. Ruzicka and E. H. Hansen, “Flow Injection Analysis Part I. A New Concept of Fast Continous Flow Analysis,” *Analytica Chimica Acta*, vol. 78. pp. 145–157, 1975.

[11] T. Ghous, “Flow Injection Analysis (FIA),” *J. Chem. Soc. Pakistan*, vol. 21, no. 4, pp. 375–381, 1999.

[12] M. Valcarcel and M. Luque de Castro, “Applications of FIA to environmental analysis,” in *Flow-Injection Analysis, Principles and Applications*, Ellis Horwood Limited, 1987, pp. 353–375.

[13] N. Dantan, W. Frenzel, and S. Kuppers, “Flow injection Analysis Coupled with HPLC and CE for Monitoring Chemical Production Processes,” *Chromatographia*, vol. 54, pp. 187–190, 2001.

[14] C. Ruiz - Capillas and L. Nollet, *Flow Injection Analysis of Food Additives*. CRC Press. Taylor & Francis Group, 2015.

[15] FIAlab®, "FIAlab Flow Cells." [Online]. Available: http://www.flowinjection.com/products/flow-cells. [Accessed: 20-Sep-2017].

[16] "Learning Solutiocs NC Community Colleges." [Online]. Available: http://www.lsteam.org/projects/videos/how-does-spectrophotometer-work. [Accessed: 10-Feb-2017].

[17] D. Harris, *Quantitative Chemical Analysis*, 7th Editio. W.H. Freeman and Company, 2007.

[18] Environment Agency, "The determination of chemical oxygen demand in waters and effluents (2007)," *Methods Exam. Waters Assiociated Mater.*, pp. 1–64, 2007.

[19] W. Boyles, "The Science of Chemical Oxygen Demand," *Tech. Inf. Ser. Bookl.*, vol. 9, 1997.

[20] R. B. Geerdink, J. Brouwer, and O. J. Epema, "A reliable mercury free chemical oxygen demand (COD) method," *Anal. Methods*, vol. 1, no. 2, p. 108, 2009.

[21] R. B. Geerdink, R. Sebastiaan van den Hurk, and O. J. Epema, "Chemical oxygen demand: Historical perspectives and future challenges," *Anal. Chim. Acta*, vol. 961, pp. 1–11, 2017.

[22] M. Kolb, M. Bahadir, and B. Teichgräber, "Determination of chemical oxygen demand (COD) using an alternative wet chemical method free of mercury and dichromate," *Water Res.*, vol. 122, pp. 645–654, 2017.

[23] National Institute of Technical Teachers' Training and Research, "Experimental on Determination of Chemical Oxygen Demand," 2011.

[24] Denver Instrument, "Chemical Oxygen Demand of Water."

[25] J. M. H. Appleton, J. F. Tyson, and R. P. Mounce, "The rapid determination of chemical oxygen demand in waste waters and effluents by flow injection analysis," *Anal. Chim. Acta*, vol. 179, pp. 267–278, 1986.

[26] "VWR We Enable Science." [Online]. Available: https://us.vwr.com/store/product/4697520/c-o-d-digestion-reagent-vials-wtw. [Accessed: 26-Sep-2017].

[27] Hach®, “Chemical Oxygen Demand for Water, Wastewater and Seawater,” pp. 425–434, 2009.

[28] Hach®, “Chemical Oxygen Demand Procedure. Reactor Digestion Method,” pp. 1–10, 2014.

[29] J. Ma, “Determination of chemical oxygen demand in aqueous samples with non-electrochemical methods,” *Trends Environ. Anal. Chem.*, vol. 14, no. April, pp. 37–43, 2017.

[30] D. Ball, S. Goode, and D. Reger, *Chemistry: Principles and Practice,* 3rd edition. Thomson Learning/Cengage Learning, 2010.

[31] International Organization for Standardization 6060, “Determination of the Chemical Oxygen Demand,” 1989.

[32] U. Latif and F. Dickert, “Chemical Oxygen Demand,” in *Enviromental Analysis by Electochemical Sensors and Biosensors*, Springer New York, 2015, pp. 719–728.

[33] A. Mahvi, E. Bazrafshan, and G. Jahed, “Evaluation of COD Determiantion by ISO, 6060 Method, Comparing with Standard Method (5220,B),” *Pakistan J. Biol. `sciences*, vol. 8, no. 6, pp. 892–894, 2005.

