Detection of Vinyl Chloride in Environmental Water Samples

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Abstract

Abstract. write toward the end

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Abbreviations

VOC	Volatile Organic Compound
VC	Vinyl Chloride
PVC	Polyvinyl Chloride
GC	Gas Chromatography
GSC	Gas-solid Chromatography
GLC	Gas-liquid Chromatography
EHP	Environmental Health Perspectives

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Background

Humans produce waste, whether that is as an individual or on an industrial level. A lot of the waste that is being produced, end up in our water supply contaminating it. An example of a toxic waste product that can end up in the water supply is vinyl chloride *(VC)*, one of the primary areas VC is found is in the production of PVC. PVC plastic is used as pipes for plumbing, bottles, and more[3]. Vinyl chloride is a highly volatile compound which makes it both hard to detect, and making it more dangerous. This has created a need for a fast and simple solution for detecting the concentration of vinyl chloride in water samples.

This has led the company Water Care Guard *(WCG)* to develop an on-site lab kit that fits in a suitcase, which is able to test a water sample for the concentration of vinyl chloride amongst other substances.

This project is done in collaboration with Roana from SDU Nano Syd and Water Care Guard.

Problem

As it stands at the moment, when you want to detect vinyl chloride in water samples you use a method of analysis called *"Gas chromatographic (GC) analysis"*[2]. Gas chromatographic analysis is an analysis method in which the sample is being heated to the point of each component in the sample is being vaporized, where it then enters a column where the different components are being separated, such that it can be detected.[4] The process of sending a water sample to a laboratory and getting the measurements made, is time-consuming and expensive. It can take up to two weeks to get a water sample analyzed in a laboratory.[5] The solution with Water Care Guard, aims to reduce this time, by creating an onsite *laboratory kit*, where you can test the water sample. It is using the fact that the refractive index of the solution with added enzyme is dependent on the concentration of vinyl chloride in the solution. The refractive index can be determined using a spectrophotometer and a cuvette with a photonic crystal applied to one side.[1]

The main part of the project is going to be about building a database for different vinyl chloride concentrations and the refractive index shift for the corresponding vinyl chloride concentration. In addition, the data from the database should be analyzed to be able to get a prediction for the concentration of vinyl chloride in the solution based on the sample given.

The second part of the project is building a simplified user interface for the suitcase, which could be installed on a tablet in the suitcase or another display that the user can interact with. It could even be used on the smartphone of the user, or something else. This could be accomplished by making a dedicated app, or by having a website that is hosted on a computer in the suitcase, this would allow the user to access the user interface from either a tablet in the suitcase or from their computer or smartphone.

Timetable and milestones

The project is divided into 3 main parts:

- Building the database
- Analyzing the data
- User interface

The different parts are going to overlap, but it is primarily going to be in the order of the first part is focusing on building the database then about finding a way to analyze the data, and lastly building the user interface.
The project is divided up with the intention of spending 8 hours per day, 5 days a week on the project i.e. 40 hours a week.