[34] T. Korenaga, T. Moriwake, and T. Takahashi, “Evaluation of Three Flow Injection Analysis Methods for the Determination of Chemical Oxygen Demand,” *Memoris Sch. Eng. Okayama Univ.*, vol. 19, no. 1, pp. 53–62, 1984.

[35] Indian Standard, “Density-Composition Tables for Aqueous Solutions of Sulphuric Acid - Specification,” pp. 1–31, 1989.

[36] R. Jawad *et al.*, “Stability of sugar solutions: A novel study of the epimerization kinetics of lactose in water,” *Mol. Pharm.*, vol. 11, no. 7, pp. 2224–2238, 2014.

Appendix I

Microprocessor program written in simplified 'C-type language' used in sample loop filling.

```
#include <LiquidCrystal.h>
#define PINVALVE1 12    //pin number to valve 1//
#define PINVALVE2 11    //pin number to valve 2
#define PINVALVE3 10    //pin number to valve 3
#define PINSWITCH 2     //pin number to switch IRQ0
#define ON HIGH         //valve on#define OFF LOW         //valve off
LiquidCrystal lcd(8, 9, 4, 5, 6, 7); // initialize the library with the numbers of the interface pins
volatile int key = 0;
volatile unsigned long previousMillis;   // will store last time updated
volatile unsigned long currentMillis;
volatile const long interval = 400;      // interval at which to blink (milliseconds)
unsigned long prevMillis;
unsigned long currMillis;
char*  Linia[]  =  {    "hello,  AGATA!",        //0    "START",                    //1
 "STOP         ",                                                                  //2
"1__            ",                                                                //3
"_2_            ",                                                                //4
"__3            ",                                                                //5
"___            ",                                                                //6
"123  "          //7
};
void setup() {  //pinMode(PINVALVE1, OUTPUT);     //pin output
pinMode(PINVALVE3, OUTPUT);
pinMode(PINSWITCH, INPUT_PULLUP);

digitalWrite(PINVALVE3, OFF);
attachInterrupt(digitalPinToInterrupt(PINSWITCH),    press_key,    FALLING);
//FALLING RISING
```

```
Serial.begin(9600);            // RS baud rate
 lcd.begin(16, 2);             // set up the LCD's number of columns and rows
 lcd.print(Linia[0]);          // Print a message to the LCD
}
void loop() {
if (key == 0) {
 monitor_LCD (2);
control_valve (OFF, OFF, OFF);
monitor_LCD (6);
Serial.print("hello, AGATA! time is: ");
prevMillis = millis();
Serial.println(prevMillis);
}
else {
monitor_LCD (1);
//step 1
control_valve (ON, ON, ON);
 currMillis = millis();
monitor_LCD (7);
Serial.print(Linia[7]);
Serial.println(currMillis - prevMillis);
prevMillis = currMillis;    delay (40000);                  // waits for 40 s
//step 2    control_valve (OFF, OFF, OFF);
currMillis = millis();    monitor_LCD (6);
Serial.print(Linia[6]);
Serial.println(currMillis - prevMillis);
prevMillis = currMillis;    delay (60000);                  // waits for 1 min
}
}                                       // *** loop end
void control_valve (int con_v1, int con_v2, int con_v3)  {
digitalWrite(PINVALVE3, con_v3);
return;
}
void monitor_LCD (int nr_text) {
```

```
switch (nr_text) {
case 1:
lcd.setCursor(0,1);
lcd.print(Linia[1]);
break;
case 2:
lcd.setCursor(0,1);
lcd.print(Linia[2]);
break;
case 3:
lcd.setCursor(8,1);
lcd.print(Linia[3]);
break;
case 4:
lcd.setCursor(8,1);
lcd.print(Linia[4]);
break;
case 5:
lcd.setCursor(8,1);
lcd.print(Linia[5]);
break;
case 6:
lcd.setCursor(8,1);
lcd.print(Linia[6]);
break;
case 7:
lcd.setCursor(8,1);
lcd.print(Linia[7]);
break;
default:
break;
 }
return;
}
```

```
void press_key() {
currentMillis = millis();
if (currentMillis - previousMillis >= interval) {
previousMillis = currentMillis;
key = !key;
}
}
```

Appendix II

Data from peak comparison in different samples, days and temperatures used in Section 4.2.
'Peak height' is 'Peak Abs' – 'Base line Abs'.

Date	Sample	Peak Abs	Base line Abs	Lowest Abs	Peak height	Peak height / Lowest Abs	Average Peak height	Temp [°C]
02/08/2017	0.9 mM	1.182	1.103	0.924	0.079	0.085	0.084	70
02/08/2017	0.7 mM	1.173	1.108	0.918	0.065	0.071		70
02/08/2017	0.5 mM	1.185	1.106	0.919	0.079	0.086		70
02/08/2017	0.3 mM	1.186	1.101	0.916	0.085	0.093		70
02/08/2017	0.1 mM	1.212	1.100	0.946	0.112	0.118		70
15/03/2017	0.1 mM	1.251	1.074	0.967	0.177	0.183	0.136	70
15/03/2017	0.3 mM	1.216	1.073	0.922	0.142	0.154		70
15/03/2017	0.5 mM	1.181	1.064	0.898	0.117	0.130		70
15/03/2017	0.7 mM	1.217	1.075	0.883	0.141	0.160		70
15/03/2017	0.9 mM	1.168	1.050	0.867	0.118	0.136		70
15/03/2017	1.1 mM	1.179	1.057	0.835	0.122	0.146		70
05/06/2017	0.1 mM	1.173	1.108	0.940	0.065	0.069	0.047	60
05/06/2017	0.3 mM	1.142	1.103	0.952	0.039	0.041		60
05/06/2017	0.5 mM	1.143	1.101	0.921	0.042	0.046		60
05/06/2017	0.7 mM	1.143	1.095	0.936	0.048	0.051		60
05/06/2017	0.9 mM	1.144	1.095	0.926	0.049	0.053		60
05/06/2017	1.1 mM	1.132	1.090	0.920	0.041	0.045		60

01/06/2017	0.1 mM	0.880	0.867	0.748	0.013	0.017	0.016	60
01/06/2017	0.3 mM	0.879	0.864	0.752	0.015	0.020		60
01/06/2017	0.5 mM	0.875	0.864	0.749	0.012	0.016		60
01/06/2017	0.7 mM	0.881	0.862	0.744	0.019	0.025		60
01/06/2017	0.9 mM	0.884	0.860	0.740	0.025	0.033		60
01/06/2017	1.1 mM	0.872	0.858	0.734	0.014	0.019		60
25/05/2017	1.1 mM	1.060	1.045	0.857	0.015	0.018	0.020	60
25/05/2017	0.9 mM	1.056	1.042	0.893	0.014	0.015		60
25/05/2017	0.7 mM	1.059	1.050	0.903	0.010	0.011		60
25/05/2017	0.5 mM	1.065	1.046	0.908	0.019	0.021		60
25/05/2017	0.3 mM	1.070	1.057	0.917	0.014	0.015		60
25/05/2017	0.1 mM	1.102	1.054	0.863	0.048	0.056		60
27/07/2017	1.1 mM	1.233	1.230	1.035	0.003	0.003	0.009	60
27/07/2017	0.9 mM	1.234	1.230	1.042	0.005	0.005		60
27/07/2017	0.7 mM	1.247	1.229	1.044	0.018	0.017		60
27/07/2017	0.5 mM	1.244	1.232	1.047	0.012	0.011		60
27/07/2017	0.3 mM	1.245	1.232	1.054	0.014	0.013		60
27/07/2017	0.1 mM	1.238	1.235	1.059	0.003	0.003		60
23/05/2017	0.1 mM	1.197	1.148	1.020	0.049	0.048	0.064	50
23/05/2017	0.3 mM	1.204	1.148	1.017	0.056	0.055		50
23/05/2017	0.5 mM	1.277	1.142	1.036	0.134	0.130		50
23/05/2017	0.7 mM	1.181	1.140	1.003	0.042	0.041		50
23/05/2017	0.9 mM	1.190	1.137	1.002	0.053	0.053		50
23/05/2017	1.1 mM	1.184	1.133	0.979	0.051	0.052		50