Gantt chart

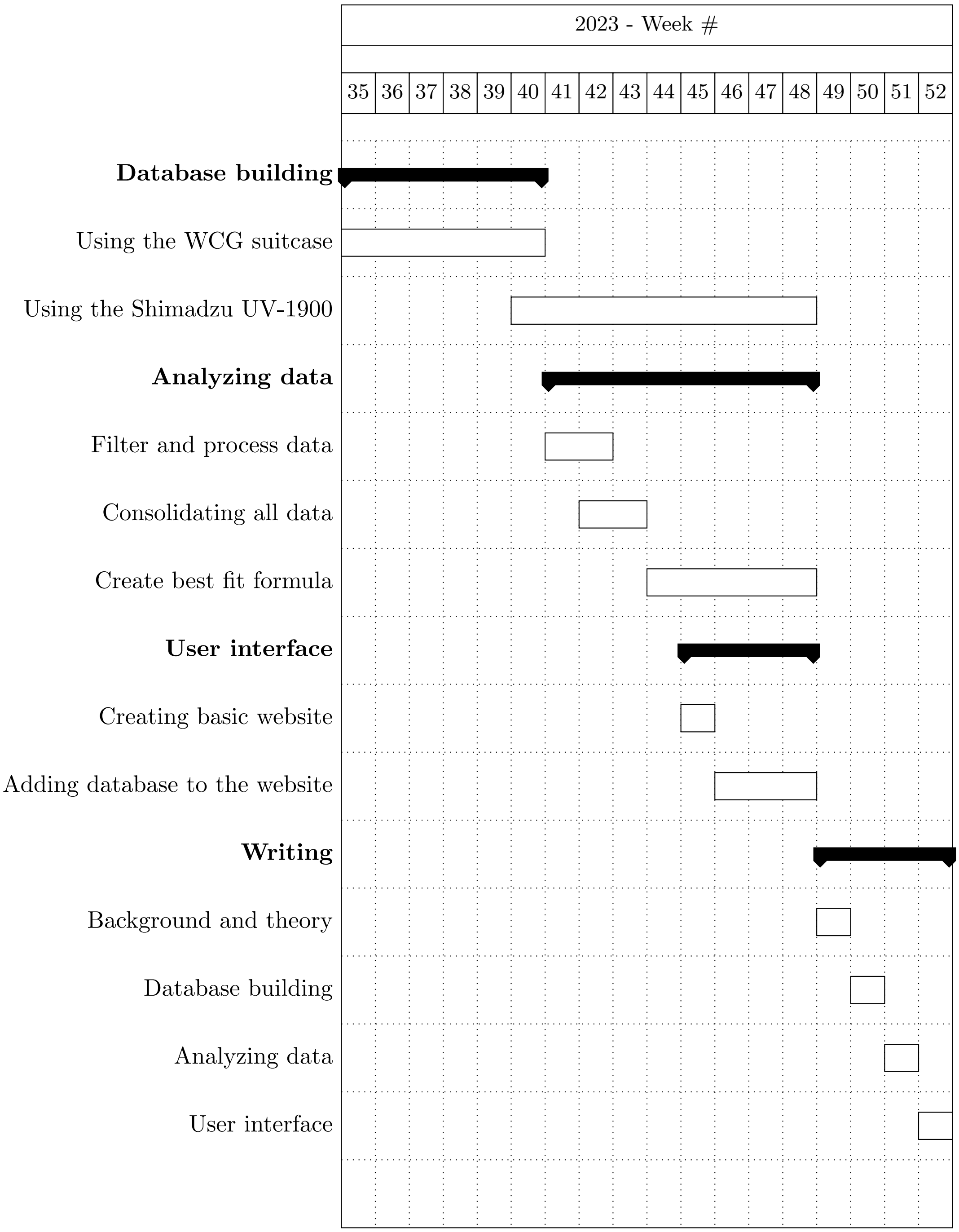

Figure 1: The timetable in a Gantt chart format, divided up in weeks

Risk assessment

The primary risk associated with the project is regards to dealing with the chemicals in making the measurements.

Issue	Who is involved	Impact/Probability	Mitigative actions
High volatility and toxicity of Vinyl Chloride. Working with the chemicals	Operator performing the measurements	High/High	Using: - labcoat - safety glasses - working in a fume hood

Table 1: The risk assessment table

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1 Introduction

1.1 Background

Humans have been producing waste for a long time, but as the materials that we use change so has our waste. In the early 20th century with the invention of fully synthetic plastic by the Belgian chemist Leo Baekeland in 1907[1], started a new type of anthropogenic pollution. One type of plastic known as Polyvinyl Chloride (PVC), which was first synthesized in 1872 by Dr. Eugen Baumann[2], it was first plastasized by Dr. Waldo L. Semon in 1926[3]. The introduction of plastics and especially PVC plastics, has introduced a new biproduct which is a highly toxic volatile organic compound (VOC), called Vinyl Chloride (VC). Vinyl chloride is a biproduct in the production of PVC plastics, from it being used as the main component in the production of PVC plastics. PVC plastics are used in a lot of different areas like construction piping, packaging, wires, toys, etc. in figure 1 some examples of PVC applications are shown.

Figure 1: Applications for PVC plastic. [4]

People can be exposed to vinyl chloride in different ways, through inhalating contaminated air, contaminated water etc. If vinyl chloride contaminate a water supply to a household, it can contaminate the air in the household leading to the inhabitants being exposed to vinyl chloride. [5] One of the main dangers with exposure to vinyl chloride is the increased risk of cancer, and in particular liver cancer.[5] In addition according to *"kemibrug.dk"*[1], vinyl chloride can also affect the central nervous system with symptoms like headache, dizziness, nausea and a possibility of loss of conscioiusness, as well as the inhalation the chemical is also easily absorbed through the skin which can lead to similar effects as of those from inhilation.[6]

At the moment the primary way to analyze whether there is vinyl chloride present in a water sample is through gas chromatography. According to an article from F.J Santos and M.T Galceran some of the advantages of gas chromatography are that is has a very high selectivity and resolution, making it easier to detect even small quantities of vinyl chloride in the sample, in addition GC has a good accuracy and precision.[7]

Gas chromatography is a physical process where a mixture of different substances are separated into their different parts.[8] There are two primary types of gas chromatography, gas-solid chromatography (GSC) and gas-liquid chromatography (GLC), in gas-solid chromatography it is about the absorbtion of the sample on the solid and with gas-liquid chromatography it is about the solubility of the sample to the liquid. [9] In the case of detecting the vinyl chloride it is GLC that is being used, since the sample to be tested for the concentration of vinyl chloride is usually water. According to the "Environmental Health Perspectives (EHP)" some of the areas that there have been shown high levels of VC are "soil, groundwater, aquifiers, and wells near landfill and industrial waste disposal sites"[10]. Gas chromatography works by having a sample that is to be analyzed, that is injected into a moving gas stream. It is then being carried down a column by a liquid with a low volatility, the sample is then separated into its different parts because the absorptivities and solubilities of the different parts differ making them arrive at different rates which makes it possible for the detector at the end to get a reading.[9] The process is illustrated in figure 2.

[1] A database for information regarding chemicals

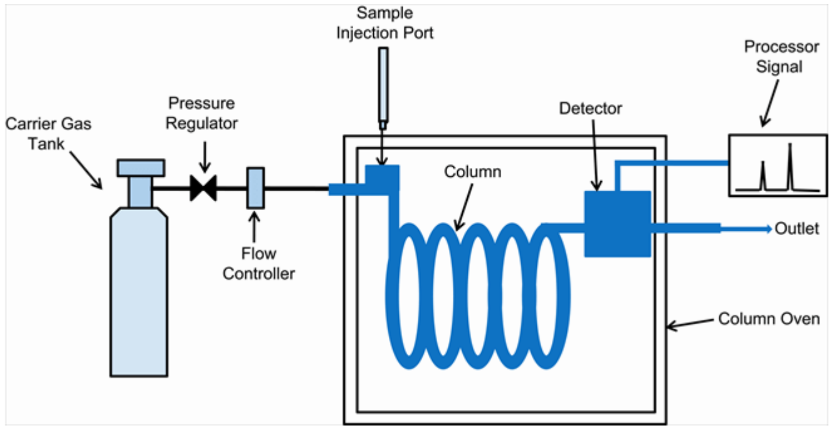

Figure 2: Schematic illustration of a gas chromatography system. [11]

A gas chromatograph is an expensive piece of laboratory equipment[12], in addition it can take up to two weeks to get a sample analyzed in a laboratory[13]. This is why there is a need to be able to get a faster on-site detection of vinyl chloride concentration in water samples. This is what the company Water Care Guard[2] is working towards, with their suitcase laboratory. Instead of using GC for detecting the vinyl chloride, it is using an enzymatic reaction between the vinyl chloride and an enzyme (Cytochromes P450), this enzymatic reaction changes the refractive index of the solution over time where the shift in the refractive index from the starting point is related to the concentration of the vinyl chloride in the solution.[14][15].

[2] `https://www.watercareguard.com/`

2 Theory

This section will be explaining the relevant concepts, for the method that is used for detecting vinyl chloride in the Water Care Guard suitcase.

2.1 What is *Refractive index*?

The definition of refractive index from The Britannica Encyclopaedia is:

”measure of the bending of a ray of light when passing
from one medium into another”[16]

The refractive index is defined as the the sine of the angle of incidence to the sine of the angle of refraction.[16] Equation 1 shows how the refractive index is calculated as either the ratio of the sine of the angles or as the ratio of the speed of light in a vacuum (c) over the speed of light in the medium (v).[17] The angles, i and r are shown in figure 3.

$$n = \frac{\sin i}{\sin r} = \frac{c}{v} \tag{1}$$

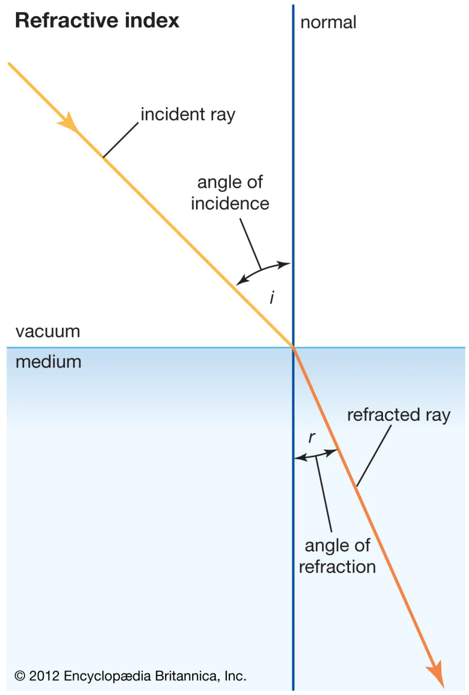

Figure 3: An illustration of refractive index[16]

When the vinyl chloride reacts with the enzyme Cytochrome P450, the refractive index of the solution changes with time as the components are reacting[14]. Thus the refractive index can be used to determine the concentration of the vinyl chloride in the solution, as a more affordable alternative to using gas chromatography.

2.2 The photonic cystals, a tool for measuring the refractive index

3 Building a database

3.1 Measuring vinyl chloride in a sample

3.1.1 General methodology

The methodology is derived from the methodology shown in the appendix 6.2, with changes depending on the specific equipment and concentration values.