19/05/2017	0.1 mM	1.280	1.234	1.083	0.046	0.042	0.050	50
19/05/2017	0.3 mM	1.270	1.238	1.074	0.033	0.031		50
19/05/2017	0.5 mM	1.277	1.228	1.077	0.050	0.046		50
19/05/2017	0.7 mM	1.290	1.234	1.085	0.057	0.052		50
19/05/2017	0.9 mM	1.307	1.235	1.088	0.072	0.066		50
19/05/2017	1.1 mM	1.274	1.233	1.077	0.041	0.038		50
18/05/2017	1.1 mM	1.240	1.203	1.051	0.037	0.035	0.049	50
18/05/2017	0.9 mM	1.272	1.205	1.070	0.067	0.063		50
18/05/2017	0.7 mM	1.268	1.212	1.061	0.056	0.053		50
18/05/2017	0.5 mM	1.248	1.209	1.024	0.039	0.038		50
18/05/2017	0.3 mM	1.243	1.202	1.027	0.042	0.041		50
18/05/2017	0.1 mM	1.257	1.202	1.028	0.056	0.054		50

Appendix III

Risk assessment for the Acid\Base experiment.

Record of a risk assessment

Task: Flow Injection Analysis evaluation experiment with acid-base titration.

Department	CHEMISTRY.	Assessment ID	
Assessor	A. MAKAS	Date of assessment	19-10-16.
Authorised by	P R FIELDEN	Review date	18-10-17

Step 1 List significant hazards	**Step 2** who might be harmed	**Step 3** determine appropriate controls	**Step 4** make it happen
Working in chemistry lab environment	All lab personnel, researchers, technical staff, postgraduate and undergraduate students.	Good laboratory practice. Ability to work safely in the lab and comply with the local rules.	All staff and students using lab should be trained in use and handling procedures and be familiar with the risk assessment.
Use of chemicals		A full COSHH assessment for the chemicals has been completed and signed by the supervisor. Using PPE (lab coat, safety glasses, gloves). Appropriate storage and labelling of the samples. Using small amount of chemicals.	
Chemical spillage		Correct handling of chemicals in the laboratory prevents spillage. Any chemicals which are used, must be lifted correctly and lids must be adequately sealed.	
Electrical –		Avoid aqueous solution Spillage on pump and Power Connector	
Mechanical		When pump is rotating keep fingers and hair away. and any loose clothing.	

Step 5 – remember to include a review date

Appendix IV

COSHH (Control of Substances Hazardous to Health) for the Acid\Base experiment.

COSHH Worksheet

Assessors and persons authorising work with hazardous substances must be familiar with;

- the list of prohibited substances
- University guidance relating to specific hazards
- University guidance relating to the use of personal protective equipment

See the University's Manual of Safety for details

Carried out by: Agata Makas	**Authorised by:** P.R. FIELDEN
Date of assessment: 19-10-16	**Due date for review:** 18-10-17.
Task Flow Injection Analysis evaluation experiment with acid-base titration.	

Step 1: Hazard Identification

Use the following table to summarise the chemical hazards associated with the task. This information can be found in the Safety Data Sheet which your chemical supplier is legally obliged to provide to you.

Substance	Risk Phrases	Exposure route and consequence	Workplace exposure limits [from safety data sheets]
Hydrochloric acid (0.1M)	H290: May be corrosive to metals	Direct contact with eyes: Flush eyes with water as a precaution. Direct contact with skin: Wash off with soap and plenty of water. Consult a physician. Ingestion: Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. Inhalation: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.	Long term exposure limit: 2 mg/m^3 Short term exposure limit: 8 mg/m^3

Sodium Hydroxide (0.1 M)	None	Direct contact with eyes: Flush eyes with water as a precaution. Direct contact with skin: Wash off with soap and plenty of water. Ingestion: Never give anything by mouth to an unconscious person. Rinse mouth with water. Inhalation: If breathed in, move person into fresh air. If not breathing, give artificial respiration.	Long term exposure limit: None Short term exposure limit: 2 mg/m^3
Bromothymol Blue	None	Direct contact with eyes: Flush eyes with water as a precaution. Direct contact with skin: Wash off with soap and plenty of water. Ingestion: Never give anything by mouth to an unconscious person. Rinse mouth with water. Inhalation: If breathed in, move person into fresh air. If not breathing, give artificial respiration.	None
Phenolphthalein	H341: Suspected of causing genetic defects. H350: May	Direct contact with eyes: Flush eyes with water as a precaution. Direct contact with skin: Wash off with soap and plenty of water. Consult a physician.	None

	cause cancer. H361f: Suspected of damaging fertility.	Ingestion: Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. Inhalation: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.	
Do any substances listed require health surveillance or workplace monitoring?			
Substance		Details of surveillance required	

Step 2: List those people who may be at risk of exposure

The following groups of people may be exposed to substances hazardous to health during normal operations; ☐ Undergraduate students ☑ Post graduate students ☑ Post doctoral researcher ☑ Technical support staff ☑ Academic staff ☑ Room occupants not carrying out the task ☐ Other (specify)
Describe the level, type and duration of exposures likely to occur during routine operations[1] (you must include the handling and disposal of any wastes generated during the work);
Describe foreseeable accidental exposure scenarios (e.g. spillage on bench). Spillage on workbench, clothing, skin, eyes, mouth. Inhalation or swallowing.
The following groups of people may be exposed to substances hazardous to health during foreseeable accident scenarios; ☐ Undergraduate students ☑ Post graduate students ☑ Post doctoral researcher ☑ Technical support staff ☑ Academic staff ☑ Room occupants not carrying out the task ☐ Other (specify)

Flammable or explosive substances:

Does the task use or produce substances which could cause fire or explosion? If so then refer to the University's guidance on the control of Dangerous Substances and Explosive Atmospheres Regulations.

Uncontrolled access to the work area:

Do the substances used or produced require the work area to be designated as a 'Hazardous Area' as per the University's Code of Practice on access to hazardous areas for Facilities personnel or contractors.

[1] A separate COSHH assessment may be necessary to cover maintenance operations

Step 3: Determine appropriate controls

Regulation 7 of COSHH stipulates that exposure to substances harmful to health MUST be prevented, or where this is not reasonably practicable, adequately controlled.

Provide a statement against each item of the following hierarchy of control giving details of the controls you will adopt or a justification as to why no controls in this category are being implemented.

Your controls must address the routine aspects of the work (including waste handling and disposal) and must reduce the risk of any accidental exposures listed in step 2.

Eliminate No materials can be eliminated from this task.
Reduce Small quantities in small concentration will be used.
Isolate: Containment Hazardous substances will be always kept in appropriate container.
Control: General ventilation to Local Exhaust Ventilation (single point extract close to source to ventilated partial containment) Fumehood will be used when required.
PPE Gloves, eye protection, closed toe footwear, lab coat.
Discipline Carry out experiments according to Good Lab Practice procedure (GLP).

Special precautions to be adopted in the event of a spillage;

Immediate actions	**e.g. deploy spill kit, evacuate the area, close the door and raise alert supervisor**
Clean-up procedure	Collect with paper towel, dispose in chemical waste.

Step 4: Complete a COSHH Summary Sheet and develop a Safe System of Work

Summarise the salient points of the assessment on a COSHH Summary sheet and append this to the front of any Operating Procedures which relate to the task.

REMEMBER:

A COSHH assessment will not protect you; it is the adoption of the control measures arising from the assessment into a 'Safe System of Work' which will keep you and your colleagues safe.

Please consider each of the following three elements of a Safe System of Work;

Procedures	Do you have a written procedure? (If not how are hazard control measures to be communicated to those undertaking the task). Are the hazards associated with the task clearly described in the procedure? Are the control measures generated by your COSHH assessment clearly identified in the procedure?
Training and Instruction	Have all those undertaking the task received an appropriate level of training in relation to any hazards and their control? Are suitable warning signs and notices displayed?
Supervision	Has an appropriate level of supervision been discussed and agreed? B – approval and advice required from supervisor prior to work starting

Appendix V

Risk assessment for the COD experiment.

Record of a risk assessment

Task:
Flow Injection Analysis of samples for chemical oxygen demand

Department	Chemistry	Assessment ID	
Assessor	Agata Makas	Date of assessment	23-01-17
Authorised by	P.R. Fielden	Review date	01-12-17.