During the measurement gathering process, there was a procedure that was followed for all the measurements, it was adapted slightly depending on what instrument the measurement was done on. Firstly the instrument was turned, and proceeding with the steps only when the instrument had heated up. With the Water Care Guard suitcase or with the Ocean Optics UV-650 UV-VIS™ the heat up wait was a fixed 15 minute wait, because those instruments did not have a heating status indicator telling when it was finished heating up. On the other hand with the Shimadzu UV-1900™ there is a light on the front indicating the status, if it is yellow it is still heating and green when it is ready, this is indicated in figure 4.

(a) Shimadzu UV-1900 warming up indicator

(b) Shimadzu UV-1900 ready and done warming up

Figure 4: Shimadzu UV-1900 status indicator

After the instrument has heated up, a water reference measurement is then completed in the SpectroWorks™ software by Copenhagen Nanosystems (cphnano), with 2-3ml of DI water in the Nanocuvette^{TM} One. The steps after this is then to prepare the measurements of vinyl chloride and DI water.

1. 10 ml of DI water is added to a glass vial
2. Added the corresponding volume of vinyl chloride to get the desired concentration. (e.g. 25 μl to get a concentration of $4\mu l/ml$)
3. Then gently shaking the vinyl chloride and DI water solution for approximately 60 seconds.
4. Takeing the enzyme Cytochrome P450 out of the freezer and start a stopwatch, to prevent the enzyme from being out of the freezer for more than 15 minutes.
5. Weighed 1.5-2mg of the enzyme.
6. Then added a proportional amount of DI water to enzyme, meaning 1.5 mg of enzyme would require 1.5 ml of DI water.
7. The enzyme and DI solution was then stirred gently for 30 seconds, until there were no visible flakes of enzyme in the solution.
8. Using a molar concentration of vinyl chloride to enzyme of 1.29×10^5, which is derived from the procedure sheet shown in the appendix 6.2. The concentration was multiplied by a factor of 1-10×, depending on the results of the previous experiment.
9. When the enzyme had been added to the vinyl chloride solution, a stopwatch was started.
10. The solution was gently shaken for approximately 30 seconds.
11. 3 ml of this solution was then pipetted into a Nanocuvette™ One.
12. The Nanocuvette™ One was then transferred into the spectrophotometer, starting with the A-side measurement, without the photonic crystal, in figure 5 an illustration shows the Nanocuvette^{TM} and the two sides of it.

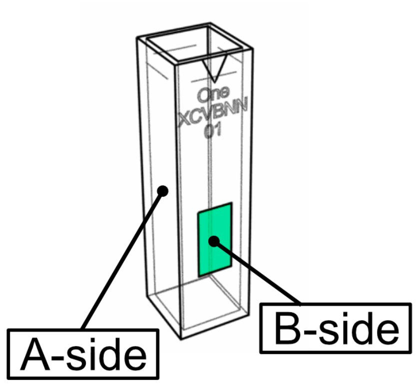

Figure 5: A Nanocuvette™ One from cphnano[18]

13. In the SpectroWorks™ software the water reference measurement done with DI water earlier, is selected.
14. The A-side measurement was then carried out, according to the steps provided by the SpectroWorks™ software.
15. The Nanocuvette™ One was then turned around to the B-side and the measurement was carried out again.
16. The details for the measurement was then entered into SpectroWorks™
    - Measurement number
    - Current time (in minutes)
    - DI volume
    - Vinyl chloride volume
    - Enzyme mass
17. The steps 12 to 16 was then carried with an approximate time gab of 1-2 minutes, until 60 minutes had passed.