Step 1 **List significant hazards**	**Step 2** **who might be harmed**	**Step 3** **determine appropriate controls**	**Step 4** **make it happen**
Working in chemistry lab environment	All lab personnel, researchers, technical and academic staff, postgraduate students and other people in the lab.	Good laboratory practice. Ability to work safely in the lab and comply with the local rules.	All staff and students using lab should be trained in use and handling procedures and be familiar with the risk assessment
Use of chemicals		A full COSHH assessment for the chemicals has been completed and signed by the supervisor. Using PPE (lab coat, safety glasses, gloves). Appropriate storage and labelling of the samples. Using small amount or diluted of chemicals.	
Chemical spillage		Correct handling of chemicals in the laboratory prevents spillage. Any chemicals which are used, must be lifted correctly and lids must be adequately sealed.	
Electricity		Avoid aqueous solutions spillage on electrical equipment. Check if PAT test labels are up to date.	
Hot surfaces		Leave enough space around the heating mat to prevent catching fire by surrounding materials. Use appropriate meter to control temperature.	
Mechanical		When pump is rotating keep fingers, hair and any loose clothing away.	

Appendix VI

COSHH (Control of Substances Hazardous to Health) for the COD experiment.

COSHH Worksheet

Assessors and persons authorising work with hazardous substances must be familiar with;

- the list of prohibited substances
- University guidance relating to specific hazards
- University guidance relating to the use of personal protective equipment

See the University's Manual of Safety for details

Carried out by: Agata Makas	**Authorised by:** P.R. Fielden
Date of assessment: 06-02-2017	**Due date for review:** 01-08-2017.
Task Flow Injection Analysis of samples for chemical oxygen demand	

Step 1: Hazard Identification

Use the following table to summarise the chemical hazards associated with the task. This information can be found in the Safety Data Sheet which your chemical supplier is legally obliged to provide to you.

Substance	Risk Phrases	Exposure route and consequence	Workplace exposure limits
Ammonium sulfate $(NH_4)_2SO_4$	None	**Direct contact with eyes:** Flush eyes with water as a precaution. **Direct contact with skin:** Wash off with soap and plenty of water. Consult a physician. **Ingestion:** Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. **Inhalation:** If breathed, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.	None
D-(+)-Glucose $C_6H_{12}O_6$	None	**Direct contact with eyes:** Flush eyes with water as precaution. **Direct contact with skin:** Wash off with soap and plenty of water. **Ingestion:** Never give anything by mouth to an unconscious person. Rinse mouth with water. **Inhalation:** If breathed in, move person into fresh air. If not breathing, give artificial respiration.	None
Iron (II) sulfate heptahydrate $FeSO_4 \cdot 7H_2O$		**Direct contact with eyes:** Rinse thoroughly with plenty of water for at least	**Long term exposure**

	H302: Harmful if swallowed. H315: Causes skin irritation. H319: Causes eye irritation.	15 min and consult a physician. **Direct contact with skin:** Wash off with soap and plenty of water. Consult a physician. **Ingestion:** Call a Poison Center/doctor if you feel unwell. Rinse mouth. **Inhalation:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.	**limit:** 1 mg/m^3 **Short term exposure limit:** 2 mg/m^3
Potassium Permanganate $KMnO_4$	H272: May intensify fire, oxidizer. H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H410: Very toxic to aquatic life with long lasting effects.	**Direct contact with eyes:** Rinse thoroughly with plenty of water for at least 15 min and consult a physician. **Direct contact with skin:** Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Consult a physician. **Ingestion:** Do not induce vomiting! Never give anything by mouth to an unconscious person. Rinse mouth with water and consult a physician. **Inhalation:** In breathed, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.	**Long term exposure limit:** 0.5 mg/m^3 **Short term exposure limit:** None
Sodium Oxalate $Na_2C_2O_4$	H302+H312: Harmful if swallowed or in contact with skin.	**Direct contact with eyes:** Flush eyes with water as a precaution. **Direct contact with skin:** Wash off with soap and	None

		plenty of water. Consult a physician. **Ingestion:** Call a Poison Center/doctor if you feel unwell. Rinse mouth. **Inhalation:** In breathed, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.	
Sulfuric Acid (6.0%) **H_2SO_4**	H290: May be corrosive to metals. H314: Causes skin burns and eye damage.	**Direct contact with eyes:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rising. Consult a physician. **Direct contact with skin:** Take off immediately all contaminated clothing. Rinse skin with water. **Ingestion:** Do not induce vomiting! Never give anything by mouth to an unconscious person. Rinse mouth with water and consult a physician. **Inhalation:** In breathed, move person into fresh air. If not breathing, give artificial respiration. Immediately call a Poison Center/doctor.	**Long term exposure limit:** 0.05 mg/m^3 **Short term exposure limit:** None
Do any substances listed require health surveillance or workplace monitoring?			
Substance		**Details of surveillance required**	

Step 2: List those people who may be at risk of exposure

The following groups of people may be exposed to substances hazardous to health during normal operations; ☐ **Undergraduate students** ☑ **Post graduate students** ☑ **Post doctoral researcher** ☑ **Technical support staff** ☑ **Academic staff** ☑ **Room occupants not carrying out the task** ☐ **Other (specify)**
Describe the level, type and duration of exposures likely to occur during routine operations[2] (you must include the handling and disposal of any wastes generated during the work); Potassium Permanganate waste must not be poured down the sink. To potassium permanganate solution add methanol and then dispose to the chemical waste container.
Describe foreseeable accidental exposure scenarios (e.g. spillage on bench). Spillage on workbench, clothing, skin, eyes, mouth. Inhalation or swallowing.
The following groups of people may be exposed to substances hazardous to health during foreseeable accident scenarios; ☐ **Undergraduate students** ☑ **Post graduate students** ☑ **Post doctoral researcher** ☑ **Technical support staff** ☑ **Academic staff** ☑ **Room occupants not carrying out the task** ☐ **Other (specify)**

[2] A separate COSHH assessment may be necessary to cover maintenance operations

Flammable or explosive substances:

Does the task use or produce substances which could cause fire or explosion? If so then refer to the University's guidance on the control of Dangerous Substances and Explosive Atmospheres Regulations.

Uncontrolled access to the work area:

Do the substances used or produced require the work area to be designated as a 'Hazardous Area' as per the University's Code of Practice on access to hazardous areas for Facilities personnel or contractors.

Step 3: Determine appropriate controls

Regulation 7 of COSHH stipulates that exposure to substances harmful to health MUST be prevented, or where this is not reasonably practicable, adequately controlled.

Provide a statement against each item of the following hierarchy of control giving details of the controls you will adopt or a justification as to why no controls in this category are being implemented.

Your controls must address the routine aspects of the work (including waste handling and disposal) and must reduce the risk of any accidental exposures listed in step 2.

Eliminate No material can be eliminated from this task.
Reduce Diluted substances would be used.
Isolate: Containment

Hazardous substances would be stored in appropriate containers and conditions.
Control: General ventilation to Local Exhaust Ventilation (single point extract close to source to ventilated partial containment) Fumehood would be used when required.
PPE Gloves, eye protection, closed toe footwear, lab coat to be worn at all the times.
Discipline Carry out experiments according to Good Lab Practice (GLP).

Special precautions to be adopted in the event of a spillage;

Immediate actions	**e.g. deploy spill kit, evacuate the area, close the door and raise alert supervisor**
Clean-up procedure	Collect with paper towel, dispose in chemical waste. In case of Fe^{2+} presence or potassium permanganate, dispose in the appropriate waste bin.

Step 4: Complete a COSHH Summary Sheet and develop a Safe System of Work

Summarise the salient points of the assessment on a COSHH Summary sheet and append this to the front of any Operating Procedures which relate to the task.

REMEMBER:

A COSHH assessment will not protect you; it is the adoption of the control measures arising from the assessment into a 'Safe System of Work' which will keep you and your colleagues safe.

Please consider each of the following three elements of a Safe System of Work;

Procedures	**Do you have a written procedure? (If not how are hazard control measures to be communicated to those undertaking the task).** **Are the hazards associated with the task clearly described in the procedure?** **Are the control measures generated by your COSHH assessment clearly identified in the procedure?**
Training and Instruction	**Have all those undertaking the task received an appropriate level of training in relation to any hazards and their control?** **Are suitable warning signs and notices displayed?**
Supervision	**Has an appropriate level of supervision been discussed and agreed?** B – approval and advice required from supervisor prior to work starting