3.1.2 Measuring with Water Care Guard suitcase

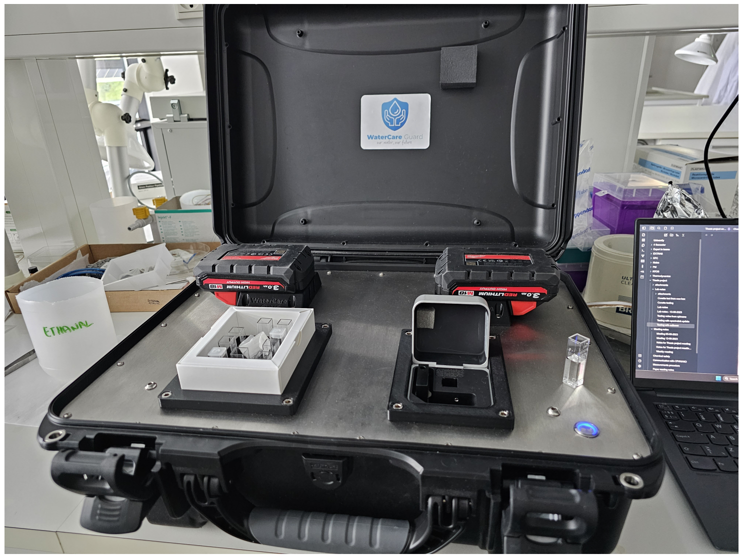

Figure 6: The Water Care Guard suitcase in the chemistry lab at SDU

3.1.3 Measuring with Shimadzu UV-1900

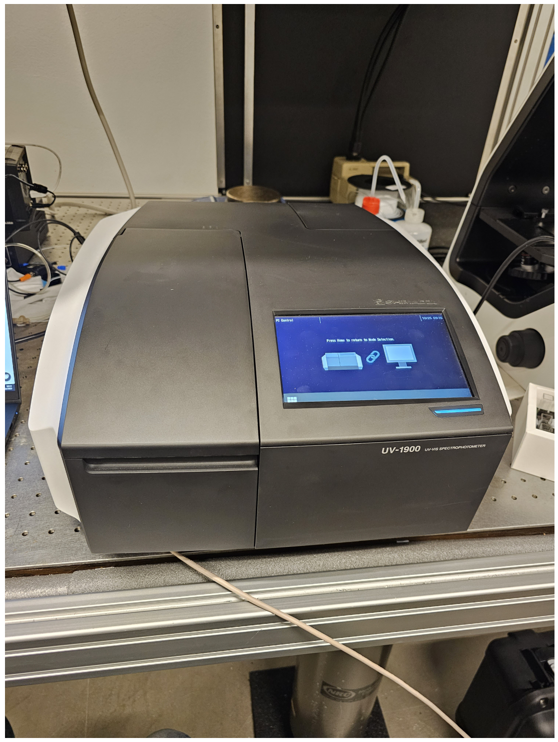

Figure 7: Shimadzu UV-1900 spectrophotometer in the photonics lab at SDU

3.2 Data analysis

3.3 Problems

4 User interface

The goal for the user interface was to have a simple solution for the user to be able to get the results of their measurements, without needing to understand the full program of SpectroWorks™ which is the backend for the handling the data, and taking the measurements.
SpectroWorks™ by cphnano is made to be able to handle a variety of measurement types and goals, like standard UV-Vis measurements, protein and enzyme concentrations, refractive index, particle size distributions etc.[19]. This is why the idea behind the user interface was to make it a simplified interface with SpectroWorks™ that was just a simple button to start the measurement and a display of the results.

4.1 API Interface

The API interface is written based on the python library for the API interface for SpectroWorks™ written by cphnano[20], it is written to be tailored to the needs of this project in an attempt to improve the performance of the system. A full flowchart explaining how the program works with both the graphical user interface and the API interface is shown in the appendix 6.1 and in figure 10.

The first thing that the API inteface does is to establish a connection with the SpectroWorks™ API, an API key has been made in the SpectroWorks™ software that is being used to ensure that we are allowed to access the data. In SpectroWorks™ we are able to define what projects the specific API key has access to, although in the API interface written, we have the option of choosing what project we are focusing on.

When the connection parameters has been set we are going to get a list of the projects available using the the API key, we then filter those projects and use only the ones we are interested in. The get_project method of the Connection class shown in code sample 1, uses the connection parameters to send a request to the SpectroWorks™ API, requesting a list of all the projects, they are then filtered based on if they are a part of the requested projects, it then returns the a Project class.

```python
def get_project(self, project_name: str = None) -> Project:
    # load projects
    ## If you haven't loaded the projects earlier
    if self.projects is None:
        # Get response
        res = requests.get(
            self.url + 'list_projects',      # Determine request
            params={'api_key': self.api_key} # Add API key
        )
        if res.status_code != 200: # Response is not OK (200)
            # Throw an error, saying the connection is not successful.
            raise ConnectionError(json.loads(res.text)['message'])
        projects = json.loads(res.text)['message']['items']
        # Loop through the projects and filter based on our parameters
        for i, project in enumerate(projects):
            if project['project_name'] == project_name:
                return Project(self, projects[i])
```

Code 1: get_project method in the Connection class, written in Python.

In the projects class we can then get the properties of the Project, as well as getting the items associated with the given project. An item is every individual measurement stored in SpectroWorks™. An example of an item as it is stored in SpectroWorks™ is shown below in figure 8, in the image you can see the inputted sample attributes on the left side and the results of the measurements on the right side.

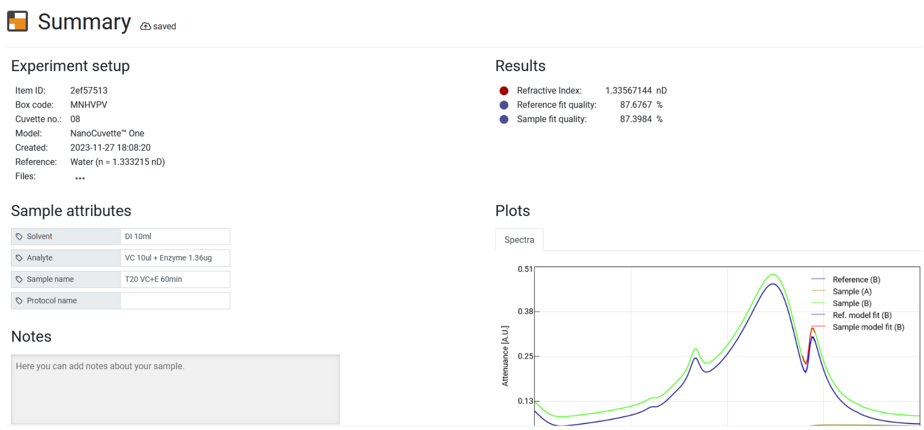

Figure 8: An item as shown in the SpectroWorks™ interface[21]

Custom parameters for an item has been written into the sample parameters, like the volume of DI water, vinyl chloride, and the amount of enzyme in the solution, in addition the number for the measurement is written in with a T prefixed e.g. *T20*, which indicates which measurements are from the same line of measurements, so that we can treat all the measurements with the same T number the same. Lastly in the Sample name the time of the specific measurement is written as well, all these values can be extracted by the api interface program, the program uses a regular expression to extract the values.

```python
## Test number
test_number = re.findall(r'T\d+', self.sample_name)
self.test_number = int(test_number[0][1:]) if test_number else None

## Measurement number
measurement_time = re.findall(r'\d+\.?\d*min', self.sample_name)
self.measurement_time = float(measurement_time[0][:-3]) if measurement_time else None
```

Code 2: Example of getting the custom parameters using a regular expression from the sample attributes of the item.

Using the `Project` class and `Item` class the program can get all the values it needs to be able to analyze the data.

The code for the SpectroWorks™ API interface and the website can be found on my github page (`https://github.com/Philip-Jorgensen/wcg_final_project`)

4.2 Graphical User Interface

The graphical user interface is written as a locally hosted website, due to it being more accessible and easier to work with as the user. As opposed to an application, a website can be accessed from any type of device assuming the device has access to the local network of the computer hosting the website. This could mean how a tablet in the suitcase could be used as the interface for operating the Water Care Guard suitcase, an example for this is shown in figure 9 or the interface could be accessed from the users phone or computer.

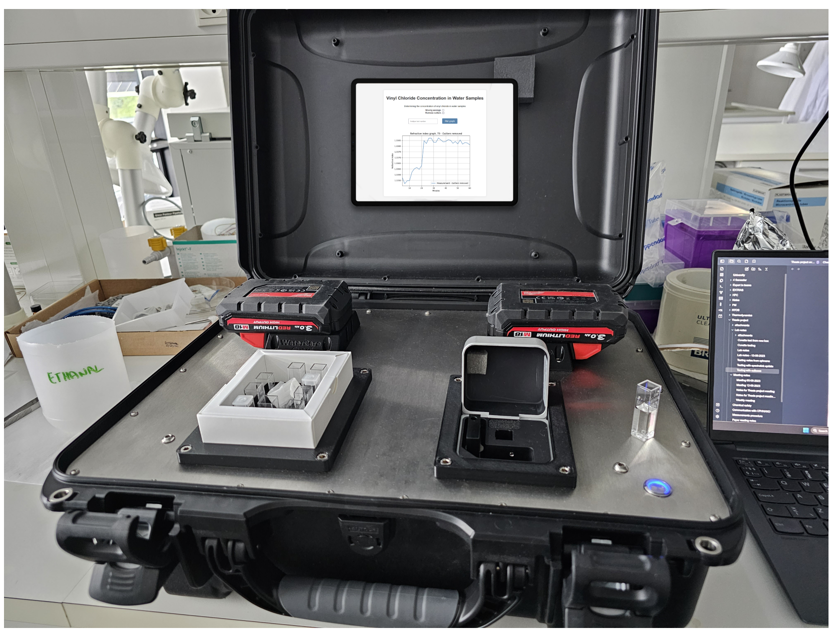

Figure 9: Illustration of the Water Care Guard suitcase with an iPad with the Graphical user interface.

The website has been written with Python as the backend with the python framework Flask[3], in addition the front end of the website is written in HTML

[3] `https://github.com/pallets/flask/`

and CSS. The code for the graphical user interface can be found on my github (`https://github.com/Philip-Jorgensen/wcg_final_project/tree/main/gui/flask_based`).

5 Conclusion

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6 Appendix

6.1 spectroworks_api_interface.py

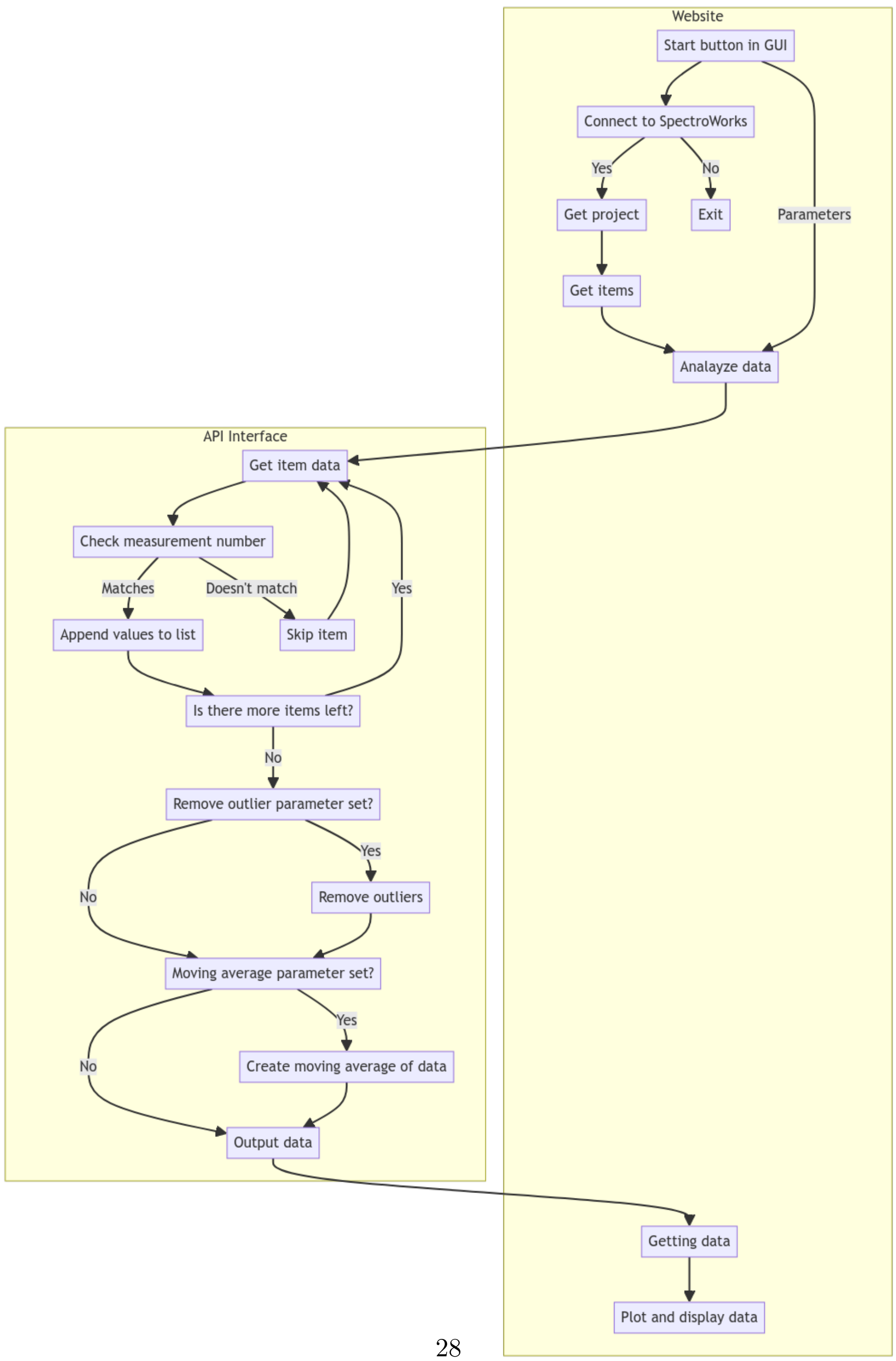

Figure 10: The flowchart for the website and SpectroWorks™ api interface

6.2 Protocol how to prepare VC + Enzyme concentration in water based on molar ratio 1:6 and measure the RI

Protocol how to prepare VC + Enzyme concentration in water based on molar ratio 1:6 and measure the RI

Working with enzyme should be done using protective clothing which include gloves, safety glasses and lab coats. Furthermore, enzyme activity can be affected by a variety of factors, such as temperature, pH and concentration and sub-optimal conditions can cause an enzyme to lose its ability to bind to a substrate. Avoid over vortexing of enzymes and prepare the fresh batch for each of your experiment. Enzymes should be stored as recommended by the suppliers. However, aliquots of enzymes should avoid constant freezing and thawing of the products.

Method for Ratio of 1:6

Materials required

1. 20 µl of Vinyl chloride (2000 µg/ml in Methanol)
2. 2.5 ml of DI water for cuvette and 2 ml of water to prepare enzyme
3. 2 mg of Enzyme PH450 in 2 ml of water from where we extract and use 0.227 µl

Equipment

1. Spectrophotometer
2. Micropipette and plastic caps
3. Nano-cuvette™ One
4. Spectro-link
5. PC/tablet/laptop

Procedure to measure refractive index using Nano-cuvette One and Spectro-works

Measure RI for water to use it as a Reference in the “Choose reference” list.

1. Turn on the spectrophotometer and stabilize it by letting it sit for at least 15 minutes before running any samples.
2. Set up the spectrophotometer for “number of scans” and “Integration time”
3. Choose “Create” and select the cuvette type.
4. Type in the cuvette batch and select the cuvette number.
5. Pipette 2,5 mL of reference DI water in Nano-cuvette™ One.
6. From the “reference list” choose the reference liquid to be water.
7. Insert the reference sample (water) cuvette into the sample chamber of spectrophotometer and run the B side measurement first (with the photonic crystal).
8. Now it requires to measure the sample, in this case the sample is water. Turn the cuvette 90 degree and run the A side measurement. Next step is to turn the cuvette with the B side again and run the measurement. Don’t forget to name the measurement in the notes as water test.
9. **OBS! Please save the measurement only if the Sample fit quality is greater than 80 %.**

Measure RI for water and VC

1. After you had measured the RI for water (see measure RI for water), you must select from the “choose reference” list the measurement for water which was done before.
2. Pipette the desire 20 µl of VC in the Nano-cuvette™ One. Gently mix the reaction mixture for 30s-1min.
3. Now it requires to measure the B side but **Pres back** to go to back to the A side measurement first and insert the cuvette (with the water and VC) into the sample chamber of spectrophotometer and run the A side measurement first (without the photonic crystal).
4. Next turn the cuvette with 90 degrees from previous position, and run the B side measurement (with the photonic crystal).
5. In the end a summary will be generated where you can find the results for water and VC, experiment setup, a graphic plot and notes. Don’t forget to name the measurement in the notes.
6. Repeat the measurement with the same cuvette re-using the water reference made in the beginning. Make the measurements in the following time points: 0min, 5min, 10min, 15min, 20min 25min, 30min, 35min, 40min, 45min, 50min, 55min and 60min.
7. Save the measurements as water + VC test.

Measure RI for water, VC and enzyme

1. After you had measured the RI for water, and RI for water + VC, you must perform the measurements adding the enzyme in the water + VC sample.
2. Prepare the enzyme (see the enzyme preparation method below)
3. Pouring the 0.227 µl of Enzyme in the cuvette which contain the water 2.5 ml and 20 µl of VC which was tested before.
4. Next step is to select from the "choose reference" list the measurement reference for water you did it before.
5. Now it requires the measure the B side but **Pres back** to go to the A side measurement first and insert the cuvette (with the water, VC and Enzyme) into the sample chamber of spectrophotometer and run the A side measurement first (without the photonic crystal).
6. Next turn the cuvette with 90 degrees from previous position, and run the B side measurement (with the photonic crystal).
7. In the end a summary will be generated where you can find the results, experiment setup, a graphic plot and notes. Don't forget to name the measurement in the notes as water + VC + Enzyme
8. Repeat the measurement with the same cuvette re-using the water reference made in the beginning. Make the measurements in the following time points: 0min, 5min, 10min, 15min, 20min 25min, 30min, 35min, 40min, 45min, 50min, 55min and 60min.
9. Save the measurements as water + VC + Enzyme with the molar ratio.

Calculation of Enzyme and Vinyl Chloride for desired molar ratio 1:6 which means 1 mol of VC to 6 mols of Enzyme

Vinyl Chloride calculation

Measure 10 ul of VC with the pipette.

10 µl = 20 µg (20 x10^{-6} gram), so 20µl of vinyl chloride = 40µg (40 x10^{-6} gram)

Convert 40µg of vinyl chloride into moles

Molar mass of vinyl chloride= 62,498 g/mole

Mass (g)=number of moles/molar mass

Number of moles= mass(g) / molar mass

=40 x10^{-6} grams / 62,498
= 6.4002 x 10^{-7} moles

So 40µg or 20µL of vinyl chloride = 6.4002 micro moles

Enzyme calculation

Take the enzyme out of the freezer and weigh 2 mg of enzyme and add 2 mL of DI water in it. (You can make this mixture in advance but don't prepare more than half an hour before you use the dissolved enzyme).

Put the rest of enzyme back in the freezer and don't leave them outside more than 15 min.

Desired molar concentration of enzyme = 6 x 2,4999 micro mole = 14,999 micro moles

Molar mass of enzyme = 55 kDa = 55000 g/mole

Mass (g)=number of moles/molar mass

$=14{,}999 \times 10^{-3} / 55000$
$= 0{,}272 \times 10^{-6}$ gram = 0,272 µg enzymes

=> 0,272 µg of enzyme = 14,999 micro moles

Enzyme stock = 1 mg in 1 mL = 1 µg in 1 µL

If 1 µg = 1µL then, 0,272µg = 0,272µL = 0,272µL enzymes

So 0,272µg = 0,272µL of enzyme= 14,99 micro moles

Caution: Working with enzyme should be done using protective clothing which include gloves, safety glasses and lab coats. Furthermore, enzyme activity can be affected by a variety of factors, such as temperature, pH and concentration and sub-optimal conditions can cause an enzyme to lose its ability to bind to a substrate.

Note: Always use a new plastic cap before using the micropipette!